

2005

Meeting of Medical Fellows

Research Training Fellowships for Medical Students

Program *and* Abstracts
May 9–11, 2005



HHMI

HOWARD HUGHES MEDICAL INSTITUTE

Office of Grants and Special Programs

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Cover: Modeling inflammation in the heart. Adoptive transfer of ovalbumin-specific cytotoxic T lymphocytes (CTLs) into mice expressing ovalbumin in cardiac myocytes results in fulminant myocarditis with prominent lymphocytic and granulocytic infiltrate, myocyte necrosis, and thrombus formation (H&E stain). Transferred CTLs can be genetically manipulated to study trafficking and activation requirements of CD8⁺ T cells in autoimmune disease and allograft rejection. See abstract on page 67. (Courtesy of Viviany Rodrigues Taqueti, Lichtman Laboratory)

INTRODUCTION

Welcome to the 2005 Meeting of Medical Student Fellows of the Howard Hughes Medical Institute. HHMI launched the Research Training Fellowships for Medical Students Program in 1989 to expand the nation's pool of medically trained researchers. The goal of the program is to afford exceptional students the opportunity to obtain high-quality training in leading laboratories and to gain new perspectives in the biomedical sciences. Since the beginning of this program, HHMI has supported more than 900 medical fellows at medical centers and other research institutions. This year, 66 medical fellows will be presenting their research. This book contains the schedule and abstracts for all the presentations.

We are delighted to have Val Sheffield, M.D., Ph.D., as our keynote speaker this year. Dr. Sheffield is an HHMI investigator and professor of pediatrics at the University of Iowa's Carver College of Medicine, where he is also director of the Division of Medical Genetics. He will present studies from his laboratory on the function and interactions of genes involved in Bardet-Biedl syndrome (BBS), an autosomal recessive disorder with primary features that include obesity and mental retardation. Since BBS families have a high incidence of diabetes and hypertension, determining the role of these genes in animal models may lead to new insights into other more common human diseases.

In addition, students from the HHMI-NIH Research Scholars Program will be joining the meeting, and 47 will present their research findings from this past year's effort. This sister program to the medical fellows program provides outstanding students at U.S. medical and dental schools the opportunity to receive a year of research training at the National Institutes of Health. The abstracts for the research scholars' posters, which will be presented Tuesday evening, are also contained in this meeting book.

The medical fellows and research scholars programs are now combined in what we believe is a unique and exciting interaction of medical student research trainees. 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 other future physician-scientists better. To enhance your careers beyond the fellowship year, we have developed the Alumni Network of current and former awardees of HHMI's research training programs. In the past two years, alumni have initiated local networks in Boston, San Francisco, Washington, D.C., Irvine, and New York City. Additional regional programs are expected to come on board soon. We invite you to become involved in the HHMI alumni group nearest you.

This meeting is held each spring so that fellows and research scholars can present their research and exchange ideas. We have grown accustomed to high-quality work from our trainees, and this year's presentations as judged by the abstracts will be no exception. We congratulate you on your scientific accomplishments. We also wish to express our appreciation to your mentors and preceptors, whose commitment to guiding you is clearly evident.

In speaking with a number of former medical student trainees, we are impressed by the pivotal effect that this research training has had on their career development. We hope that you will view your training experience similarly and that you will pursue further research training opportunities and, ultimately, rewarding careers as physician-scientists.

Finally, we are interested in your comments and suggestions regarding both this meeting and the fellowship program in general. Please direct feedback to Anh-Chi Le at (301) 215-8879. You may also send e-mail to grantmed@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 Science Education and
Medical Research Training Program*

PROGRAM SCHEDULE

2005 MEETING OF MEDICAL FELLOWS
HHMI HEADQUARTERS AND CONFERENCE CENTER, CHEVY CHASE, MARYLAND

Monday, May 9, 2005

- 5:30–6:15 p.m. Welcoming Reception, *Great Hall*
- 6:15–7:15 p.m. Dinner, *Dining Room*
- 7:15 p.m. **Welcoming Remarks**, *Auditorium*
Peter J. Bruns, Ph.D., Vice President for Grants and Special Programs,
Howard Hughes Medical Institute
- 7:30 p.m. **Keynote Speaker**, *Auditorium*
*From Human Patients to Animal Models and Back:
The Study of a Human Obesity Syndrome*
Val C. Sheffield, M.D., Ph.D., Investigator, Howard Hughes Medical Institute;
Professor, Department of Pediatrics; and Director, Division of Medical Genetics,
University of Iowa Carver College of Medicine
- Rathskeller open until 11:00 p.m.
-

Tuesday, May 10, 2005

- 7:30 a.m. Breakfast, *Dining Room*
- 8:30 a.m. **Fellows' Presentations**
Molecular Cell Biology, *Room A*
Pharmacology and Physiology, *Room B*
- 10:15–10:30 a.m. Break
- 10:30 a.m. **Fellows' Presentations**
Molecular Cell Biology, *Room A*
Pharmacology and Physiology, *Room B*
- 11:15 a.m. **Fellows' Presentations**
Cancer Biology, *Room A*
Molecular Genetics, *Room B*
- 12:15–12:30 p.m. Break
- 12:30 p.m. **Fellows' Presentations**
Cancer Biology, *Room A*
Molecular Genetics, *Room B*
- 1:45 p.m. Lunch, *Dining Room*
- 6:00–7:00 p.m. **HHMI-NIH Research Scholars' Poster Presentations and Reception**, *Great Hall*
- 7:00–8:00 p.m. Dinner (casual attire) and presentation of certificates, *Dining Room*
- Rathskeller open until 11:00 p.m.

Wednesday, May 11, 2005

- 7:15 a.m. Breakfast, *Dining Room*
- 8:00 a.m. **Fellows' Presentations**
Immunology and Microbiology, *Room A*
Neuroscience, *Room B*
- 9:45–10:15 a.m. **President's Remarks, Auditorium**
The Aviator
Thomas R. Cech, Ph.D., President, Howard Hughes Medical Institute
- 10:30 a.m. **Fellows' Presentations**
Immunology and Microbiology, *Room A*
Cardiovascular Biology, *Room B*
- 12:15 p.m. Lunch, *Dining Room*
- 1:00 p.m. Adjournment

From Human Patients to Animal Models and Back: The Study of a Human Obesity Syndrome**VAL C. SHEFFIELD, M.D., PH.D.**

Investigator, Howard Hughes Medical Institute, and Professor of Pediatrics and Director of the Division of Medical Genetics, University of Iowa Carver College of Medicine, Iowa City, Iowa

■ The identification of genes, sequence variations, and mechanisms involved in complex human disorders holds great promise for improving health care, but at the same time presents a difficult challenge to the scientific community. In order to better understand the genetics of complex human disorders, my laboratory has studied isolated human populations and Mendelian disorders that share a phenotypic component with common complex disorders. We have used isolated human populations to map dozens of disorders and have used positional cloning methods to identify numerous disease-causing genes. This work has provided insight into the types of genes, mutational events, and gene product interactions that are likely to contribute to common complex disorders, and has influenced our approach toward identifying genes involved in polygenic disorders. Recent progress in the laboratory has resulted in the identification of genes involved in a heterogeneous autosomal recessive disorder known as Bardet-Biedl syndrome (BBS).

BBS has the primary features of obesity, retinal degeneration, polydactyly, hypogonadism, renal anomalies, and mental retardation. Secondary features of BBS include diabetes mellitus, hypertension, and congenital heart defects. Obesity generally begins in childhood, and nearly half of all BBS patients develop type II diabetes. The phenotypic features of BBS and the finding that carriers of the disorder may be predisposed to hypertension, diabetes mellitus, and obesity suggest that BBS genes and the biological systems they identify may contribute to common disorders.

Little was known about the etiology of BBS and no BBS genes or loci were identified until we began studying the disorder in isolated Bedouin Arab populations. Surprisingly, BBS proved to be genetically heterogeneous, mapping to three separate loci in this isolated Bedouin population. Studies by us and others have now shown that there are at least eight BBS loci, and my laboratory has used positional cloning methods and mutation analysis of candidate genes to identify five BBS genes (*BBS1*, *BBS2*, *BBS3*, *BBS4*, and *BBS6*) that account for ~80% of known BBS cases. The use of



Photo: Denise Aguiar Crouch

population isolates and the use of haplotype analysis were keys to the identification of BBS genes by making it possible to map the individual loci and narrow the disease intervals even in the presence of extensive genetic heterogeneity. Our studies illustrate the value of haplotype comparisons between affected and unaffected individuals in population isolates, as well as the value of a large collection of unrelated probands from diverse populations for mutation analysis to confirm disease causation.

The extensive genetic heterogeneity of BBS raises the possibility of complex interactions between BBS genes. Sequence analysis of *BBS1*, the gene most commonly involved in this disorder, and the other known BBS genes in a large patient cohort indicates that BBS penetrance is explained by autosomal recessive inheritance. However, the existence of inter- and intra-familial clinical variation suggests that there are genes that modify the BBS phenotype. These modifier genes may be the other BBS genes or genes that encode products that interact with BBS proteins. Identification of modifier genes may contribute to the understanding of common disorders associated with BBS, such as obesity and diabetes.

Current efforts in the laboratory are aimed at determining the function and interactions of the known BBS genes and developing BBS animal models, which will be valuable in identifying interactions between the protein products of these genes, as well as interactions with other proteins. We have generated knockout mouse models for *BBS2*, *BBS4*, and *BBS6*. We show that mice lacking gene expression have major components of the human phenotype, including obesity and hypertension. In addition, these mice have phenotypes associated with cilia dysfunction, including retinopathy, renal cysts, male infertility, and a deficit in olfaction. We demonstrate that *BBS2* retinopathy involves apparently normal retina development,

followed by apoptotic death of photoreceptors, the primary ciliated cells of the retina. Photoreceptor cell death is preceded by mislocalization of rhodopsin, indicating a defect in intracellular transport. The evaluation of *Bbs* gene knockout mice indicates additional phenotypes that should be evaluated in human patients. Finally, using zebrafish, we have demonstrated that knocking down the expression of BBS genes leads to abnormal intracellular transport, a finding that suggests a common mechanism involved in BBS pathogenesis.

Dr. Sheffield received his M.D. and Ph.D. degrees from the University of Chicago. He completed a pediatric residency, a medical genetics fellowship, and

postdoctoral work at the University of California, San Francisco. He continues to practice clinical medicine. His research interests are focused on the identification of genes and disease mechanisms involved in Mendelian and complex human genetic disorders, including glaucoma, macular degeneration, autism, obesity, and cardiovascular disorders. Early in his career, Dr. Sheffield developed and improved methods for identifying single-base variation in DNA. His laboratory contributed to the completion of the first major goal of the Human Genome Project, the creation of a high-density genetic map of the whole genome. His work on human genetic disease has resulted in the mapping of over 35 genetic disorders and the identification of 16 disease-causing genes.

SCHEDULE OF PRESENTATIONS

TUESDAY
ROOM A

Molecular Cell Biology

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- 8:30 a.m.** Characterization of *peroxisome proliferator activated receptor gamma coactivator-1 α* (PGC-1 α)-mediated mitochondrial biogenesis
Lauren Marie Kim, Washington University School of Medicine (Daniel P. Kelly, M.D.)
- 8:45 a.m.** Suppression of diacylglycerol acyl transferase 2 expression using antisense oligonucleotides improves hepatic steatosis and protects against fat-induced insulin resistance
Ameya Ravindrakumar Kulkarni, Yale University School of Medicine (Gerald I. Shulman, M.D., Ph.D.)
- 9:00 a.m.** Effects of ghrelin and insulin on phosphoinositide 3-kinase signaling in the hypothalamus
Tracy Susan Tylee, University of Washington School of Medicine (David E. Cummings, M.D.)
- 9:15 a.m.** Evidence of differential gene expression in various tissues and their correlation to insulin resistance
Oscar I. Gonzalez, Stanford University School of Medicine (Philip S. Tsao, Ph.D., and Gerald M. Reaven, M.D.)
- 9:30 a.m.** Characterization of the MAP functions of the RP1 protein
Nicole Benitah, University of California, San Francisco, School of Medicine (Eric A. Pierce, M.D., Ph.D.)
- 9:45 a.m.** Ankyrin-B syndrome: physiological and molecular studies
Hui Xue, Duke University School of Medicine (Vann Bennett, M.D., Ph.D.)
- 10:00 a.m.** The role of the integrin $\alpha\text{v}\beta\text{6}$ in biliary fibrosis
Bruce Mao Zheng Wang, University of California, San Francisco, School of Medicine (D. Montgomery Bissell, M.D.)
- 10:15-10:30 a.m.** Break
- 10:30 a.m.** Mitochondrial uncoupling protein 2 modifies the response to oxidative stress in neural stem cells
Neal Matsumori Rao, University of Colorado School of Medicine (Michael W. Zawada, Ph.D.)
- 10:45 a.m.** Biliverdin reductase and cell stress
Daniel Smith Higginson, Johns Hopkins University School of Medicine (Solomon H. Snyder, M.D.)
- 11:00 a.m.** Cytoprotective mechanisms of heme oxygenase-1 in bronchopulmonary dysplasia
Sheila Naghshineh, Harvard Medical School (Stella Kourembanas, M.D.)

- 11:15 a.m.** Mechanistic studies of the radiosensitizing properties of NESP
Kevin Forsythe, Stanford University School of Medicine (Susan J. Knox, M.D., Ph.D.)
- 11:30 a.m.** Hypoxia-inducible factor-1 upregulation is mediated by the phosphatidylinositol-3-kinase signaling pathway in head and neck squamous cell carcinoma
Charles J. Lin, University of Pittsburgh School of Medicine (Jennifer R. Grandis, M.D., and Stephen Y. Lai, M.D., Ph.D.)
- 11:45 a.m.** Aromatase inhibitors in human lung cancer therapy
Olga K. Weinberg, Vanderbilt University School of Medicine (Richard J. Pietras, M.D., Ph.D.)
- Noon** Interrogation and targeting of epidermal growth factor receptors and integrins in glioblastomas
Yolanda T. Chik, Duke University School of Medicine (Henry S. Friedman, M.D., and Jeremy N. Rich, M.D.)
- 12:15–12:30 p.m.** Break
- 12:30 p.m.** Targeted delivery of cationic nanoparticles against the vasculature of a glioblastoma multiforme
Leroy Sims, Stanford University School of Medicine (Griffith R. Harsh, M.D., and Samira Guccione, Ph.D.)
- 12:45 p.m.** The state of eIF4GI and eIF4GII in malignant tissues and the role of protease 2A in internal ribosome entry site-mediated translation
Rachel Nicole Grisham, Duke University School of Medicine (Matthias Gromeier, M.D.)
- 1:00 p.m.** Evaluation of HER2 expressing viral vectors in a tumor-bearing human HER2 transgenic mouse model
Gabriel Tsing-Tzong Chong, Duke University School of Medicine (Herbert K. Lyerly, M.D., and Timothy M. Clay, Ph.D.)
- 1:15 p.m.** Examination of the c-Myc pathway
Clara Kim, Brown Medical School (John Sedivy, Ph.D.)
- 1:30 p.m.** Immune profile in axillary lymph nodes predicts disease-free survival in breast cancer

SCHEDULE OF PRESENTATIONS

TUESDAY
ROOM B

Pharmacology and Physiology

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- 8:30 a.m.** Distinct structural determinants govern the directional preference for human 17 β -hydroxysteroid dehydrogenases types 1 and 2 in intact cells
Daniel Paul Sherbet, University of Texas Southwestern Medical Center at Dallas Southwestern Medical School (Richard J. Auchus, M.D., Ph.D.)
- 8:45 a.m.** Characterization of the estrogen-related receptor alpha through the use of designer coactivators
Christiane Haeffle, Duke University School of Medicine (Donald P. McDonnell, Ph.D.)
- 9:00 a.m.** Parathyroid hormone regulation of notch signaling during osteoblast maturation
Laura Marie Bevelock, University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School (Nicola C. Partridge, Ph.D.)
- 9:15 a.m.** Optimization of murine adipose-derived mesenchymal cell osteogenesis stimulated with retinoic acid
Matthew Thomas Siedhoff, Stanford University School of Medicine (Michael T. Longaker, M.D.)
- 9:30 a.m.** Age-related changes in murine fracture healing: a biochemical, radiographic, and histomorphometric analysis
Amish Atulbhai Naik, University of Rochester School of Medicine and Dentistry (Regis J. O’Keefe, M.D., Ph.D.)
- 9:45 a.m.** Modulation of endothelial nitric oxide synthase phosphorylation as a molecular mechanism of endothelial dysfunction
Annie Misung Wang, University of Rochester School of Medicine and Dentistry (Paul L. Huang, M.D., Ph.D.)
- 10:00 a.m.** Connexin43 and the cardiac sodium channel SCN5a: effects on impulse propagation and safety factor in cardiac ventricular tissue
Rajan Bahl, New York University School of Medicine (Glenn I. Fishman, M.D.)
- 10:15–10:30 a.m.** Break
- 10:30 a.m.** Aquaporin-4 enhances clearance of fluid from the brain extracellular space: implications for edema associated with cerebral infection and hydrocephalus
Orin Bloch, University of California, San Francisco, School of Medicine (Alan S. Verkman, M.D., Ph.D., and Geoffrey T. Manley, M.D., Ph.D.)
- 10:45 a.m.** P2Y receptor-mediated calcium responses are altered in senescent trabecular meshwork cells
Jessica Chow, Duke University School of Medicine (Fulton Wong, Ph.D., and Pedro Gonzalez, Ph.D.)
- 11:00 a.m.** Shear stress and cyclic strain induce two different vascular cell phenotypes from one mesenchymal cell line
Gordon Miles Riha, Baylor College of Medicine (Johnny Chen, M.D., Ph.D.)

- 11:15 a.m.** Genetic analysis of preterm labor
Lisanne Palomar, Dartmouth Medical School (Louis J. Muglia, M.D., Ph.D.)
- 11:30 a.m.** Androgenetic alopecia: a novel role for liver x receptor β in the regulation of 5α -reductase type 1
Bryan Allan Ong, University of Texas Southwestern Medical Center at Dallas Southwestern Medical School (David J. Mangelsdorf, Ph.D.)
- 11:45 a.m.** Mutations in nuclear-encoded complex III chaperone BCS1L cause Bjornstad syndrome, sensorineural deafness, and pili torti
John Travis Hinson, Harvard Medical School (Jonathan G. Seidman, Ph.D.)
- Noon** CLN8 impacts sphingolipid synthesis
Adam Zucker, Duke University School of Medicine (Rose-Mary Boustany, M.D.)
- 12:15–12:30 p.m.** Break
- 12:30 p.m.** Wnt5a is required for the septation of the cardiac outflow tract in mice
J. Robert Schleiffarth, University of Minnesota Medical School–Twin Cities (Anna Petryk, M.D., and Michael B. O'Connor, Ph.D.)
- 12:45 p.m.** Functional evidence of mismatch repair deficiency in non-obstructive azoospermia
Lei Chu, Baylor College of Medicine (Dolores J. Lamb, Ph.D.)
- 1:00 p.m.** Elucidating the nature of pancreatic β -cell regeneration after the reversal of autoimmunity in spontaneously diabetic NOD mice
Corinna C.D. Franklin, Harvard Medical School (Boris Nikolic, M.D.)
- 1:15 p.m.** RNA-based gene modification of T lymphocytes for adoptive immunotherapy
Isaac O. Karikari, Duke University School of Medicine (John H. Sampson, M.D., Ph.D., and Joseph Nevins, Ph.D.)
- 1:30 p.m.** Deciphering the estrogen receptor histone code
Anna Jadwiga Szary, Harvard Medical School (Myles A. Brown, M.D.)

SCHEDULE OF PRESENTATIONS

TUESDAY
GREAT HALL

Poster Session, 6:00–7:00 p.m.

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Note: Posters presented by HHMI-NIH Research Scholars.

- Poster 1** Identification of differentiation inducing gene products in Glioblastoma multiforme tumor stem cells
Oluwaseun Akeju (John Park, M.D., Ph.D., National Institute of Neurological Disorders and Stroke)
- Poster 2** The role of Wiskott Aldrich syndrome protein in T helper cell function
Joseph Aoki (Pamela Schwartzberg, M.D., Ph.D., National Human Genome Research Institute)
- Poster 3** Triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone): a ribonucleotide reductase inhibitor with potent radiosensitizing properties
Christopher Andrew Barker (Kevin Camphausen, M.D., National Cancer Institute)
- Poster 4** Cancer-wide tissue microarray survey of ezrin and merlin expression
Benjamin Bruce (Chand Khanna, D.V.M., Ph.D., National Cancer Institute)
- Poster 5** The potential of farnesyltransferase inhibitors in Hutchinson-Gilford progeria syndrome
Brian C. Capell (Francis S. Collins, M.D., Ph.D., National Human Genome Research Institute)
- Poster 6** Mining of drug database from developmental therapeutic programs reveals antineoplastics that selectively target P-glycoprotein overexpressing cell lines
Benjamin Fu-Han Chu (Michael M. Gottesman, M.D., National Cancer Institute)
- Poster 7** Characterization of the M-current in hippocampal stratum oriens-alveus lacunosum-moleculare projecting interneurons
Joseph F. Churchill (Chris J. McBain, Ph.D., National Institute of Child Health and Human Development)
- Poster 8** Bortezomib effects on irradiation-induced oral mucositis
Ingrida Dapkute-Marcus (Carter Van Waes, M.D., Ph.D., National Institute on Deafness and Other Communication Disorders)
- Poster 9** Minimizing respiratory variations improves functional connectivity delineation
Jason Diamond (Peter Bandettini, Ph.D., National Institute of Mental Health)
- Poster 10** The role of Rap1 and RalA in Bax activation and translocation to the mitochondria during apoptosis
Brian J. Dlouhy (Richard J. Youle, Ph.D., National Institute of Neurological Disorders and Stroke)
- Poster 11** Exploring the role of EBV-specific CD4⁺ T cells in the pathogenesis of multiple sclerosis
Nancy Edwards (Roland Martin, M.D., National Institute of Neurological Disorders and Stroke)
- Poster 12** Systems-based gene expression analysis highlights the role of NFκB as a mediator of hepatic fibrosis
Eldad Elnekave (Thomas A. Wynn, Ph.D., National Institute for Allergy and Infectious Disease)
- Poster 13** Hereditary spastic paraplegia and spartin: what is the link?
Parvin Fatheddin (Craig Blackstone, M.D., Ph.D., National Institute of Neurological Disorders and Stroke)
- Poster 14** Comprehensive DNA copy number analysis of breast cancer cell line genomes by microarray comparative genomic hybridization
Pauline Funchain (Paul S. Meltzer, M.D., Ph.D., National Human Genome Research Institute)
- Poster 15** Sublethal doses of chemotherapy and irradiation to human squamous cell carcinoma of the head and neck modulate phenotype resulting in enhanced killing by CTL
Alexander Gelbard (James W. Hodge, Ph.D., National Cancer Institute)
- Poster 16** Low-dose nitrite ameliorates myocardial ischemia/reperfusion injury and reduces infarct size in a canine model
Felix M. Gonzalez (Andrew E. Arai, M.D., National Heart, Lung, and Blood Institute)
- Poster 17** Differential effects of full-length and truncated forms of CAIR-1/BAG-3 on cell proliferation and migration and migration-associated gene expression
Elizabeth A. Guancial (Elise C. Kohn, M.D., National Cancer Institute)
- Poster 18** Genetic association of BDNF, DISC1, and the serotonin transporter gene with brain structure in schizophrenic patients
Katherine B. Hobbs (Daniel R. Weinberger, M.D., National Institute of Mental Health)

- Poster 19** The Reelin-Dab 1 signaling pathway: a role in postnatal development of the cerebellum
LeRon Celeste Jackson (Brian W. Howell, Ph.D., National Institute of Neurological Disorders and Stroke)
- Poster 20** Attention-deficit/hyperactivity disorder and behavioral comorbidities in a genetic isolate: linkage between traits and to loci at 4q13, 13q14, 18q21, and 19p13
Mahim Jain (Maximilian Muenke, M.D., National Human Genome Research Institute)
- Poster 21** Proteomic and functional differences in mitochondria of different tissues
D. Thor Johnson (Robert Balaban, Ph.D., National Heart, Lung, and Blood Institute)
- Poster 22** Does the interval between Papanicolaou tests influence the quality of cytology?
Michelle Khan (Mark Schiffman, M.D., Ph.D., National Cancer Institute)
- Poster 23** Induction of a graft-versus-host-like skin disease by intradermal injection of CD8⁺ T-cell receptor (OT-1) transgenic T cells into keratin 14-ovalbumin-expressing transgenic mice
Brian S. Kim (Stephen I. Katz, M.D., Ph.D., National Cancer Institute)
- Poster 24** Differential signaling pathways for low- versus high-dose interleukin-7 in human T cells
Thomas Krupica Jr. (Crystal L. Mackall, M.D., National Cancer Institute)
- Poster 25** Nonsense-mediated decay gene expression analysis in diffuse large B-cell lymphoma
John Lee (Louis Staudt, M.D., Ph.D., National Cancer Institute)
- Poster 26** Characterization of POTE, a prostate and germ-cell specific protein expressed in several breast cancers
Yoomi Lee (Ira Pastan, M.D., National Cancer Institute)
- Poster 27** Heparan sulfate modulates fibroblast growth factor function during submandibular gland branching morphogenesis
Karen M. Likar (Matthew P. Hoffman, Ph.D., National Institute of Dental and Craniofacial Research)
- Poster 28** Plexin D1 signals to guide endothelial cells
Yuliya Linhares (J. Silvio Gutkind, Ph.D., National Institute of Dental and Craniofacial Research)
- Poster 29** Analysis of mammalian SIRT proteins during adipocyte differentiation
Stanley Liu (J. Carl Barrett, Ph.D., National Cancer Institute)
- Poster 30** Activated Rheb regulates growth and cell migration through TOR and DGAP1/IQGAP mediated pathways
Amit R. Majithia (Alan R. Kimmel, Ph.D., National Institute of Diabetes and Digestive and Kidney Diseases)
- Poster 31** Erythropoietin and hypoxic effects on the erythropoietin receptor in HL-1 cardiomyocytes
Kevin P. Martinez (Constance T. Noguchi, Ph.D., National Institute of Diabetes and Digestive and Kidney Diseases)
- Poster 32** Activation of AMP-activated protein kinase (AMPK) by phosphatidylinositol ether lipid analogues (PIAs)
Regan Memmott (Phillip A. Dennis, M.D., Ph.D., National Cancer Institute)
- Poster 33** Transvenous access to the pericardial space: a novel approach to epicardial lead implantation for cardiac resynchronization therapy
Steven R. Mickelsen (Eliot McVeigh, Ph.D., National Heart, Lung, and Blood Institute)
- Poster 34** Intestinal epithelial cell-dendritic cell interactions in the induction of immunity to type 1 reovirus
Carmen Ruzica Mikacenic (Brian L. Kelsall, M.D., National Institute of Allergy and Infectious Diseases)
- Poster 35** Actions of interleukin-15 on natural killer and CD8⁺ memory T cells *in vivo*
Hiral Patel (Thomas A Waldmann, M.D., National Cancer Institute)
- Poster 36** Functional detection of tumor epithelial cell growth via *in vivo* multispectral optical imaging
Jade Quijano (King C. Li, M.D., National Cancer Institute/SAIC-Frederick)
- Poster 37** CD4-dependent and -independent enhancement of CD8 T cell proliferation and effector function induced by CTLA-4 blockade
Anjana Ranganathan (Nicholas P. Restifo, M.D., National Cancer Institute)

SCHEDULE OF PRESENTATIONS

TUESDAY
GREAT HALL

- Poster 38** Isolation of a population of neurogenic, platelet-derived growth factor-responsive progenitors from the embryonic cortex
Rajesh Rao (Ronald McKay, Ph.D., National Institute of Neurological Disorders and Stroke)
- Poster 39** Histone acetylation at the survival motor neuron gene: identifying a potential target for spinal muscular atrophy therapeutics
Melissa L. Russo (Kenneth H. Fischbeck, M.D., National Institute of Neurological Disorders and Stroke)
- Poster 40** TRAIL-expressing adenovirus gene therapy combined with cisplatin results in supra-additive cytotoxicity in thoracic carcinoma cells
Susan Shamimi-Noori (Dao M. Nguyen, M.D., National Cancer Institute)
- Poster 41** Electrospun three-dimensional polycaprolactone nanofibers: candidate scaffold for skeletal muscle tissue engineering
Rabie M. Shanti (Rocky S. Tuan, Ph.D., and Wan-Ju Li, National Institute of Arthritis and Musculoskeletal and Skin Diseases)
- Poster 42** Telomere lengths in tumor infiltrating lymphocytes used for adoptive cell transfer immunotherapy
Xinglei Shen (Richard Hodes, M.D., National Cancer Institute)
- Poster 43** Pulsed-high intensity focused ultrasound enhanced thrombolysis: from *in vitro* mechanisms to *in vivo* evaluation in a novel thrombosis model
Michael J. Stone (Bradford J. Wood, M.D., Clinical Center, National Institutes of Health)
- Poster 44** FKBP-8 is a potential mediator of the pro-survival effects of Notch-1 in glioma cells
Tilak K. Sundaresan (Howard A. Fine, M.D., National Cancer Institute)
- Poster 45** Recombinant human parainfluenza virus type 1 C protein mutants demonstrate increased sensitivity to interferon- β
William C. Van Cleve (Brian R. Murphy, M.D., National Institute of Allergy and Infectious Diseases)
- Poster 46** Characterization of macrophage subpopulations in atherosclerotic disease
Stephen W. Waldo (Howard S. Kruth, M.D., National Heart, Lung, and Blood Institute)
- Poster 47** Self-organization of salivary gland epithelium during branching morphogenesis
Cindy Hsin-yao Wei (Kenneth M. Yamada, M.D., Ph.D., National Institute of Dental and Craniofacial Research)

Immunology and Microbiology

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- 8:00 a.m.** The influence of regulatory T cells on gender- and age-associated susceptibility to experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis
Eytan Moshe Stein, Northwestern University Feinberg School of Medicine (Stephen D. Miller, Ph.D.)
- 8:15 a.m.** Development of non-myeloablative hematopoietic cell transplantation protocols for the treatment of the murine model of the autoimmune disease multiple sclerosis
Mary-Elizabeth Anselmo Muchmore, Stanford University School of Medicine (Judith A. Shizuru, M.D., Ph.D.)
- 8:30 a.m.** The effects of immunomodulatory therapy on FOXP3+ regulatory T cells in autoimmune diabetes
Jason Y. Adams, University of California, San Francisco, School of Medicine (Jeffrey A. Bluestone, Ph.D., and Qizhi Tang, Ph.D.)
- 8:45 a.m.** T cell homing to metastatic melanoma: immune evasion by tumor vessel dysregulation
Karla Nicole Muñoz, Harvard Medical School (Thomas S. Kupper, M.D., and Robert C. Fuhlbrigge, Ph.D., M.D.)
- 9:00 a.m.** Inhibition of HLA-DM: defining the active site and mechanism of HLA-DO
James Joseph Harding, Albert Einstein College of Medicine of Yeshiva University (Elizabeth D. Mellins, M.D.)
- 9:15 a.m.** BCAP serves an immunoregulatory role in macrophage TNF-alpha production in response to LPS stimulation
James Peacock, Vanderbilt University School of Medicine (Steven Greenberg, M.D.)
- 9:30 a.m.** Identification of autoantigens in autoimmune uveitis by subtractive phage display
Anil Vedula, Yale University School of Medicine (Wei Li, Ph.D., and M. Elizabeth Fini, Ph.D.)
- 9:45-10:15 a.m.** President's Remarks, *Auditorium*
- 10:30 a.m.** Role of virulence pathways in *Candida albicans*
Emily Dawn Eads, Duke University School of Medicine (Joseph Heitman, M.D., Ph.D.)
- 10:45 a.m.** CD4⁺ T cell and anti-basal ganglia antibody characterization in response to streptococcal virulence factor immunization
Kyle Allen Williams, University of Minnesota Medical School–Twin Cities (Patrick M. Schlievert, Ph.D.)
- 11:00 a.m.** Dendritic cells transduced with an HIV-derived vector encoding Gag-Pol are able to induce a potent *in vivo* CD4 T cell response
Jeremy Blaine Wingard, Duke University School of Medicine (Drew Weissman, M.D., Ph.D.)
- 11:15 a.m.** T-bet controls CD8⁺ T cell-mediated inflammation in the heart
Viviany Rodrigues Taqueti, Harvard Medical School (Andrew H. Lichtman, M.D., Ph.D.)
- 11:30 a.m.** A novel approach to tolerance induction in cynomolgus monkeys
Bidhan Bihari Das, Yale University School of Medicine (Joren C. Madsen, M.D., D.Phil.)
- 11:45 a.m.** Macrophage depletion suppresses cardiac allograft vasculopathy
William Henry Kitchens Jr., Harvard Medical School (Joren C. Madsen, M.D., D.Phil., and Paul S. Russell, M.D.)
- Noon** The role of mutations in the CXCR4 gene in the pathogenesis of WHIM (warts, hypogammaglobulinemia, infections, myelokathexis) syndrome
Kyle Eash, Washington University School of Medicine (Daniel C. Link, M.D.)

SCHEDULE OF PRESENTATIONS

Neuroscience

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- 8:00 a.m.** Survival of new neurons in the dentate gyrus after trauma to the developing brain
Matthew Bryan Potts, University of California, San Francisco, School of Medicine (Linda J. Noble, Ph.D., and John R. Fike, Ph.D.)
- 8:15 a.m.** Spatio-temporal patterns of mitral and tufted cell dendritic development in the mouse main olfactory bulb
Masha Rand, Yale University School of Medicine (Charles A. Greer, Ph.D.)
- 8:30 a.m.** Evidence for the role of PSD-95 in dopamine receptor signaling and behavioral plasticity
Mark Howard Neely, Duke University School of Medicine (Marc G. Caron, Ph.D.)
- 8:45 a.m.** Characterizing the potential for neurogenesis in the cortex of adult mammals after stroke
Paulina Barbara Sergot, Columbia University College of Physicians and Surgeons (James E. Goldman, M.D., Ph.D., and E. Sander Connolly Jr., M.D.)
- 9:00 a.m.** Selective isolation and gene expression analysis of sprouting neurons after focal cortical stroke
Diana Katsman, University of California, Irvine, College of Medicine (Stanley Thomas Carmichael, M.D., Ph.D.)
- 9:15 a.m.** 12-Lipoxygenase and matrix metalloproteinase-9: potential therapeutic targets for stroke
Sophia Wang, Mount Sinai School of Medicine (Eng H. Lo, Ph.D.)
- 9:30 a.m.** Intracerebrally administered anti-amyloid- β antibodies clear amyloid- β deposition in a transgenic murine model of Alzheimer's amyloidosis
Lewis Zhiyuan Leng, University of Pennsylvania School of Medicine (Virginia M.-Y. Lee, Ph.D.)
- 9:45-10:15 a.m.** President's Remarks, *Auditorium*

Cardiovascular Biology

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- 10:30 a.m.** Gene transfer to tissue engineered blood vessels
Kelley Amber Hutcheson, Duke University School of Medicine (Jeffrey H. Lawson, M.D., Ph.D.)
- 10:45 a.m.** Notch signaling mediates the control of arterial identity by fluid mechanical forces
Johannes R. Kratz, Harvard Medical School (Guillermo Garcia-Cardena, Ph.D.)
- 11:00 a.m.** The role of CX₃CR1 in the arterial response to injury
Sarita Ulhas Patil, Duke University School of Medicine (Dhavalkumar D. Patel, M.D., Ph.D.)
- 11:15 a.m.** STRO-1 positive human mesenchymal stem cells delivered in a fibrin scaffold enhance myocardial neovascularization and cardiogenesis after acute ischemia
Allan Wiley Tulloch Jr., Columbia University College of Physicians and Surgeons (Silviu Itescu, M.D.)
- 11:30 a.m.** Cardioprotective effects of selective β estrogen receptor agonist
Ivana Nikolic, Duke University School of Medicine (Charles Steenbergen, M.D., Ph.D., and Elizabeth Murphy, Ph.D.)
- 11:45 a.m.** Characterization of Z-band alternatively spliced PDZ-motif protein in development of ventricular dysfunction
William Buck Kyle, Cornell University John and Sanford I. Weill Medical College and Graduate School of Medical Sciences (Jeffrey A. Towbin, M.D., and Matteo Vatta, Ph.D.)
- Noon** Amyloid toxicity in heart failure: characterization of the pathology associated with the R120G missense mutation in α B-crystallin
Chet Ridall Villa, University of Cincinnati College of Medicine (Jeffrey Robbins, Ph.D.)

ABSTRACTS OF PRESENTATIONS

TUESDAY
ROOM A

8:30 A.M.

Characterization of peroxisome proliferator activated receptor gamma coactivator-1 α (PGC-1 α)-mediated mitochondrial biogenesis**LAUREN MARIE KIM,*** Washington University School of Medicine

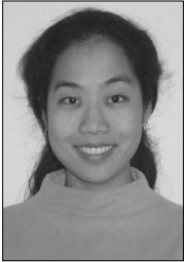
Mentor: Daniel P. Kelly, M.D., Alumni Endowed Professor in Cardiovascular Diseases, Departments of Medicine, of Molecular Biology and Pharmacology, and of Pediatrics, Washington University School of Medicine, St. Louis, Missouri

■ The ability to regulate mitochondrial number and function of mitochondria is essential in supporting the metabolic requirements of postnatal life. Consequently, mitochondrial dysfunction is a central feature of a variety of inherited and acquired diseases. The transcriptional coactivator *peroxisome proliferator activated receptor gamma coactivator-1 α* (PGC-1 α) has been shown to play a regulatory role in mitochondrial biogenesis. One potential mechanism whereby PGC-1 α regulates this process is through the coactivation of *nuclear respiratory factor-1* (NRF-1), a transcription factor known to initiate mitochondrial DNA replication. Additionally, nuclear receptors, including *estrogen-related receptor α* (ERR α) and *peroxisome proliferator-activated receptor α* (PPAR α), have been shown to play a role in PGC-1 α -mediated mitochondrial biogenesis.

We hypothesized that PGC-1 α -mediated mitochondrial biogenesis requires concerted coactivation of nuclear receptors and NRF-1. To localize the region of PGC-1 α essential for NRF-1 coactivation, we used (i) transcriptional assays in cell culture and (ii) GST-protein "pulldowns," an assay to assess direct physical interactions. We determined that NRF-1 maps to the C-terminal region of PGC-1 α (amino acids 338-797), which does not include the nuclear receptor interaction region of PGC-1 α . This knowledge informed the design of PGC-1 α mutants whose interaction with either nuclear receptors or NRF-1 was abolished. These mutants are presently being evaluated in adenoviral-mediated overexpression studies in primary neonatal cardiac myocytes in culture. Our preliminary data suggest that NRF-1 is not required for PGC-1 α -mediated mitochondrial biogenesis. In contrast, PGC-1 α mutants incapable of interacting with ERR α and PPAR α do not support mitochondrial biogenesis.

We are currently performing nuclear receptor gain-of-function studies to complement the PGC-1 α mutant studies. Our results to date indicate that whereas nuclear receptors are necessary for PGC-1 α -mediated mitochondrial biogenesis, NRF-1 is dispensable.

*Second-Year Medical Fellow



L.M. KIM



A.R. KULKARNI

8:45 A.M.

Suppression of diacylglycerol acyl transferase 2 expression using antisense oligonucleotides improves hepatic steatosis and protects against fat-induced insulin resistance**AMEYA RAVINDRAKUMAR KULKARNI,** Yale University School of Medicine

Mentor: Gerald I. Shulman, M.D., Ph.D., Investigator, Howard Hughes Medical Institute; Professor, Departments of Internal Medicine and of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, Connecticut

■ As type 2 diabetes grows in prevalence, finding novel therapeutic targets is becoming increasingly important. Recently, the focus of this search has been the major steps of triglyceride synthesis, because the accumulation of triglycerides, especially within the liver, seems to induce insulin resistance. Specifically, acyl CoA:diacylglycerol acyl transferase 1 (DGAT1) and DGAT2 have been studied as potential targets for new intervention.

In this study, we investigated the role of DGAT1 and DGAT2 in triglyceride synthesis by reducing their expression in liver and fat. To do this, we used optimized antisense oligonucleotides (ASO) in SD rats fed high-fat diet (27% safflower oil). Four groups were injected with either saline, control ASO (25 mg/kg), DGAT1 ASO (25 mg/kg), or DGAT2 ASO (37.5 mg/kg) twice a week for four weeks. There was also a saline group that received regular chow. DGAT1 and DGAT2 ASO treatment reduced DGAT1 and DGAT2 mRNA expression by 95% and 57% in liver, but only DGAT2 ASO treatment reduced triglyceride levels. The effect on insulin action was tested using a 135-minute hyperinsulinemic (4 mU/kg/min)-euglycemic clamp. Glucose turnover and uptake were determined using [3 -H] glucose and [14 -C]2-deoxyglucose injection during clamps.

DGAT2-treated rats showed protection from fat-induced insulin resistance as demonstrated by an 80% increase in glucose infusion rate during the clamp (24.0 \pm 0.9 vs 13.4 \pm 1.1 mg/kg/min, $p < 0.001$). This was accounted for by improved insulin action in liver (suppression of hepatic glucose production: 82 \pm 6% vs 53 \pm 11%, $p < 0.05$) and peripherally (54% increase in insulin-stimulated whole body glucose uptake). Suppression of plasma free fatty acids during the clamp was also improved in the DGAT2 group (64 \pm 4% vs 48 \pm 5%, $p = 0.015$).

This study demonstrates that the reduction of DGAT2 using an ASO protects against insulin resistance in liver and peripheral tissue, and suggests its use in the treatment of type 2 diabetes.

9:00 A.M.

Effects of ghrelin and insulin on phosphoinositide 3-kinase signaling in the hypothalamus**TRACY SUSAN TYLEE**, University of Washington School of Medicine

Mentor: David E. Cummings, M.D., Associate Professor, Department of Medicine, University of Washington School of Medicine, VA Puget Sound Health Care System, Seattle, Washington

■ Body weight is regulated in part by opposing CNS actions of anorexigenic hormones, such as leptin and insulin, and the orexigenic hormone, ghrelin. Ghrelin increases food intake, body weight, and NPY/AgRP neuronal activity, whereas insulin exerts the opposite effects. Since insulin and ghrelin have antagonistic physiologic and cellular effects, we hypothesized that ghrelin inhibits insulin-mediated intracellular signaling. The anorexigenic effects of insulin are mediated by hypothalamic phosphoinositide 3-kinase (PI3K) signaling. Ghrelin could plausibly act at several points in this pathway. We sought to determine if ghrelin can inhibit insulin-induced hypothalamic protein kinase B (PKB) activation, a downstream consequence of PI3K signaling.

Male Wistar rats were implanted with 3rd intracerebroventricular (ICV) cannulas one week before studies. Animals received 2 μ l ghrelin (1 nmol) or vehicle ICV, followed by 2 μ l insulin (10 mU) or vehicle. Animals were sacrificed and a wedge of mediobasal hypothalamus was isolated. Levels of phosphorylated PKB (PKB-PO₄) were determined using an ELISA and normalized for total protein content. In separate rats we measured food intake to correlate biochemical findings with feeding behavior. Cannulated rats were given 2 μ l ICV injections of vehicle, ghrelin (1 nmol), or ghrelin and insulin (10 mU). Food was weighed every 30 minutes for 2 hours.

Regardless of pre-treatment, animals that received insulin showed an increase in PKB-PO₄ (6.7×10^{-3} U PKB-PO₄/ μ g total protein (vehicle), 11.9×10^{-3} U/ μ g (insulin), $P=0.001$). Pre-treatment with ghrelin did not affect insulin-induced PKB-PO₄ (13.2×10^{-3} U/ μ g (ghrelin), 10.8×10^{-3} U/ μ g (vehicle), $P=0.329$). Ghrelin stimulated 2-hour food intake, unaffected by co-injection of insulin (1.5 g [vehicle], 4.6 g [ghrelin], 5.1 g [ghrelin+insulin]).

Our results show that ghrelin increases food intake without a corresponding decrease in insulin-induced PKB-PO₄. These findings do not support the hypothesis that competitive convergence of ghrelin and insulin on hypothalamic PI3K signaling underlies these hormones' antagonistic actions in energy balance.

9:15 A.M.

Evidence of differential gene expression in various tissues and their correlation to insulin resistance**OSCAR I. GONZALEZ**, Stanford University School of Medicine

Mentors: Philip S. Tsao, Ph.D., Associate Professor, and Gerald M. Reaven, M.D., Emeritus Professor, Department of Medicine, Division of Cardiovascular Medicine, Stanford University School of Medicine, Stanford, California

■ **Purpose:** Insulin resistance increases the risk for cardiovascular disease. In fact, atherosclerosis is accelerated in the diabetic milieu in humans and murine models; however, the exact mechanisms are not clear. Gene expression changes at the tissue level can be assessed to give clues to initiating factors that lead to cardiovascular disease, especially when evaluated after therapeutic intervention.

Methods: In one study, apolipoprotein E-null mice were fed a high-fat diet and subsequently treated with rosuvastatin. In another experiment, C57Bl/6J mice were fed a high-fat diet and subsequently treated with rosuvastatin, rosiglitazone, or reversal to normal chow diet. In a separate human study, adipose tissue biopsies were obtained from individuals who were either insulin resistant or sensitive individuals and had similar body mass index. Various tissues were harvested and gene expression levels were determined by quantitative real-time polymerase chain reaction.

Results: In the apolipoprotein E-null murine study, there were no significant differences in gene expression of *Dimethylarginine dimethylaminohydrolase 1 and 2*, *Tumor Necrosis Factor- α* , *Vascular Cell Adhesion Molecule 1*, *Mast Cell Proteinase-1*, *CD36 Antigen*, *Peroxisome proliferator-activated receptor gamma (PPAR γ)* in both cardiac and aortic tissues. In the second murine study with C57Bl/6J mice, *Apelin-angiotensin receptor-like 1 (APJ)* expression was significantly reduced with rosiglitazone treatment in cardiac tissue, while no differences in *Apelin* were detected. In the human study, *Adiponectin*, *PPAR γ 1*, *PPAR γ 2*, and *Glucose Transporter 4* all showed 2–3-fold lower levels of expression in insulin-resistant individuals compared to those that were insulin sensitive.

Conclusions: Our studies support the notion that insulin resistance plays an important part in the development of cardiovascular disease. This is evidenced most by our human study where there is clearly a correlation between differential gene expression and the degree of insulin sensitivity. Further work will help clarify the role of other genes in adipose tissue.



T.S. TYLEE



O.I. GONZALEZ

TUESDAY
ROOM A

9:30 A.M.

Characterization of the MAP functions of the RP1 protein**NICOLE BENITAH**, University of California, San Francisco, School of Medicine**Mentor:** Eric A. Pierce, M.D., Ph.D., Assistant Professor of Ophthalmology, F.M. Kirby Center for Molecular Ophthalmology, University of Pennsylvania, Philadelphia, Pennsylvania

■ **Purpose:** Mutations in the *RP1* gene are a common cause of dominant retinitis pigmentosa (RP). All of the mutations in *RP1* identified to date are predicted to produce mutant proteins that contain the N-terminal 1/3 to 1/2 of RP1. The mechanism by which these truncated proteins cause photoreceptor cell death is unclear. The RP1 protein has been shown to be a photoreceptor-specific microtubule associated protein (MAP). Both the full-length and N-RP1 proteins stabilize the photoreceptor axoneme *in vivo*. However, *in vitro* tests have been limited to the N-RP1 protein due to difficulty expressing the full-length protein in heterologous systems. I used adenoviral vectors to express full-length RP1 in cultured cells, and tested the MAP activities of the full-length protein.

Methods: Three recombinant adenoviral vectors were created containing the coding sequences for full-length human RP1 (FL), a truncated N-terminal form with previously demonstrated MAP activity (N-RP1), and a form from which the microtubule binding domain was deleted ($\Delta 2-3$). All three viruses contain a C-terminal V5 epitope tag. COS-7 cells were infected with the viruses at an MOI of 10^4 PFU/cell for 48 hours, followed by treatment with varying concentrations of the microtubule-destabilizing drug nocodazole. The cells were then fixed and stained with antibodies to V5 and α -tubulin.

Results: Twenty percent of cells infected with the N-RP1 virus expressed the protein, compared to 3% of cells infected with the FL virus and <0.1% of cells infected with the $\Delta 2-3$ virus. As shown previously, the truncated N-RP1 protein associated closely with microtubules and stabilized them against the depolymerizing effects of nocodazole. The FL-RP1 protein was also associated with microtubules, but did not appear to prevent nocodazole-induced depolymerization to the same degree as N-RP1.

Conclusions: Adenovirus is capable of generating expression of the full-length RP1 protein, although with less efficiency than the truncated N-RP1 protein. The full-length RP1 protein does not appear to stabilize microtubules in heterologous cells as well as the N-RP1 protein. This suggests that domains in the C-terminal portion of RP1 could modulate the MAP activity of the N-terminal microtubule-binding domain. This may help explain how mutations in *RP1* cause retinal degeneration.

9:45 A.M.

Ankyrin-B syndrome: physiological and molecular studies**HUI XUE**, Duke University School of Medicine**Mentor:** Vann Bennett, M.D., Ph.D., Investigator, Howard Hughes Medical Institute; James B. Duke Professor of Cell Biology, Biochemistry, and Neuroscience, Department of Cell Biology, Duke University, Durham, North Carolina

■ Ankyrin isoforms -R, -B, and -G belong to a family of cytoskeletal adaptor proteins that bind to diverse integral membrane proteins. Mutations of ankyrin-B cause a fetal cardiac arrhythmia termed type 4 long-QT syndrome/sick sinus syndrome. The focus of my study has been on the ankyrin-B syndrome. Ankyrin-B is found in the brain, heart, kidney, retina, skeletal muscle, pancreatic beta cells, and various other tissues. Little is known about the function of ankyrin-B in the kidneys, and the heterozygous ankyrin-B (\pm) knockout mice have shortened life spans. The ankyrin-B (\pm) mice exhibit signs of premature aging in thinning of the skin, thinning of the bone and cartilage, replacement of red marrow with white marrow, and fibrotic changes and loss of pulp organization of the spleen. In the adult mouse kidney, I've localized ankyrin-B to the thin ascending loop of Henley, the distal convoluted tubules, the macula densa cells, and potentially mesangial cells. The physiologic roles of those nephron segments implicate possible altered renal function in the ankyrin-B (\pm) mice. Upon assessment, ankyrin-B (\pm) mice were found to have a 10 mmHg increase in baseline systolic blood pressure and to have intact renal functions with normal urine creatinine and protein levels and appropriate concentrating ability post 12 hour water deprivation. The significance of these findings was further evaluated in human patient for ankyrin-B mutations and balanced polymorphisms. In the alleles screened thus far, no protein level change of ankyrin-B has been detected.



N. BENITAH



H. XUE

10:00 A.M.

The role of the integrin $\alpha\beta6$ in biliary fibrosis**BRUCE MAO ZHENG WANG**, University of California, San Francisco, School of Medicine

Mentor: D. Montgomery Bissell, M.D., Professor and Chief, Division of Gastroenterology; Director, University of California–San Francisco Liver Center, Department of Medicine, University of California–San Francisco, San Francisco, California

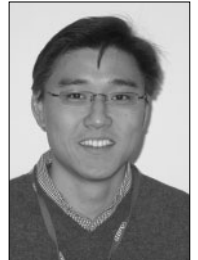
■ Injuries affecting the biliary tract and the hepatic parenchyma differ in important ways. They involve distinct etiologies and have a characteristic histopathology. While much is known about parenchymal fibrosis, little is known about biliary fibrosis. Given the currently limited options for treating biliary fibrosis, understanding its pathogenesis is a crucial step towards developing effective treatment options. In this report, we present evidence that the integrin $\alpha\beta6$ regulates biliary fibrosis by locally activating latent TGF β . We use selective bile duct ligation (SBDL) as a model of biliary injury and show that following SBDL, biliary epithelial cells (BEC) and oval cells proliferate and express $\alpha\beta6$ integrin. Blocking antibody to $\alpha\beta6$ reduced biliary fibrosis to a similar degree as soluble TGF β receptor antagonist. $\beta6$ knockout mice are protected from biliary fibrosis following bile duct ligation. They also exhibit increased bile duct proliferation compared to wild-type mice. Co-localization of $\alpha\beta6$ and TGF β will be shown using immunohistochemistry staining in the *ova-bil* transgenic model of autoimmune biliary injury. A method to isolate BECs using FACS analysis is presented that allows for the study of BECs in culture. The proliferative effects of $\alpha\beta6$ will be tested on cultured BECs as well signaling intermediates of the TGF β pathway. Lastly, the SBDL model will be compared with autoimmune biliary injury in the transgenic *ova-bil* mouse to explore the cell types and cytokines responsible for inducing $\alpha\beta6$ expression. We conclude that in response to bile duct injury, biliary epithelial cells express the integrin $\alpha\beta6$ that locally activates latent TGF β leading to biliary fibrosis.

10:30 A.M.

Mitochondrial uncoupling protein 2 modifies the response to oxidative stress in neural stem cells**NEAL MATSUMORI RAO**, University of Colorado School of Medicine

Mentor: Michael W. Zawada, Ph.D., Associate Professor, Department of Clinical Pharmacology, University of Colorado, Health Sciences Center, Denver, Colorado

■ Although oxidative stress is an important mediator of neural cell death in neurodegenerative disorders, its effects on neural stem cells (NSCs) are virtually unknown. We hypothesize that reactive oxygen species (ROS) induce the expression of immune recognition molecules on the surface of NSCs, making them vulnerable targets for destruction by the immune system. By examining the mouse C17.2 NSC line using flow cytometry, we found that NSCs express the Fas (CD95) death receptor and B7.1 co-stimulatory molecule. Furthermore, hydrogen peroxide and rotenone (ROS-forming pesticide) upregulate B7.1 expression. To investigate whether NSCs' response to ROS can be attenuated, we examined the role of mitochondrial uncoupling protein 2 (UCP-2), a protein that increases anti-oxidative capacity and reduces expression of immune recognition molecules on tumor cells. For this purpose, we developed a C17.2 cell line stably expressing mouse UCP-2. Stable transfectants had a decreased upregulation of B7.1 in response to rotenone when compared to untransfected controls. We conclude that NSCs upregulate surface expression of immune recognition molecules in response to oxidative stress and that UCP-2 moderates this response.



B.M.Z. WANG



N.M. RAO

TUESDAY
ROOM A

10:45 A.M.

Biliverdin reductase and cell stress**DANIEL SMITH HIGGINSON**, Johns Hopkins University School of Medicine

Mentor: Solomon H. Snyder, M.D., Chair, Department of Neuroscience; Professor, Department of Psychiatry; Professor, Department of Pharmacology and Molecular Sciences, Johns Hopkins School of Medicine, Baltimore, Maryland



D.S. HIGGINSON

■ The production of intracellular bilirubin is now understood to be protective in a wide variety of disease states, particularly ischemia-reperfusion injuries. Bilirubin is produced via the heme degradation pathway and its two enzymes, heme oxygenase and biliverdin reductase. Heme oxygenase-1 (HO1) is a highly inducible enzyme, and overexpression or pre-induction of HO1 is protective in animal models of cerebral ischemia, ALS, and pulmonary fibrosis. HO1 induction leads to an increase in intracellular bilirubin, a potent antioxidant that quenches harmful reactive oxygen species. While toxic in high concentrations, bilirubin in the blood may prevent LDL oxidation and atherosclerosis. The focus of this research effort is to better understand the biliverdin reductase (BVR) enzyme, its regulation, cellular localization, and binding partners, in hopes of learning more about basic bilirubin biology.

We report the first BVR binding protein, Hsp70, and postulate that the Hsp70-BVR interaction may mediate nuclear translocation of BVR. Heat shock treatment of HeLa cells, well known to cause nuclear translocation of Hsp70, also led to increased BVR nuclear localization. Hsp70 is an inducible cell stress related protein, and here we report amplification of BVR activity in the presence of Hsp70.

Nuclear localization of BVR implies that bilirubin may prevent DNA oxidation, an important factor in telomere shortening and cellular senescence.



S. NAGHSHINEH

11:00 A.M.

Cytoprotective mechanisms of heme oxygenase-1 in bronchopulmonary dysplasia**SHEILA NAGHSHINEH**, Harvard Medical School

Mentor: Stella Kourembanas, M.D., Associate Professor of Pediatrics; Chief, Division of Newborn Medicine, Children's Hospital Boston, Boston, Massachusetts

■ Oxidative injury is a major component of the pulmonary damage characteristic of bronchopulmonary dysplasia (BPD) of preterm infants. Recent availability of animal models relevant to BPD, including hyperoxia, has permitted elucidation of its pathogenesis. Heme oxygenase-1 (HO-1), a stress inducible enzyme, catalyzes the production of iron, carbon monoxide, and biliverdin that is subsequently converted to bilirubin. Iron induces the production of the antioxidant ferritin, and both biliverdin and bilirubin have potent antioxidant properties, whereas CO has anti-inflammatory effects. We hypothesize that HO-1 is critical to the immature lung's defenses against injury and serves a protective, anti-inflammatory, antioxidant role in BPD through the action of its enzymatic products.

In this study, lungs were isolated from neonatal mice genetically engineered to express high levels of human cDNA HO-1 transgene in lung epithelium and its wild-type prototype exposed to 75% oxygen for 16–18 days. We used potassium ferrocyanide to highlight iron deposition and mRNA analysis to assess ferritin heavy chain mRNA levels.

Neither wild-type nor transgenic neonatal lungs show iron deposition at normoxia. Wild-type mice exposed to hyperoxia demonstrated significant numbers of iron-laden alveolar macrophages as well as prominent perivascular and peribronchiolar iron deposition. In contrast, transgenic HO-1 overexpressing mice under hyperoxia exhibited almost no iron in the interstitium or in lung macrophages. However, despite the marked difference in iron deposition between the two genotypes under hyperoxia, no difference in ferritin heavy chain mRNA levels was detected between lungs exposed to varying oxygen tensions.

We speculate that constitutive lung HO-1 expression may increase ferritin protein levels and reduce oxidative stress in hyperoxia through intracellular iron sequestration. We demonstrate that HO-1 does not transcriptionally regulate the expression of the ferritin heavy chain gene. Future investigations into translational or light chain transcript regulation are key to understanding ferritin's role in HO-1 defense against BPD.

TUESDAY
ROOM A

11:15 A.M.

Mechanistic studies of the radiosensitizing properties of NESP

KEVIN FORSYTHE, Stanford University School of Medicine

Mentor: Susan J. Knox, M.D., Ph.D., Associate Professor of Radiation Oncology, Stanford University, Stanford, California

■ Anemia is a serious complication faced by a majority of cancer patients and has been associated with poorer survival outcomes for patients receiving radiation therapy. One reason for this may be due to decreased delivery of oxygen to the tumor, since hypoxic tissues are generally more resistant to radiation-induced cell killing when compared to normoxic tissues. We have shown that administration of the erythropoietic stimulating drug NESP (a derivative of the glycosylated protein hormone erythropoietin, or Epo) to anemic mice bearing tumors can potentiate the effects of radiation therapy. Interestingly, this observed radiosensitizing effect of NESP may in part be due to properties of the drug other than its erythropoietic stimulating effect.

The erythropoietin receptor is known to be expressed outside of the hematopoietic compartment and has recently been shown to be expressed on the surface of various cancer cell types. Our initial studies have failed to show a direct radiosensitizing effect of NESP on cancer cells *in vitro*, so we are currently considering potential stromal interactions for the cause of NESP's radiosensitizing abilities. We are also investigating potential differences in the biological effects of NESP and its parent compound Epo.

11:30 A.M.

Hypoxia-inducible factor-1 upregulation is mediated by the phosphatidylinositol-3-kinase signaling pathway in head and neck squamous cell carcinoma

CHARLES J. LIN, University of Pittsburgh School of Medicine

Mentors: Jennifer R. Grandis, M.D., Professor, and Stephen Y. Lai, M.D., Ph.D., Head and Neck Fellow, Department of Otolaryngology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

■ The transcription factor, hypoxia-inducible factor 1 (HIF-1), controls expression of many genes responsible for adaptation to hypoxia. HIF-1 is a heterodimer of HIF-1 α and HIF-1 β regulated by the oxygen-dependent degradation of HIF-1 α . In head and neck squamous cell carcinoma (HNSCC), hypoxia and HIF-1 α overexpression correlate with poor patient prognosis. Extracellular factors and cytokines have been shown to regulate HIF-1 activity and expression in an oxygen-independent manner in many cancer types. We examined the effects of epidermal growth factor (EGF) and erythropoietin (EPO) on HIF-1 in the paired UM-22A and UM-22B HNSCC cells. These cell lines were derived from the primary tumor (UM-22A) and metastatic cervical lymph node disease (UM-22B) of the same HNSCC patient. Preliminary studies show that EGF or EPO treatment of UM-22A and UM-22B demonstrates a dose-dependent increase in HIF-1 α expression by immunoblot analysis. Treatment with these growth factors also increased Akt phosphorylation (pAkt), supporting a role for the phosphatidylinositol-3-kinase (PI3K) signaling pathway in HIF-1 upregulation by EGF and EPO. UM-22A and UM-22B HNSCC cell lines treated with LY294002 (20 μ M) and wortmannin (250 nM), chemical inhibitors of the PI3K pathway, expressed lower levels of HIF-1 α by immunoblot analysis than untreated cells under normoxic and hypoxic conditions. These findings were confirmed with blockade of the PI3K pathway by transient transfection of p85 siRNA in UM-22A and UM-22B cells. These findings suggest that the PI3K pathway is important for mediating oxygen-dependent and -independent upregulation of HIF-1 in HNSCC.



K. FORSYTHE



C.J. LIN

TUESDAY
ROOM A

11:45 A.M.

Aromatase inhibitors in human lung cancer therapy**OLGA K. WEINBERG**, Vanderbilt University School of Medicine

Mentor: Richard J. Pietras, M.D., Ph.D., Associate Professor of Medicine, Department of Medicine and Pathology, University of California, Los Angeles, David Geffen School of Medicine, Los Angeles, California

■ Lung cancer is the most common cause of cancer mortality in both male and female patients in the United States. Current survival rates are unacceptably low, and new therapeutic options are urgently needed. The etiology of lung cancer is not well-defined, but several studies suggest a major role of growth factors and estrogens in non-small cell lung cancer, with estrogen receptor (ER) pathways implicated in the rising incidence among women. Aromatase catalyzes conversion of androstenedione and testosterone to estrone and estradiol, thus providing a local source of ligand for ER activation. We assessed expression of aromatase in twelve lung cancer cells using gel electrophoresis and Western blot methods with anti-aromatase antibody (CYP19). Aromatase expression occurred in all cell lines, with the highest expression level, comparable to that in SKBR3 breast cancer cells, in A549 cells. Furthermore, cellular aromatase activity assayed in lung tumor cells averaged 1.49 ± 0.23 pmol/mg/h as compared with 5.84 pmol/mg/h in SKBR3 cells. To assess the prevalence of aromatase, archival lung tumor specimens (including adenocarcinoma, squamous, bronchoalveolar, and adenosquamous) from 56 patients were analyzed by immunohistochemistry. Aromatase localized primarily in tumor epithelial cells. Using a cutoff score of 15% total cell staining for positivity, 86% of samples expressed aromatase, and this finding was relatively uniform among different histologic lung tumor types. To test antitumor effects of the aromatase inhibitor, anastrozole, A549, and H23 lung cancer cells were grown *in vitro* for 48 h with or without anastrozole. At doses of 0.1–50 μ M, anastrozole elicited a marked suppression of lung cell growth as compared with controls ($P < 0.001$). Using an *in vivo* tumor xenograft model with A549 lung cells in ovariectomized nude mice, supplemented with androstenedione substrate daily, anastrozole (0.1 mg/kg PO daily) elicited a significant decrease in tumor size to 89% of controls after three weeks of treatment ($P < 0.001$). Moreover, both *in vitro* and *in vivo* data showed greater antitumor effects when anastrozole was combined with receptor tyrosine kinase inhibitor, gefitinib.



O.K. WEINBERG



Y.T. CHIK

Noon

Interrogation and targeting of epidermal growth factor receptors and integrins in glioblastomas**YOLANDA T. CHIK**, Duke University School of Medicine

Mentors: Henry S. Friedman, M.D., Associate Professor, Department of Neurology, and Jeremy N. Rich, M.D., James B. Powell Professor, Department of Surgery, Duke University Medical Center, Durham, North Carolina

■ Glioblastoma multiforme is an aggressive malignancy of the CNS, with mean survival of less than one year. Novel therapies aim to increase specificity and decrease toxicity by targeting critical molecular pathways known to play a role in generating gliomas.

This project investigated the potential synergistic therapeutic benefits in targeting gliomas cell lines with ZD1839 (a selective inhibitor of the epidermal growth factor receptor) and EMD121974 (an integrin antagonist). Thymidine incorporation assays showed that cellular proliferation was potentially inhibited by EMD121974 and to a lesser degree by ZD1839, with the combination of drugs having an additive effect. Autonomous cellular proliferation and survival assayed through colony formation was inhibited by EMD121974. The addition of ZD1839 contributed a combinatorial effect. Migration through an artificial matrix was greatly inhibited by ZD1839 and to a lesser extent by EMD121974, with the combination of drugs having an additive effect. The same results were found with invasion through an artificial matrix. An Annexin V assay demonstrated that ZD1839 induced apoptosis in gliomas cells while EMD121974 displayed no change compared to control. These results were confirmed by cell cycle arrest flow cytometry, which was notable for increased percentage of cells gated during the sub-G0 phase with ZD1839 treatment. Lastly, activation of downstream effector molecules common to the EGFR and integrin pathways was induced with EGF and other growth factors. Increased activation was observed with FAK, Erk, and Akt. Diminished phosphorylation of FAK, ERK, and Akt was noted when treated with ZD1839. Decreased phosphorylation of FAK was observed when treated with EMD121974. When treatment consisted of both drugs, a combinatorial effect on inhibition was seen upon Akt and FAK.

These results show that targeting gliomas cells with EMD121974 and ZD1839 inhibits specific parameters, which give rise to tumor formation, with an additive effect observed with the agents in combination.

12:30 P.M.

Targeted delivery of cationic nanoparticles against the vasculature of a glioblastoma multiforme**LEROY SIMS**, Stanford University School of Medicine

Mentors: Griffith R. Harsh, M.D., Professor, Department of Neurosurgery, and Samira Guccione, Ph.D., Assistant Professor, Department of Radiology, Stanford University School of Medicine, Stanford, California

■ Glioblastoma multiforme (GBM) is a malignant brain tumor that is highly angiogenic. Endothelial cells of GBMs uniquely express $\alpha\beta3$ integrin. This integrin plays an important role in angiogenesis. We pursued antiangiogenic therapy in hopes of increasing survival. We assessed a cationic nanoparticle (NP), which was coupled to a ligand that specifically binds the $\alpha\beta3$ integrin and becomes endocytosed into the cell, as a potential specific delivery vehicle against GBMs. We used the rat GBM model RT2 to test our NP. Our first aim was to demonstrate the presence of the $\alpha\beta3$ integrin on the endothelial cells within the tumor vessels of the RT2 tumor. Our second aim was to demonstrate the specificity of our NP for the endothelial cells within the RT2 tumor.

Using a monoclonal antibody against $\alpha\beta3$, we successfully demonstrated the expression of $\alpha\beta3$ integrin in the vessels of the RT2 tumor. We are currently using NPs containing green fluorescence protein (GFP) to address the second aim. We hope to see GFP expression within the endothelial cells of the vessel to confirm successful targeting and transfection. Additional experiments used NPs containing ATP^R-Raf (a potent gene that induces apoptosis) against the RT2 tumor in hopes of increasing survival. Preliminary results demonstrated 100% survival in the treatment group (n=4) and 100% mortality (median 14 days) in the untreated group (p=0.0048, n=4) at 45 days post implantation. Serial MRIs demonstrated tumor regression in the treated group. Terminal deoxynucleotidyl transferase mediated dUTP nick-end-labeling (TUNEL) assays confirmed increased apoptosis compared to the controls. H&E staining demonstrated a marked reduction of tumor vessel density in the treated cohort. Larger studies with added controls are under way.

Targeted NPs offer a novel specific delivery vehicle against the vasculature of GBMs that may complement or perhaps even replace current modes of therapy.

12:45 P.M.

The state of eIF4GI and eIF4GII in malignant tissues and the role of protease 2A in internal ribosome entry site-mediated translation**RACHEL NICOLE GRISHAM**, Duke University School of Medicine

Mentor: Matthias Gromeier, M.D., Assistant Professor, Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina

■ We have genetically modified the human pathogen poliovirus (PV) to achieve selective virus replication and cytotoxicity in tumor cells without collateral damage to normal CNS or extraneural tissues. All picornaviruses depend on the internal ribosome entry site (IRES) within their 5' untranslated region for translation initiation of their uncapped (+) strand RNA genome. By exchanging the PV IRES for its counterpart from human rhinovirus 2 (HRV2), the PV-RIPO chimera has been constructed. The selective activity of PV-RIPO in malignant cells suggests fundamental differences in the control of translation in cancerous cells as compared to non-malignant cells. Translation of conventional mRNAs occurs after the cap is bound by the cap-binding protein complex eIF4F, which consists of three subunits, eIF4E, eIF4A, and eIF4G. Since the cellular translation initiation factor eIF4G is cleaved by PV protease 2A following picornavirus infection. This has led some to speculate that the cleavage of eIF4G itself directly stimulates IRES-mediated translation. With this current work we investigate the integrity of eIF4GI and its functional homologue eIF4GII in primary and metastatic brain tumors as compared to levels of intact protein found in normal brain tissue. We also examine the effects of viral infection, isolated protease 2A activity, and eIF4G overexpression on IRES and cap-mediated translation.

Research findings to date indicate that isolated protease 2A exerts a powerful stimulatory effect on IRES-mediated translation. This effect is not inhibited by overexpression of eIF4GI or expression of cleavage resistant eIF4GI. In addition, eIF4GI is present in primary and metastatic brain tumor tissues at a level similar to that seen in normal brain and extraneural tissue.

Although stimulation of IRES-mediated translation is known to coincide with the cleavage of eIF4G, our studies implicate protease 2A as an independent stimulator of IRES-mediated translation.



L. SIMS



R.N. GRISHAM

TUESDAY
ROOM A

1:00 P.M.

Evaluation of HER2 expressing viral vectors in a tumor-bearing human HER2 transgenic mouse model

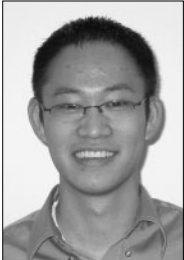
GABRIEL TSING-TZONG CHONG, Duke University School of Medicine

Mentors: Herbert K. Lyerly, M.D., Professor, Department of Surgery; Director, Duke Comprehensive Cancer Center, and Timothy M. Clay, Ph.D., Associate Professor, Department of Surgery; Director, Clinical Biologics Manufacturing and Cell Culture Core, Duke Comprehensive Cancer Center, Duke University Medical Center, Durham, North Carolina

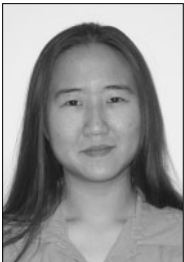
■ **Background:** Despite considerable promise in animal models of breast cancer, human clinical trials of cancer vaccines targeting tumor antigens such as HER2/*neu* have thus far activated only modest antigen-specific immune responses *in vivo*. We and others have observed that the most potent platform for immunization involves modifying dendritic cells (DC) with viral vectors. We propose to modify DC with adenoviral vectors encoding the tumor antigen HER2/*neu*, which is often overexpressed in aggressive breast cancers. The effectiveness of these vectors in a preclinical model is being examined using a human HER2-transgenic mouse model (Piechocki et al., *J. Immunol.*, 109, 259–264, 2003).

Methods/Results: Mouse bone marrow progenitors were differentiated *in vitro* into DC using GM-CSF/IL-4 and infected for 48 hours with Ad[E1-]HER2 vector at a multiplicity of infection (MOI) of 0–4000 particles per cell. Ad[E1-]HER2 vector reproducibly achieved transduction efficiencies of 10% up to 65% in these DC at higher MOI (3500, 4000). Using ELISPOT analysis, we demonstrated that a single vaccination with Ad[E1-]HER2 vector activated human HER2-specific T cell responses in the human HER2-transgenic mice, but successive vaccination with Ad[E1-]HER2 vector did not increase the magnitude of the immune response. In order to demonstrate the anti-cancer effect of these vaccines, we are developing a murine model of HER2/*neu* expressing mammary carcinoma using 4T1 cells that we have successfully transfected with a human HER2 cDNA containing plasmid.

Conclusion: Ad[E1-]HER2 vector can break tolerance to human HER2 in transgenic mice expressing human HER2. Repeated usage of such vector does not boost the immune response, likely due to an anti-adenoviral neutralizing antibody response. A heterologous prime-boost derived viral vector strategy using both adenovirus and Venezuelan equine encephalitis (VEE)-virus vectors could avoid anti-vector neutralizing antibody responses and enhance the immune response to human HER2.



G.T.T. CHONG



C. KIM

1:15 P.M.

Examination of the c-Myc pathway

CLARA KIM, Brown Medical School

Mentor: John Sedivy, Ph.D., Professor of Medical Science, Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, Rhode Island

■ The Myc oncogene is one of the most important cancer causing genes in humans, and is believed to be involved in the genesis of over 30% of all human cancers. As a transcription factor, c-Myc has been found to bind to thousands of target gene promoters. Although the identities of the genes that c-Myc regulates are rapidly coming into focus, the biochemical mechanisms by which c-Myc accomplishes this task are largely unknown. Throughout this year, I have applied a variety of methods to analyze protein interactions with c-Myc.

SIRT1, an NAD-dependent protein deacetylase has been found to deacetylate, and thereby alter function of, p53, FOXO3, and other proteins. It is a strong candidate for interaction with c-Myc. Immunoprecipitations with an HA-tagged c-Myc cell line were inconclusive as to the involvement of SIRT1 with c-Myc. I pursued manual antibody arrays, but after a month of troubleshooting was still unable to formulate a useful assay.

Currently, I am pursuing two paths which have promise. I found E-box sequences in the promoter region of SIRT1, making it a possible target gene of C-myc. Real-time PCR has initially shown a two-fold increase in SIRT1 mRNA in rat cell lines with c-Myc function knocked out. I intend to repeat this experiment and also test it in human cell lines.

While testing the manual antibody array, I used formaldehyde to stabilize the proteins for the array. Vasilescu et al. published a paper that suggests that cross-linking with paraformaldehyde stabilizes transient interactions between proteins which can then be analyzed with immunoprecipitation or mass spectrometry (*Proteomics*, 4, 3845–3854, 2004). Initial results show low yield but altering lysis conditions may improve yield. Through these methods, I intend to gain a better understanding of the c-Myc pathway.

1:30 P.M.

Immune profile in axillary lymph nodes predicts disease-free survival in breast cancer**HOLBROOK EDWIN KOHRT**, Stanford University School of Medicine

Mentor: Peter P. Lee, M.D., Assistant Professor, Department of Medicine, Division of Hematology, Stanford University School of Medicine, Stanford, California

■ While lymph node metastasis is among the strongest predictors of disease-free and overall survival for patients with breast cancer, the immunological nature of tumor-draining lymph nodes has not been rigorously examined to determine if a significant association also exists between nodal immune profile and clinical outcome. We performed immunohistochemical analysis of sentinel and axillary (nonsentinel) nodes from 29 breast cancer patients with five years of follow-up to determine if alterations in CD4, CD8, and CD1a cell populations predict nodal metastasis or disease-free survival.

Sentinel and axillary node CD4 and CD8 T cells were decreased compared to controls. CD1a den-

dritic cells were similarly diminished in sentinel, but increased in axillary nodes. Accuracy of CD4 T cell, dendritic cell, and CD4:CD8 ratio to predict nodal metastasis from a single nodal section was superior to detection by multilevel hematoxylin and eosin staining (accuracy 93.1%, 96.6%, 98.3%, vs. 74.1%, respectively). Axillary node CD4 T cell and dendritic cell populations were highly correlated with disease-free survival independent of axillary metastasis (both $P < 0.001$). Patients stratified by axillary node CD4 T cell populations $\geq 7.0\%$ had a five-year disease-free survival rate of 88.2% versus 25.0% for CD4 populations $< 7.0\%$ ($P = 0.003$). Similarly, patients stratified by axillary node dendritic cell populations $\geq 0.6\%$ had a disease-free survival rate of 76.2% versus 25.0% for populations $< 0.6\%$ ($P = 0.015$). ALN immune profile was a stronger predictor of disease-free survival than extent or size of nodal metastasis. Moreover, axillary node CD4 T cell population stratified disease-free survival of patients with T2 tumors superior to all other characteristics ($P = 0.007$).

These findings demonstrate that lymph node immune profile accurately reflects nodal metastasis and, within axillary nodes, predicts disease-free survival among breast cancer patients.



H.E. KOHRT

TUESDAY
ROOM B

8:30 A.M.

Distinct structural determinants govern the directional preference for human 17 β -hydroxysteroid dehydrogenases types 1 and 2 in intact cells**DANIEL PAUL SHERBET**, University of Texas Southwestern Medical Center at Dallas Southwestern Medical School**Mentor:** Richard J. Auchus, M.D., Ph.D., Assistant Professor, Department of Internal Medicine, Division of Endocrinology and Metabolism, University of Texas Southwestern Medical Center, Dallas, Texas

■ The 17 β -hydroxysteroid dehydrogenases (17 β -HSDs) types 1 and 2 interconvert the weak and potent estrogens estrone and 17 β -estradiol. In intact cells, each enzyme exhibits a strong directional preference that favors either oxidation (17 β -HSD2) or reduction (17 β -HSD1). A positively charged arginine (R37) adjacent to the 2'-phosphate stabilizes NADP(H) binding to 17 β -HSD1 and favors reduction due to the high cytoplasmic NADPH/NADP⁺ ratio. In contrast, 17 β -HSD2 has a negatively charged glutamate (E116) at the position corresponding to R37 of 17 β -HSD1, which presumably repels the 2'-phosphate of NADP(H) and favors oxidation by harnessing the high cytoplasmic NAD⁺/NADH ratio. Substitution of a negatively charged aspartate, but not neutral glycine, for R37 of 17 β -HSD1 markedly reduces the affinity for NADP(H) and reverses the directional preference to oxidation. We hypothesized that E116 confers oxidative preference to 17 β -HSD2 and substitution of either a neutral or a positively charged residue for E116 reverses the directional preference to favor reduction.

Mutations E116G, E116R, and the double mutation E116G+N117R failed to attenuate the >95% oxidative preference of 17 β -HSD2 in intact cells. Affinity for all cofactors was measured for wild-type and mutant 17 β -HSD2 enzymes in yeast microsomes. For wild-type 17 β -HSD2, affinity for NAD/NADH is nearly 500-fold greater than for NADP/NADPH, and the mutant enzymes demonstrate similar poor affinities for NADP(H).

We conclude that the directional preference of 17 β -HSD1 is governed by electrostatic interactions with the 2'-phosphate of NADP(H), but the oxidative preference of 17 β -HSD2 is not solely due to E116 in the cofactor-binding domain. These data suggest that the directional preference of 17 β -HSD2 is controlled by other aspects of its cofactor-binding domain, such as the size of the cofactor-binding pocket. We are creating additional mutations and chimeras targeting other residues that may contribute to cofactor selectivity and thus directional preference for 17 β -HSD2.



D.P. SHERBET



C. HAEFFELE

8:45 A.M.

Characterization of the estrogen-related receptor alpha through the use of designer coactivators**CHRISTIANE HAEFFELE**, Duke University School of Medicine**Mentor:** Donald P. McDonnell, Ph.D., Associate Professor, Departments of Pharmacology and Cancer Biology and of Medicine; Director, Graduate Studies, Department of Pharmacology, Duke University, Durham, North Carolina

■ Under the conventional paradigm for steroid hormone receptor action, steroid receptors are thought to mediate their actions through a binary modality within the cell. This model proposes that the receptors reside within the cell in a quiescent "off" state until bound by a ligand. However, recent studies of the estrogen receptor have led to the discovery of estrogen-related receptors (ERRs), a class of steroid hormone receptors that appear to function in complete contradiction to the currently accepted "switch" model. ERRs are classified as nuclear orphan receptors because they do not respond to estrogen stimulation and have no known endogenous ligand. Currently, there are three known isoforms of ERR: ERR α , ERR β , and ERR γ . In the absence of a ligand, the ERRs adopt a transcriptionally active conformation, able to interact with coactivators and DNA-binding elements and affect gene expression.

To better understand the role of these receptors, we have developed specific peptide antagonists against the ERR α isoform. These antagonists were developed using the M13 phage display technology to identify high-affinity peptides that specifically interact ERR α . PGC-1 α , a known coactivator of ERR α , was modified using these peptides to create "designer coactivators." By replacing the receptor interaction domains in PGC-1 α with peptides identified in the M13 screen, we were able to create coactivator proteins that specifically manipulate the activity of ERR α . Using these designer coactivators, we then identified a number of downstream targets that are strongly upregulated in cells expressing the customized PGC-1 α . These target genes involve a diverse range of cellular processes, including metabolism, mitochondria biogenesis, and fatty acid β -oxidation. Use of these specific coactivators has positive implications for further characterizing the role of the ERRs, as well as other orphan nuclear receptors. Once these pathways are better characterized, pharmacological interventions can then be designed to modulate their activity.

9:00 A.M.

Parathyroid hormone regulation of notch signaling during osteoblast maturation**Laura Marie Bevelock**, University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School**Mentor:** Nicola C. Partridge, Ph.D., Professor and Chair, Department of Physiology and Biophysics, University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School, Piscataway, New Jersey

■ Prolonged exposure to parathyroid hormone (PTH) results in bone resorption, while intermittent low doses of PTH produce an anabolic effect. The mechanism of this anabolic effect is largely unknown. We first identified that PTH increases the expression of Jagged1, a ligand of the Notch signaling pathway, in osteoblastic cells. We hypothesized that Jagged1 stimulates proliferation of osteoblasts, while inhibiting their further differentiation. The objective of this study was to characterize the regulation of Jagged1 by PTH *in vitro* and *in vivo* and to determine the effect of Jagged1 stimulation on the osteoblast.

UMR 106-01 osteoblastic cells treated with PTH increased Jagged1 mRNA expression eightfold after 2 h of 10^{-8} M rPTH(1–34) treatment. This increase in Jagged1 mRNA expression translates into increased Jagged1 protein expression. PTH treatment resulted in an increase in mRNA levels of Notch target genes, Hes-1 and Hey1, which suggests that PTH stimulation of Jagged1 increases Notch signaling. We also found that 5 d intermittent injections of 10^{-8} M hPTH(1–34; 8 μ g/100 g) in three-month-old female rats increases Jagged1 mRNA levels fourfold. These results demonstrate that PTH increases Jagged1 mRNA levels both *in vitro* and *in vivo*. The upregulation of Jagged1 by PTH in osteoblastic cells is a primary response since it does not require *de novo* protein synthesis. In rat primary calvarial osteoblasts, we found that Jagged1 promotes growth while inhibiting mRNA levels of known markers of osteoblast differentiation.

These results suggest that Jagged1 stimulates proliferation of osteoblasts, while inhibiting their further differentiation. PTH stimulation of Jagged1 on the osteoblast may result in increased signaling by Jagged1 to preosteoblasts to increase their proliferation, resulting in overall bone growth. The increase in the preosteoblast pool may be one mechanism by which intermittent injection of PTH increases bone mass.

9:15 A.M.

Optimization of murine adipose-derived mesenchymal cell osteogenesis stimulated with retinoic acid**Matthew Thomas Siedhoff**, Stanford University School of Medicine**Mentor:** Michael T. Longaker, M.D., Professor, Department of Surgery; Director, Children's Surgery Research, Stanford University School of Medicine, Lucile Salter Packard Children's Hospital, Stanford, California

■ *In vivo* studies demonstrate that murine adipose-derived mesenchymal cells (AMCs) “pre-differentiated” with retinoic acid regenerate bone in critically-sized calvarial defects more rapidly than AMCs alone, but the new bone is quickly resorbed due to osteoclast recruitment. Retinoic acid is a known stimulator of osteoclast activity, and, in this study, we investigate the effects of shorter retinoic acid exposure times for optimal *in vitro* bone formation.

AMCs harvested from 3-week-old mice were expanded in culture with osteogenic differentiation medium (ODM) containing beta-glycerophosphate and ascorbic acid-2-phosphate plus 2.5 μ M retinoic acid. Cells were switched to ODM alone after 5, 10, 15, and 20 days. Alkaline phosphatase staining, a measure of preliminary osteogenesis, was performed at 15 days, and terminal differentiation was assessed at 25 days by alizarin red quantification and QRT-PCR for early (*runx2* and *osteopontin*) and late (*osteocalcin*) osteogenic genes.

Alkaline phosphatase staining intensified with increasing proportion of time with retinoic acid. Alizarin red staining and quantification was consistent, with a 3.60-, 3.66-, 32.16-, 33.00-, and 69.09-fold increase with 5, 10, 15, 20, and 25 days of retinoic acid, respectively ($p < 0.05$). *Runx2* expression was highest among cells switched from retinoic acid to ODM after 5 days, and *osteopontin* among cells switched after 10 days. *Osteocalcin* was induced only after 25 days of retinoic acid.

While continual retinoic acid stimulation enhances mineralization, our results demonstrate that shorter exposure to retinoic acid may be adequate to prime AMC osteogenesis. The data, therefore, suggest an abbreviated time course in culture with retinoic acid (e.g., 15 days) followed by growth in standard osteogenic medium might accelerate subsequent *in vivo* bone formation without concurrently promoting osteoclastogenic resorption.



L.M. BEVELOCK



M.T. SIEDHOFF

TUESDAY
ROOM B

9:30 A.M.

Age-related changes in murine fracture healing: a biochemical, radiographic, and histomorphometric analysis**AMISH ATULBHAI NAIK**, University of Rochester School of Medicine and Dentistry

Mentor: Regis J. O'Keefe, M.D., Ph.D., Dean's Professor of Orthopaedics, University of Rochester School of Medicine and Dentistry, Rochester, New York

■ **Introduction:** Fracture healing involves inflammatory, reparative, and remodeling phases. During the inflammatory phase, mesenchymal stem cells proliferate and migrate to the fracture site. This is followed by a reparative phase consisting of chondrocytic and osteoblastic differentiation. A cartilage template is formed and subsequently ossified. Initially, woven bone is formed but is replaced by well-organized lamellar bone during the remodeling phase.

Hypothesis/Aim: Our overall hypothesis is that fracture healing is impaired in aged (1-year-old) versus young (6- to 8-week-old) mice. Using a standardized murine femoral fracture model, we compare fracture healing in young and aged mice and investigate factors involved in these differences.

Methods: Mice are fractured and allowed 0, 3, 5, 7, 10, 14, 18, 21, 25, 30, or 35 days to heal. Histomorphometric and radiographic analysis are performed at each time point. *In situ* hybridization and real-time PCR localize and quantify gene expression associated with mesenchymal stem cell differentiation/proliferation (*VEGF*, *BMP2*, *4*, and *6*, *noggin*, and *chordin*), cartilage formation (*Collagen types I, II, IX, and X*), and bone formation (*osterix*, *osteocalcin*, and *runx2*). Differences in vascular tissue and total callus volumes are quantified by micro-CT.

Progress/Results: Radiographic evidence demonstrates delayed fracture healing and remodeling in the aged mice. *Col X*, a marker of chondrocyte maturation, has both reduced and delayed expression in aged mice, consistent with impaired endochondral bone formation. Similarly, *osteocalcin* expression, a marker of bone formation, is reduced in older mice. These findings demonstrate important temporal changes in bone repair with aging that are related to the differentiation of precursor cells and patterns of gene expression.

Near Future Directions: Include histomorphometric analysis to quantitatively compare mesenchymal, cartilaginous, and bony composition of fracture callus. We will also continue real-time PCR, *in situ* hybridization analysis of gene expression, and micro-CT analysis of healing patterns in young and aged mice.



A.A. NAIK



A.M. WANG

9:45 A.M.

Modulation of endothelial nitric oxide synthase phosphorylation as a molecular mechanism of endothelial dysfunction**ANNIE MISUNG WANG**, University of Rochester School of Medicine and Dentistry

Mentor: Paul L. Huang, M.D., Ph.D., Associate Professor of Medicine, Harvard Medical School, Departments of Cardiology and of Cardiovascular Research, Massachusetts General Hospital, Charlestown, Massachusetts

■ Endothelial dysfunction plays a pivotal role in the pathogenesis of many cardiovascular diseases. We studied modulation of eNOS phosphorylation as a molecular mechanism that mediates a common pathway for many forms of endothelial dysfunction. We tested the hypothesis that abnormalities in eNOS phosphorylation at serine 1179 (S1179) underlie vascular dysfunction and that modulation of eNOS phosphorylation can reverse negative effects. We generated transgenic and knockin eNOS mice carrying a constitutively active form (S1179D) and a non-phosphorylatable form (S1179A) of eNOS. We used these mice to test the effects of eNOS phosphorylation on hemodynamics and vascular reactivity in intact mutant mice and in mutant mice models of diabetes and obesity (*db/db* and *ob/ob* mice).

Our hemodynamic studies showed that S1179D mice had lower blood pressures than wild-type and eNOS knockout mice. Mean arterial pressure (MAP) of S1179D mice was 60 ± 3 mmHg, while wild-type mice had MAP of 75 ± 2 mmHg and eNOS knockout mice had MAP of 120 ± 4 mmHg. In contrast, S1179A mice had higher than normal blood pressures, 90 ± 3 mmHg. Using vascular reactivity studies, we found that S1179D mice had greater vasodilatory responses to acetylcholine and carbachol compared to S1179A mice. Maximal responses to ACh were $61 \pm 7\%$ for S1179D mice ($n=8$) and $18 \pm 8\%$ for S1179A mice ($n=4$). Similarly, maximal responses to carbachol were $50 \pm 7\%$ for S1179D mice ($n=8$) and $32 \pm 6\%$ for S1179A mice ($n=4$). There were no significant changes in diameter to ACh ($5 \pm 2\%$) or carbachol ($3 \pm 2\%$) in eNOS knockout mice.

These results provide evidence that abnormalities in eNOS phosphorylation at serine 1179 mediate endothelial dysfunction. Furthermore, they suggest that we may be able to correct vascular dysfunction by modulating eNOS phosphorylation. Further studies with pathologic mice models of diabetes and obesity are warranted to determine the pathophysiological association of metabolic diseases to endothelial dysfunction.

10:00 A.M.

Connexin43 and the cardiac sodium channel SCN5a: effects on impulse propagation and safety factor in cardiac ventricular tissue**RAJAN BAHL**, New York University School of Medicine

Mentor: Glenn I. Fishman, M.D., Professor, Departments of Medicine and of Physiology, Neuroscience and Pharmacology; Director, Division of Cardiology, New York University School of Medicine, New York, New York

■ The cardiac sodium channel SCN5A and the primary connexin in the heart ventricle, connexin43 (Cx43), play an important role in normal impulse propagation, making them important in clinically relevant cardiac arrhythmias. Both help determine the success of propagation through their opposing effects on the safety factor of propagation. Computer modeling has shown that decreased expression of SCN5A decreases safety factor, while decreased conductivity between myocytes, a result of decreased Cx43, increases safety factor. To test this modeling *in vivo*, we studied mice with a heterozygous SCN5A germline knockout, an inducible Cx43 knockdown, with both effects and two sets of wild-type controls, given and not given tamoxifen. The inducible knockdown was achieved with homozygous floxed alleles of Cx43 and a tamoxifen-inducible cre recombinase (MerCreMer). These mice and appropriate controls were optically mapped to find sinus activation patterns, ventricular effective refractory periods, and conduction velocities for both the right and left ventricles. The Cx43 expression level was verified using Western immunoblotting and immunostaining of sections.

Consistent with reduced Cx43 expression, subcutaneous ECGs of the tamoxifen-induced mice showed QRS amplitude reductions and widening. Difficulties with the induction protocol made a full study of the groups not yet possible. However, preliminary data has shown reduced conduction velocities, increased ventricular effective refractory periods, and an increased anisotropic ratio between the controls and the combination sodium channel heterozygous knockouts/Cx43 knockdown mice. For verification of knockdown, preliminary immunostaining data were ambiguous. Western immunoblotting showed decreased Cx43 expression in the inducible mice compared to the induced controls, but interestingly increased expression in both induced groups as compared to not induced controls.

Lack of statistically significant numbers makes it difficult to interpret these data. Knockdown of Cx43 and 50% knockout of SCN5A seem to cause marked changes in the aforementioned parameters.

10:30 A.M.

Aquaporin-4 enhances clearance of fluid from the brain extracellular space: implications for edema associated with cerebral infection and hydrocephalus**ORIN BLOCH**, University of California, San Francisco, School of Medicine

Mentors: Alan S. Verkman, M.D., Ph.D., Professor, Departments of Medicine and of Physiology, and Geoffrey T. Manley, M.D., Ph.D., Associate Professor, Department of Neurological Surgery, University of California, San Francisco, San Francisco, California

■ The water channel aquaporin-4 (AQP4) is expressed in foot processes of perivascular astrocytes at the blood-brain barrier (BBB), and at the ependymal and glial limiting surfaces of the brain in contact with cerebrospinal fluid (CSF). In vasogenic brain edema, extracellular fluid accumulation from increased permeability of the BBB can cause elevated intracranial pressure (ICP), cerebral ischemia, herniation, and death. Two clinically relevant mouse models of brain edema were developed to determine the role of AQP4 in clearance of excess fluid from the brain—bacterial brain abscess and communicating hydrocephalus. To create a focal brain abscess, live *Staphylococcus aureus* was injected into the striatum of mice. Wild-type and AQP4-deficient mice had comparable immune responses measured by abscess size, bacterial count, and cytokine levels. While BBB permeability was comparably increased in both groups, AQP4 null mice had a much greater increase in brain water content and significantly higher ICP (27 ± 2 vs. 17 ± 2 mmHg, $p < 0.001$) as compared to wild-type mice. To investigate the role of AQP4 in fluid reabsorption when normal CSF pathways are blocked, we developed a mouse model of communicating hydrocephalus by injection of kaolin into the cisterna magna. AQP4-deficient mice had 50% greater ventriculomegaly and a 2-fold greater increase in ICP compared to wild-type mice. Together, these results establish a new paradigm in how excess water is removed from the brain and suggest increasing AQP4 expression as a non-surgical treatment for brain edema associated with cerebral infections and hydrocephalus.



R. BAHL



O. BLOCH

TUESDAY
ROOM B

10:45 A.M.

P2Y receptor-mediated calcium responses are altered in senescent trabecular meshwork cells**JESSICA CHOW**, Duke University School of Medicine

J. CHOW

Mentors: **Fulton Wong, Ph.D.**, Professor, Departments of Ophthalmology and Neurobiology, and **Pedro Gonzalez, Ph.D.**, Assistant Professor, Department of Ophthalmology, Duke University School of Medicine, Durham, North Carolina

■ Cellular senescence of the trabecular meshwork (TM), the primary outflow pathway for aqueous humor, may contribute to the pathophysiology of glaucoma by increasing outflow resistance. Proposed mechanisms of outflow regulation include cell volume and extracellular matrix regulation, which may be affected by P2Y receptor-mediated calcium homeostasis. We set out to determine which P2Y receptor subtypes are involved in TM calcium regulation and to characterize how P2Y receptor-mediated calcium responses might be altered in senescent TM cells. Primary porcine TM cell cultures were used to assess the effects of P2Y agonists/antagonists on intracellular calcium $[Ca^{2+}]_i$ levels and P2Y receptor expression. Calcium responses were compared in TM cells cultured in standard conditions, oxidative stress, and replicative stress.

RT-PCR analysis of DNA sequences revealed porcine TM cell expression of the P2Y₁, P2Y₂, P2Y₄, and P2Y₆ subtypes. ATP, UTP, and ADP each induced an increase in $[Ca^{2+}]_i$, while UDP, a P2Y₆ agonist, did not evoke a response. Pretreatment with the nonspecific antagonist suramin blocked the responses to the above agonists, while MRS2179, a specific P2Y₁-receptor antagonist, did not block the UTP-induced response. Compared to cells incubated in physiological conditions, the average calcium response to all agonists in both replicative and oxidative stress-induced senescent cells decreased significantly (by 34–54% and 32–42%, respectively, with all P values <0.05).

The agonist/antagonist pharmacological profile supported the presence of functional P2Y₁ and P2Y₂ receptors in porcine TM cells. Compared to cells incubated in physiological conditions, the decrease in P2Y agonist-induced $[Ca^{2+}]_i$ rise in both oxidative and replicative senescent cells was statistically significant, suggesting that these cells may have altered calcium homeostasis; such changes might contribute to the pathophysiology of glaucoma.



G.M. RIHA

11:00 A.M.

Shear stress and cyclic strain induce two different vascular cell phenotypes from one mesenchymal cell line**GORDON MILES RIHA**, Baylor College of Medicine

Mentor: **Johnny Chen, M.D., Ph.D.**, Professor, Departments of Surgery and of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas

■ Hemodynamic forces within the vasculature play crucial roles in the modulation of vascular development, remodeling, healing, and atherosclerotic lesion formation. The forces of cyclic strain and shear stress may influence progenitor cell differentiation along the two respective lineages of smooth muscle cells (SMCs) and endothelial cells (ECs). To test this hypothesis, a murine embryonic mesenchymal cell line (C3H/10T1/2) was cultured separately under conditions of shear stress (15 dyn/cm²) for 6 and 12 hours and under conditions of cyclic equiaxial strain on collagen-coated membranes at 10% stretch (30 cycles/min) for 4 days. Expression of specific EC markers CD31 and von Willebrand Factor (vWF) and smooth muscle marker smooth muscle myosin heavy chain (SMMHC) was determined quantitatively with real-time PCR for shear and strain experiments, respectively. Additionally, levels of transforming growth factor- β (TGF- β) and vascular endothelial growth factor (VEGF) were quantitatively determined under conditions of shear, and 2 ng/ml TGF- β was supplemented to a group of cells subjected to strain.

Under conditions of shear stress, the mRNA levels of CD31 and vWF were increased 757- and 108-fold as compared to static controls at 12 hours. Additionally, after 12 hours of shear, levels of VEGF were increased seven-fold over static control, and TGF- β was decreased by 52% as compared to a 100% static control. Under conditions of cyclic strain, the same cell line did not express an increase of SMMHC with strain alone; however, when TGF- β was supplemented with strain, cellular expression of SMMHC increased 18% over both static TGF- β culture and strain-only controls.

This study suggests that differential expression of an EC or SMC phenotype may depend on the exposure of progenitor cells to the respective hemodynamic forces of shear stress and cyclic strain. Furthermore, VEGF and TGF- β may help to drive this hemodynamic-influenced differentiation toward ECs and SMCs, respectively.

11:15 A.M.

Genetic analysis of preterm labor

LISANNE PALOMAR, Dartmouth Medical School

Mentor: Louis J. Muglia, M.D., Ph.D., Associate Professor, Departments of Pediatrics, of Molecular Biology and Pharmacology, and of Obstetrics and Gynecology; Director, Division of Pediatric Endocrinology and Diabetes, Washington University School of Medicine, St. Louis, Missouri

■ **Context:** We hypothesize that there are genetic factors involved in the timing of labor, and alterations can lead to preterm labor in some women. We tested this hypothesis with the following aims: 1) Analyze transgenic mice to be used for deletion of genes likely to be involved in parturition; 2) Identify pedigrees with multiple members affected by preterm labor and create a biologic repository to be analyzed for candidate genes; and 3) Analyze births in a large population for familial and non-familial factors associated with recurrence of preterm delivery.

Results: We demonstrated that deletion of loxP-flanked target genes in a novel line of transgenic mice initiated by postnatal week two and completed by approximately one month with expression specific to uterine myometrium and bladder smooth muscle. Twenty families with multiple members affected by preterm birth have been identified, and detailed pedigrees and obstetric histories have been obtained. Thirty-four DNA samples to be analyzed for linkage and association studies have been obtained. Analysis of births in Missouri showed African Americans accounting for larger proportions of very early preterm birth, independent of many environmental factors, and found small variations of gestational ages within individual families.

Conclusions: The loxP-flanked telokin gene is successfully deleted starting at postnatal week two and the mice are appropriate for analyzing genes likely to be involved in parturition. There is evidence for familial preterm labor in the pedigrees with multiple affected members. Further evaluation of these families and identification of additional families is necessary to determine a mode of inheritance and candidate genes involved in preterm labor. The overrepresentation of African American births in earliest gestation when morbidity and mortality most likely occur and the clustering of early gestational ages within a given family support the need to discover genetic contributors involved in preterm birth.

11:30 A.M.

Androgenetic alopecia: a novel role for liver x receptor β in the regulation of 5 α -reductase type I

BRYAN ALLAN ONG, University of Texas Southwestern Medical Center at Dallas Southwestern Medical School

Mentor: David J. Mangelsdorf, Ph.D., Investigator, Howard Hughes Medical Institute; Professor, Department of Pharmacology; Doris and Bryan Wildenthal Distinguished Chair in Medical Science, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas

■ **Androgenetic alopecia**, or male pattern baldness, results from follicle exposure to testosterone and, more importantly, dihydrotestosterone (DHT). DHT is produced from testosterone by 5 α -reductase type 1 (5 α R1) in target tissues, thus making the inhibition of 5 α R1 activity an important pharmacologic aim. Previous reports have shown that mice lacking the oxysterol nuclear receptor liver x receptor β (LXR β) have decreased epidermal proliferation and increased sebocyte differentiation similar to skin treated with androgens. To investigate the possibility that LXR β regulates expression of 5 α R1 in skin, we have conducted a survey of 5 α R1 expression and function in male wild-type and LXR-null mice that had been castrated at 12 weeks and treated dorsally with a subcutaneous 15 mg testosterone pellet.

Our initial LXR genotype survey revealed a remarkable ninefold higher 5 α R1 expression in LXR $\beta^{-/-}$ mice compared to wild types, while LXR $\alpha^{-/-}$ mice exhibited no difference. Upon further analysis using castrated, testosterone-treated animals, LXR $\alpha^{-/-}\beta^{-/-}$ mice exhibited notable hair loss at the site of pellet implantation compatible with androgenetic alopecia (increased telogen shedding-stage follicles). Hair loss did not occur in testosterone-treated wild types or placebo-treated mice. 5 α R1 mRNA expression relative to placebo-treated wild type increased as follows: 10-fold in testosterone-treated wild type, 19-fold in placebo-treated LXR $\alpha^{-/-}\beta^{-/-}$, and 22-fold in testosterone-treated LXR $\alpha^{-/-}\beta^{-/-}$ mice. Testosterone-treated LXR $\alpha^{-/-}\beta^{-/-}$ mice possessed a twofold higher serum DHT concentration than similarly treated wild types. Though elevated, no differences in skin DHT concentrations were observed. Additionally, 5 α R1 promoter analysis revealed one putative LXR response element 6 kb upstream of the human 5 α R1 gene and two others 12 kb upstream of the mouse 5 α R1 gene.

Our findings support LXR β negative regulation of 5 α R1. Although promoter analysis is pending, we propose that pharmacologic LXR β activation could lead to 5 α R1 repression, thus attenuating *de novo* scalp DHT synthesis and further protecting an individual from androgenetic alopecia.

TUESDAY
ROOM B

L. PALOMAR



B.A. ONG

TUESDAY
ROOM B

11:45 A.M.

Mutations in nuclear-encoded complex III chaperone BCS1L cause Bjornstad syndrome, sensorineural deafness, and pili torti**JOHN TRAVIS HINSON**, Harvard Medical School

Mentor: Jonathan G. Seidman, Ph.D., Investigator, Howard Hughes Medical Institute; Henrietta B. and Frederick H. Bugher Professor of Cardiovascular Genetics, Department of Genetics, Harvard Medical School, Boston, Massachusetts

■ Bjornstad syndrome is a recessive human disease characterized by sensorineural hearing loss and pili torti, a hair shaft defect. We have previously mapped the Bjornstad disease gene in a consanguineous kindred to a 3 cM region at 2q34-36. By a candidate approach sequencing 44 of 47 genes, we identified a homozygous, missense mutation in BCS1L. Analysis of BCS1L in five smaller families with Bjornstad syndrome revealed seven novel mutations. Notably, an infant with BCS1L mutations had Bjornstad syndrome with developmental delay and growth retardation. Yeast BCS1L is localized to the mitochondrial inner membrane and functions as a chaperone for the assembly of the Rieske Fe/S protein into complex III of the respiratory chain. Work by others has identified mutations in BCS1L in two lethal neonatal syndromes, GRACILE syndrome and complex III deficiency. Comparing mitochondrial function in a yeast complementation model showed that both complex III deficiency/GRACILE mutations and Bjornstad mutations similarly alter yeast mitochondrial function. The complex clinical heterogeneity of BCS1L mutations may be explained by the clustering of Bjornstad mutations around residues 183-184 and within the AAA-ATPase domain at 302-306. Mutation clustering and the lack of yeast genotype/phenotype correlation implicate a novel role for BCS1L in the inner ear and hair follicle that is not conserved to yeast. Moreover, specific regions of the human hair follicle are enriched with BCS1L protein and complex III by immunofluorescence. Within human mitochondria, we have identified BCS1L within two protein complexes with the reduction of the larger 450 kD complex correlating with an accumulation of sub-assembly complex III supercomplexes without the Rieske Fe/S protein. We conclude that BCS1L is the Bjornstad syndrome gene, hearing loss and hair shaft defects can be due to mitochondrial diseases, and complex III of the respiratory chain plays a yet undefined role in hair follicle morphogenesis and sensorineural hearing.



J.T. HINSON



A. ZUCKER

NOON

CLN8 impacts sphingolipid synthesis**ADAM ZUCKER**, Duke University School of Medicine

Mentor: Rose-Mary Boustany, M.D., Professor, Department of Pediatrics and Neurobiology, Duke University Medical Center, Durham, North Carolina

■ Neuronal ceroid lipofuscinoses (NCLs) are inherited neuronal cell storage disorders characterized by retinitis pigmentosa, seizures, cognitive and motor decline, and death by the late teens. Of the nine clinical variants, six have known genetic defects. The genes for variants CLN3, CLN6, and CLN8 code for hydrophobic proteins. CLN3 impacts sphingolipid metabolism. Different mutations in CLN8 cause epilepsy with mental retardation (EMPR) and Turkish variant late infantile NCL. The *mnd* mouse is a naturally occurring mouse model for the disease. *Mnd* fibroblasts have increased rates of growth and apoptosis compared to controls. They also have decreased levels of glucosylceramide, lactosylceramide, galactosylceramide, ceramide trihexoside, and globoside. Transfection of human CLN9 fibroblasts (an NCL disease with low levels of SM and ceramide) with CLN8 causes partial correction of both ceramide and SM. This suggests that CLN8 may regulate sphingomyelin or ceramide synthesis. Two forms of SMS exist in mammalian cells: SMS1 and SMS2. SMS1 is located in the golgi while SMS2 is specific for the plasma membrane. siRNA specific for sphingomyelin synthase 2 (SMS2) was used to knock down gene expression in *mnd* and normal mouse fibroblasts. Growth rates of normal mouse fibroblasts increased, mimicking the growth of CLN8-deficient mouse cells. Knocking down SMS2 exaggerated the *mnd* fibroblast growth rate pattern. This data suggests the following hypothesis: *CLN8 protein may positively regulate SMS2 in normal cells.* This hypothesis will be tested by 1) characterizing SM and ceramide synthesis in Golgi and plasma membrane fractions of normal vs. *mnd* cells before and after a) knock down of SMS2 and b) overexpression of SMS2 and by 2) determining effect of SMS2 knock down or overexpression in normal and *mnd* cells on a) SMS1 RNA levels and b) apoptosis and cell growth.

12:30 P.M.

Wnt5a is required for the septation of the cardiac outflow tract in mice**J. ROBERT SCHLEIFFARTH**, University of Minnesota Medical School—Twin Cities

Mentors: Anna Petryk, M.D., Assistant Professor, and Michael B. O'Connor, Ph.D., Investigator, Howard Hughes Medical Institute; Professor, Departments of Pediatrics, and of Genetics, Cell Biology, and Development, University of Minnesota—Twin Cities, Minneapolis, Minnesota

■ The cardiac neural crest (CNC) is a migratory population of cells important for the formation of the cardiac outflow tract and remodeling of the aortic arches. Wnt5a is a secreted ligand that interacts with the transmembrane receptor Fz2 at the cell surface. In this report we characterize the cardiac outflow tract abnormalities found in *Wnt5a* mutant mice. These abnormalities are consistent with those observed in CNC ablated chick embryos including persistent truncus arteriosus (PTA) with or without coarctation of the aorta or interruption of the aortic arch, and double outlet right ventricle (DORV). *Fz2* and *Wnt5a* expression was detected in the cardiac outflow tract of wild type mice at the time of outflow tract septation, suggesting a possible role for Wnt5a/Fz2 in the formation of the cardiac outflow tract. Expression of the neural crest marker *Sox10* and hindbrain neural crest marker *Crabp1* was normal in the *Wnt5a* mutant mice at E9.5 and E10.5, respectively, suggesting normal initial specification and migration of CNC cells to the level of the brachial arches. However, expression of the CNC markers *PlexinA2* and *Sema3C* at the time of CNC invasion of the outflow tract was greatly reduced in the *Wnt5a* mutant mice. These data suggest Wnt5a is needed for the proper migration of the CNC into the cardiac outflow tract.

12:45 P.M.

Functional evidence of mismatch repair deficiency in non-obstructive azoospermia**LEI CHU**, Baylor College of Medicine

Mentor: Dolores J. Lamb, Ph.D., Professor, Department of Molecular and Cell Biology, and The Scott Department of Urology, Baylor College of Medicine, Houston, Texas

■ Genomic integrity requires DNA mismatch repair (MMR) protein expression. MMR-deficient rodents are prone to tumorigenesis and exhibit testicular pathologies similar to testicular failure (TF) patients. Abnormalities in two MMR proteins, MSH2 and MLH1, have been shown to account for 90% of hereditary nonpolyposis colon cancer and 20% of endometrial cancers. Recently, our lab showed that abnormal MSH2 and MLH1 expressions lead to TF.

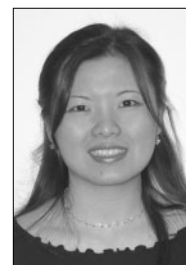
In this study, we tested the hypothesis that men with idiopathic, primary non-obstructive azoospermia exhibit higher predisposition to tumorigenesis, microsatellite instability (MI), and MSH2 and MLH1 abnormalities. Study design involves a prospective analysis of cultured testicular fibroblasts from these patients compared to patients with normal spermatogenesis and obstructive azoospermia (negative control) and known MMR-deficient cancer cell lines (positive control) based on tolerance to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG)—the survival response to alkylating agents provides an excellent functional assay to detect MMR abnormalities. Immunohistochemistry analysis was then done on MNNG-tolerant cell lines to determine abnormalities in MSH2 and MLH1. Finally, peripheral blood genomic DNA from these patients was analyzed for MI.

Results indicate that fibroblasts from sixteen out of seventeen patients studied with primary, idiopathic non-obstructive azoospermia exhibited MMR failure as evidenced by their tolerance to MNNG exposure: nine out of ten patients with Sertoli cell-only syndrome (SCO), three out of four with hypospermatogenesis, and three out of three with maturation arrest. Three SCO patients exhibited abnormal MSH2 or MLH1 protein expressions, all of whom also showed moderate MI in genomic DNA. Finally, two patients had a history of cancer: one testicular and one colon.

In conclusion, genetic defects associated with cancer predisposition in TF patients are more common than previously expected. As these men were candidates for testicular sperm extraction and ICSI-IVF, their “mutator” phenotype may have other unrecognized systemic consequences for the offspring.



J.R. SCHLEIFFARTH



L. CHU

TUESDAY
ROOM B

1:00 P.M.

Elucidating the nature of pancreatic β -cell regeneration after the reversal of autoimmunity in spontaneously diabetic NOD mice**CORINNA C.D. FRANKLIN**, Harvard Medical School

Mentor: Boris Nikolic, M.D., Assistant Professor of Medicine, The Renal Unit, Harvard Medical School and Massachusetts General Hospital, Boston, Massachusetts

■ Mixed hematopoietic chimerism induces tolerance toward donor pancreatic islets and reverses established autoimmunity in spontaneously diabetic NOD mice. Mixed chimeric NOD mice can then accept islet grafts, which normalize blood glucose levels; these NOD mice are thus cured from autoimmune diabetes. In some mixed chimeric mice in which donor- and self-tolerance was induced, signs of insulin-producing beta cell regeneration in the native pancreata have been observed. The purpose of this project has been to study the islet regeneration in these chimeric NOD mice. We have been investigating both the extent of this islet regeneration following the reversal of autoimmunity and elucidating its genetic and epigenetic profile. We have detected firm evidence of native β -cell regeneration in the native pancreata of mixed chimeric mice and are preparing to more fully characterize it via immunofluorescence and confocal microscopy. We have also optimized two methods for the detection of epigenetic changes in regenerated vs. normal pancreata, and are in the process of generating and analyzing microarray data from these specimens, in addition to microarray gene expression data. The information gained from these investigations will develop our understanding of the capacity of adult pancreatic islets to regenerate after autoimmune destruction. Regenerative therapy might then be combined with other therapeutic strategies, including the transplantation of islets, to promote both the proliferation and regeneration of insulin producing cells, to produce a cure for type 1 diabetes.



C.C.D. FRANKLIN



I.O. KARIKARI

1:15 P.M.

RNA-based gene modification of T lymphocytes for adoptive immunotherapy**ISAAC O. KARIKARI**, Duke University School of Medicine

Mentors: John H. Sampson, M.D., Ph.D., Associate Professor of Surgery and Assistant Professor of Pathology, Department of Surgery (Neurosurgery), and Joseph Nevins, Ph.D., Investigator, Howard Hughes Medical Institute; Director, Center for Genome Technology, Institute for Genome Sciences and Policy, Duke University Medical Center, Durham, North Carolina

■ Adoptive immunotherapy with antigen-specific T lymphocytes is a potential treatment against cancer and viral diseases. Despite recent success in phase I clinical trials using tumor infiltrative lymphocytes in the context of non-myeloablative chemotherapy in patients with malignant melanoma, adoptive immunotherapy continues to face significant impediments (Dudley et al., *Journal of Immunotherapy*, 25, 243–251, 2002). One of the biggest challenges facing adoptive immunotherapy lies in the enhancement of the T cell function. The advent of effective methods of gene transfer in T lymphocytes provides a novel means of enhancing various T cell functions. Recently, our lab and others have observed efficient gene transfer into T cells using mRNA electroporation, which offers a valuable and relatively safer alternative to retroviral-based gene transfer (Smits et al., *Leukemia*, 18(11), 1898–1902, 2004). In this project, we sought to 1) determine the efficiency and duration of mRNA electroporation in T cells assessed by green fluorescent protein (GFP), 2) demonstrate the ability to target antigen-specific T cells using RNA gene transfer, and 3) enhance T cell trafficking and proliferation/memory differentiation using mRNA encoding the chemokine receptor CXCR2 (IL8R β) and Interleukin 15, respectively.

Using flow cytometry analysis, we found a transfection efficiency of 65% and a duration of GFP expression of approximately 5 days post-electroporation. Moreover, we were able to demonstrate antigen-specific targeting with 75% of the GFP expressing T cells belonging to the antigen-specific group. The results for enhancement of T cell trafficking, proliferation/memory differentiation are currently being evaluated.

These studies will lead to a rationale approach to the enhancement of T cell function for adoptive immunotherapy.

1:30 P.M.

Deciphering the estrogen receptor histone code**ANNA JADWIGA SZARY**, Harvard Medical School

Mentor: Myles A. Brown, M.D., Chief, Division of Molecular and Cellular Oncology; Associate Professor of Medicine, Harvard Medical School, Dana-Farber Cancer Institute, Boston, Massachusetts

■ Estrogen is essential for the normal development of the breast and also plays a significant role in the development and progression of breast cancer. Estrogen interacts with the estrogen receptor (ER), causing ER to bind to various regions of the genome. However, the organization of these cis-regulatory elements has yet to be fully elucidated. Furthermore, ER binding results in complex interactions between chromatin and various regulatory factors, including histone modifying factors. Posttranslational histone modifications are thought to supply a code for the regulation of gene expression through chromatin remodeling and the interaction of non-histone regulatory factors with

chromatin. However, the role of specific histone modifications during estrogen-induced proliferation remains unclear. With the advent of the completion of the human genome, it is now possible to utilize an unbiased approach to identify all regulatory regions that may play a role in ER-mediated transcription. ER binding and changes in histone modifications on the complete non-repetitive sequences of human chromosomes 21 and 22 were investigated using chromatin immunoprecipitation (ChIP) coupled with tiled microarrays. Microarray data revealed a limited number of *in vivo* ER binding sites, in which the majority of sites are found greater than 100 kb from the transcription start site of regulated genes. Furthermore, these sites share common motifs and are involved in transcriptional regulation. Analysis of histone modifications on chromosomes 21 and 22 suggests a discrete and specific set of signature histone modifications that occur prior to and following estrogen-induced transcriptional activation. These data will allow the analysis of the biological significance of histone modifications in estrogen action.



A.J. SZARY

Note: Posters presented by HHMI-NIH Research Scholars.

POSTER 1

Identification of differentiation inducing gene products in Glioblastoma multiforme tumor stem cells

OLUWASEUN AKEJU, University of Medicine and Dentistry of New Jersey, New Jersey Medical School

Preceptor: John Park, M.D., Ph.D., Surgical and Molecular Neuro-oncology Unit, Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland

■ Similarities in cell morphology and expression of markers exist between cancerous and normal cells in the brain, suggesting a common cellular origin. Recent advances in the field of neuro-oncology has led to the identification of *in vivo* brain tumor stem cells (BTSC) from human brain tumors which possess markers for normal neural stem cells such as nestin and CD133. These BTSC, which represent a minority of the entire brain tumor cell population, possess the marked capacity for proliferation, self-renewal, and differentiation that has been postulated to be responsible for maintenance of the tumor bulk.

Using two-dimensional differential gel electrophoresis and chromatin immuno-precipitation techniques on a glioma *in vitro* model in which BTSC were isolated from Glioblastoma multiforme and maintained in an undifferentiated state in culture by the addition of epidermal growth factor and fibroblast growth factor-2, and then modulated by extracellular cues such as ciliary neurotrophic factor and bone morphogenic protein-2, we have shown that these BTSC differentiate to recapitulate cells from their tumor of origin. We have also identified candidate gene products that may play pivotal roles in the process of differentiation of these BTSC.

Recent evidence dictates that these core population of BTSC are responsible for generating and maintaining the tumor mass, and this may in part explain why bulk tumor therapy has had limited success. At the molecular level, our focus is to better understand the mechanisms by which these BTSC integrate the multiple growth factor induced signals to ultimately develop a differentiated phenotype. Given the fact that cell differentiation status affects glioma formation, further characterization of our candidate gene products by gene transfer and RNA interference techniques will afford a significant contribution to the design of glioma treatment therapy, effective in achieving cell cycle arrest of tumor growth by the activation of a differentiation program.

POSTER 2

The role of Wiskott Aldrich syndrome protein in T helper cell function

JOSEPH AOKI,* University of Hawaii, John A. Burns School of Medicine

Preceptor: Pamela Schwartzberg, M.D., Ph.D., Cell Signaling Section, Genetic Disease Research Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland

■ Wiskott Aldrich syndrome (WAS) is characterized by immune deficiency, thrombocytopenia, eczema, and increased incidence of autoimmune disease. The protein deficient in this syndrome, WASp, is required for actin-based cytoskeletal rearrangement and T cell activation. Recent data reveal that the Tec kinase, Itk, is required for WASp activation and actin polymerization. Additionally, Itk-deficient mice show defective T helper cell differentiation. Regulation of T helper cell differentiation and cytokine production is essential for mounting appropriate responses against pathogens, while dysregulation of these pathways has been implicated as a possible cause of hypersensitivity and autoimmunity. Given the increased incidence of eczema and autoimmune disease in WAS patients, we asked whether WASp might also be involved in T helper cell differentiation and function.

We have found that murine WAS^{-/-} CD4⁺ T cell cultures exhibit marked reductions in secretion of both the Th1 cytokine IFN- γ and the Th2 cytokine IL-4. Nonetheless, intracellular staining revealed that WASp-deficient CD4 cells produce normal to elevated levels of IFN- γ and IL-4. These data suggest that WASp may be involved in the secretion, but not production, of IL-4 and IFN- γ in CD4⁺ T cells.

Surprisingly, following challenge with the Th2 inducing agent, *Schistosoma mansoni*, WAS^{-/-} mice show heightened responses, including larger pulmonary granulomas and higher production and secretion of Th2 cytokines. Moreover, WAS^{-/-} mice show fewer numbers of brain cysts and increased production and secretion of IFN- γ following infectious challenge to the Th1 inducing parasite *Toxoplasma gondii*. Thus, WASp-deficient mice can mount both a Th1 and Th2 response *in vivo* that may be exaggerated. Together, these findings suggest that WASp deficiency has complex effects on T helper cell effector function, which may provide insight into altered immune responses and autoimmunity in patients with Wiskott Aldrich syndrome.

*Advanced Scholar

POSTER 3

Triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone): a ribonucleotide reductase inhibitor with potent radiosensitizing properties**CHRISTOPHER ANDREW BARKER**, University of Florida College of Medicine

Preceptor: Kevin Camphausen, M.D., Imaging and Molecular Therapeutics Section, Radiation Oncology Branch, Radiation Oncology Sciences Program, Molecular Radiation Therapeutics Branch, Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

■ Ribonucleotide reductase is the rate-limiting enzyme of *de novo* DNA synthesis. The enzyme is composed of two homodimer subunits, hRRM1 and hRRM2. p53R2, a recently discovered functional alternative to the hRRM2 subunit, appears to be induced by DNA damage through p53 binding. Clinically, the enhancement of tumor control through radiation therapy and ribonucleotide reductase inhibition was first attempted using hydroxyurea; however, efficacy in chemoradiation trials was limited. *In vitro* studies later showed that these limitations may be due to differential enzyme sensitivity whereby the p53R2 holoenzyme is less sensitive to hydroxyurea than the holoenzyme involving hRRM2. Triapine, a novel ribonucleotide reductase inhibitor, exhibits an equivalent sensitivity profile for the holoenzymes containing p53R2 or hRRM2. We hypothesized that following radiation, Triapine would more effectively inhibit DNA precursor synthesis and repair, thus imparting a radiosensitizing effect.

To test our hypothesis, we conducted a series of experiments using *TP53* wild-type and mutant human tumor lines and human fibroblasts. Western blot confirmed the increase in p53R2 following irradiation. DNA synthesis assays demonstrated the inhibitory effect of Triapine in a time-dependent manner. Clonogenic assays demonstrated the radiosensitizing effect of Triapine, with a dose-enhancement ratio between 1.43 and 1.97. Flow cytometry confirmed that cell cycle redistribution did not account for the radiosensitization. *In vivo*, radiation and Triapine delayed tumor growth more than the sum effect of both modalities. These data suggest that following DNA damage, Triapine inhibits DNA precursor synthesis and enhances the effect of radiation.

POSTER 4

Cancer-wide tissue microarray survey of ezrin and merlin expression**BENJAMIN BRUCE**, Stanford University School of Medicine

Preceptor: Chand Khanna, D.V.M., Ph.D., Tumor and Metastasis Biology Section, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

■ We have previously demonstrated that suppression of ezrin resulted in the inhibition of metastasis in murine models of osteosarcoma and rhabdomyosarcoma. Although they both localize at the membrane cytoskeleton interface, merlin's function may differ and may be antagonistic to that of ezrin. To date, little is known about the expression and role of ezrin and merlin in cancers. To determine whether ezrin's demonstrated role in sarcoma metastasis extends to other cancers, particularly cancers of epithelial origin, and to define the expression of merlin, a putative competitor of ezrin, we performed a cancer-wide survey of ezrin and merlin expression using tissue microarrays (TMA). Immunohistochemistry on 326 different tumor samples (including breast, colon, lung, ovarian, and prostate cancers) and over 200 different normal samples were analyzed for both merlin and ezrin staining intensity. TMA analysis revealed ezrin is expressed in both cancer and normal tissues. High ezrin expression was seen in 51% (n=42) of breast cancers, 34% (n=60) of ovarian cancers, 87% (n=82) of lung cancers, 95% (n=73) of colon cancers, and 100% (n=69) of prostate cancers. Similarly, merlin was found to be expressed in cancer and normal tissues. Merlin was highly expressed in 53% (n=41) of breast cancers, 25% of ovarian cancers (n=36), 29% (n=86) of lung cancers, 54% (n=76) of colon cancers, and 32% (n=69) of prostate cancers. There was no significant difference in expression of either protein between normal tissue and its corresponding cancer. These data suggest that it is not likely that either gain of ezrin or loss of merlin expression is involved in early tumorigenesis of breast, colon, lung, or prostate cancer; however, analysis of ezrin, merlin, and ezrin:merlin ratio expression as a function of clinical outcome in patients is currently under way.

POSTER 5

The potential of farnesyltransferase inhibitors in Hutchinson-Gilford progeria syndrome**BRIAN C. CAPELL**, New York University School of Medicine

Preceptor: Francis S. Collins, M.D., Ph.D., Molecular Genetics Section, Genome Technology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland

■ Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic disorder characterized by dramatic premature aging. Death occurs at an average age of 12, most commonly due to myocardial infarction or stroke. Classical HGPS cases are almost always caused by a *de novo* point mutation in the lamin A (*LMNA*) gene that activates a cryptic splice donor site and produces a truncated mutant protein, termed “progerin.” Progerin acts as a dominant negative to disrupt the nuclear scaffold, resulting in significant nuclear blebbing, nuclear pore clustering, and disruption of the underlying heterochromatin. In normal cells, prelamin A is anchored to the nuclear envelope by the addition of a farnesyl group to the C-terminal CAAX motif. The terminal 18 amino acids and the farnesyl group are then cleaved, releasing mature lamin A from this tether. In progerin, this cleavage site is deleted. We hypothesize that retention of the farnesyl group causes progerin to become permanently anchored in the nuclear membrane, disrupting the nuclear scaffolding and leading to the characteristic nuclear blebbing seen in HGPS cells. We therefore hypothesized that blocking farnesylation might decrease the toxicity of progerin. To test this, site directed mutagenesis was performed on the CAAX sequence. When the normal CSIM sequence of progerin was mutated to SSIM (which should block all prenylation), all the progerin was relocalized from the nuclear periphery into nucleoplasmic aggregates and nuclear blebbing was prevented. In the CSIL mutant, which is predicted to be geranylgeranylated, nuclear blebbing was reduced. These results indicate that preventing farnesylation by treatment with farnesyltransferase inhibitors might prevent or even reverse the nuclear blebbing seen in HGPS, and thus serve as a potential therapeutic option for patients. Current experiments are exploring the efficacy of the farnesyltransferase inhibitors SCH6636 (lonafarnib) and R115777 (tipifarnib) in both cell culture studies and in an HGPS mouse model.

POSTER 6

Mining of drug database from developmental therapeutic programs reveals antineoplastics that selectively target P-glycoprotein overexpressing cell lines**BENJAMIN FU-HAN CHU**, Texas Tech University Health Sciences Center School of Medicine

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

■ P-glycoprotein (Pgp), an ABC (ATP-binding cassette) transporter capable of effluxing functionally and structurally diverse chemotherapies from cancerous cells, is an important mediator of intrinsic and acquired multidrug resistance (MDR). Recently, Szakacs et al. has used a bioinformatic approach to correlate patterns of drug sensitivity with expression profiles of Pgp, and forty-seven remaining transporters, among sixty cancer cell lines (NCI60.) Putative P-gp substrates, those with strong negative correlations between drug efficacy and P-gp expression, were validated using human KB epidermoid carcinoma cell lines and tetracycline-regulated Pgp on-off systems. This approach also identified a thiosemicarbazone derivative with a strong positive correlation between drug sensitivity and P-gp expression, suggesting that P-gp, paradoxically, may potentiate rather than suppress cytotoxicity of select thiosemicarbazones. In an effort to screen for compounds that behaved similarly (those with strong positive correlations), the analysis was extended beyond the initial 1,400 compounds used for the prior analysis to include a larger dataset of approximately 17,000 compounds tested by the Developmental Therapeutic Program (DTP) of NCI. The extended screening of a larger dataset enabled the identification of four additional molecules demonstrating higher toxicity in cancer cell lines that over-expressed P-gp. Interestingly, two of the four molecules are structurally similar to the initially identified thiosemicarbazone derivative, suggesting a possible pharmacophore. Further evaluation of hypothesized P-gp sensitizers and their structural analogs may provide clues to elucidate their mechanism of action, and may serve as a basis for potential therapy against multidrug resistant cancers.

POSTER 7

Characterization of the M-current in hippocampal stratum oriens-alveus lacunosum-moleculare projecting interneurons

JOSEPH F. CHURCHILL, State University of New York at Buffalo School of Dental Medicine

Preceptor: Chris J. McBain, Ph.D., Laboratory of Cellular and Synaptic Neurophysiology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland

■ Action potential firing properties amongst select cell types are strongly regulated by the M-current (I_M), a muscarine inhibited potassium conductance that activates near threshold. Given that interneurons in the hippocampus are positioned to influence excitability of large populations of pyramidal cells, neuromodulation of I_M on hippocampal interneurons is an attractive mechanism for regulating large-scale network oscillations—a key feature of active learning states observed within hippocampus. Electrophysiological and immunohistochemical techniques were utilized to uncover the distribution of I_M and its molecular correlate, the KCNQ family of voltage-gated K^+ channels, in CA1 stratum oriens–lacunosum-moleculare (O-LM) interneurons of mouse hippocampus. The electrophysiological studies employed XE991, a potent KCNQ channel blocker as well as tetraethylammonium (TEA), which exhibits marked selectivity for KCNQ2 homomers. Whole-cell voltage clamp data revealed a subset of interneurons with cell bodies in stratum oriens/alveus of CA1 that contain significant outward current sensitive to XE991 block (1.36 ± 0.736 nA for $n=8$ of 17). Further voltage clamp experiments demonstrated a clear reduction of I_M following wash-in of XE991 (24.04 ± 5.58 to 6.13 ± 1.07 pA, $p=0.02$, $n=7$) or TEA (24.98 ± 8.63 to 9.07 ± 3.70 pA, $p=0.03$, $n=8$), when hyperpolarizing the cell from -30 to -50 mV. Consistent with electrophysiological findings, immunohistochemical studies revealed somatodendritic localization of KCNQ2 and KCNQ3 channel subunits amongst a subset of O-LM hippocampal interneurons. This powerful marriage between immunohistochemistry and electrophysiology of I_M provides the quantitative, kinetic, and morphological information required to integrate this conductance into a working computerized O-LM interneuron model. Threading this model into a virtual hippocampal network will lend insight into understanding normal and pathological hippocampal network processing—effectively toward understanding the very nature of memory processing.

POSTER 8

Bortezomib effects on irradiation-induced oral mucositis

INGRIDA DAPKUTE-MARCUS, University of Pennsylvania School of Dental Medicine

Preceptor: Carter Van Waes, M.D., Ph.D., Head and Neck Surgery Branch, National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Bethesda, Maryland

■ **Background:** Oral mucositis (OM) is a common adverse effect of radiation therapy in patients with head and neck squamous cell carcinoma (HNSCC). OM is associated with activation of transcription factor nuclear factor kappa B (NF- κ B) and cytotoxic cytokine genes in irradiated human and animal mucosa. NF- κ B can promote survival of malignant cells, but it also can induce cytokine-mediated toxicity in normal tissues.

Bortezomib is a proteasome inhibitor that acts on constitutive and radiation-induced activation of NF- κ B and sensitizes HNSCC to treatment. Clinical observations of the effects of Bortezomib in patients undergoing chemoradiotherapy for recurrent HNSCC showed lower grade OM. Therefore, in this study we examined if Bortezomib has radioprotective effects on normal mucosa in a murine OM model.

Materials and Methods: Kinetics of activation of NF- κ B target genes and mucositis in irradiated tongues of normal mice were determined via reverse transcription-polymerase chain reaction (RT-PCR). NF- κ B DNA binding assay and immunohistochemistry were conducted to validate NF- κ B expression. Next, 40 mice were injected with a single dose of 1.3 mg/kg ps-341 24 hours before irradiation. The animals were sacrificed on days 7 and 8 after radiation treatment to assess ulcer formation. Finally, mice were injected with ps-341 twice weekly (24 hrs before and 48 hrs after irradiation) to closer reflect the human regimen.

Results: By day 7, NF- κ B regulated cytokine genes IL-1 β , TNF- α , and Gro-1 were significantly overexpressed in irradiated tissues concurrent with mucositis and ulcer formation. Pretreatment with Bortezomib 24 hours before radiation delayed ulcer formation by 1 day. Results of twice weekly Bortezomib treatment used in clinical studies are pending. Additional experiments are under way to determine if Bortezomib inhibits activation of NF- κ B, apoptosis and expression of toxic cytokines. Also, further investigation will show if PS-341 has radiosensitizing or radioprotective effects against SCC in a murine model.

POSTER 9

Minimizing respiratory variations improves functional connectivity delineation

JASON DIAMOND, Nova Southeastern University, College of Osteopathic Medicine

Preceptor: Peter Bandettini, Ph.D., Functional Imaging Methods Unit, Laboratory of Brain and Cognition, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland

■ Low-frequency blood oxygen level-dependent (BOLD) signal fluctuations are present in fMRI during rest. While the interregional temporal correlations of these spontaneous fluctuations may determine functional connectivity, the extent to which the fluctuations represent neuronal, vascular, or metabolic mechanisms is currently unknown. A recent study found BOLD signal fluctuations correlated to fluctuations in end-tidal CO₂. Carbon dioxide has also been shown by studies of breath-holding to increase the BOLD signal. Recently, functional connectivity has been shown between brain regions that show task-independent decreases in activity, suggesting a “default mode” network in the resting brain. In this study, we examine the extent to which functional connectivity in this network is influenced by respiratory physiology.

MR scans of four healthy volunteers were performed 1) during a lexical decision task, 2) at rest, and 3) while regulating their breathing pattern based on a visual stimulus oscillating at a constant rate. Respirations were measured using a belt positioned at the level of the abdomen, and were used to estimate respiration volume/time, an approximation of end-tidal CO₂. Functional connectivity analysis was performed on the resting and regulated respiration runs based on a region that showed lexical decision task induced deactivation.

During the resting runs, significant connectivity was found between areas associated with the “default mode” network as well as a variety of other brain regions. During runs where variations in the respiration volume/time measurements were minimized, the resulting connectivity maps more closely resembled the spatial pattern depicted during the task state, and thus may be reflective of true neuronal connectivity. Therefore, for accurate delineation of functional networks to be possible, contamination of the BOLD signal by respiratory variations must be corrected for. An accurate characterization of such functional networks and their low frequency fluctuation constituents may provide important insights to brain physiology and pathology.

POSTER 10

The role of Rap1 and RalA in Bax activation and translocation to the mitochondria during apoptosis

BRIAN J. DLOUHY, New York University School of Medicine

Preceptor: Richard J. Youle, Ph.D., Biochemistry Section, Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland

■ Apoptosis describes the genetically designed cellular process whereby the cell undergoes staging membrane blebbing, cell shrinkage, protein fragmentation, chromatin condensation, and DNA degradation followed by engulfment of cellular remains with little inflammation. Bax is a proapoptotic protein that resides mainly in the cytosol in healthy cells and translocates to the mitochondria, inserts and oligomerizes during apoptosis. However, the method by which this occurs is unknown, and the purpose of this work was to further elucidate this mechanism.

A cytosolic component was deemed necessary for Bax conformational change and translocation to the mitochondria during apoptosis. Ral-GDS, a protein in the cytosol associated with the small G binding proteins Ras, Rap, and Ral, was found to be a candidate protein possibly involved in Bax translocation. Through Rap and RalA pulldown assays, Rap1, RalA, and Bax were found in transient complexes in apoptotic cells but not in healthy cells. Overexpression of Rap1 and RalA sensitized HeLa cells to apoptotic inducers. Knockdown of either Rap1 or RalA through RNAi significantly decreased the number of apoptotic cells after apoptotic induction compared to control cells. We are currently investigating a wider role of Rap1 and RalA in apoptosis to determine if the activation of RalA would allow Bak, another pro-apoptotic protein, to oligomerize at the mitochondrial membrane. Bak and RalA are located in complexes of the same molecular weight at the mitochondrial membrane in healthy cells suggesting a possible interaction between the two proteins. The activation of RalA could trigger a conformational change in Bak allowing its oligomerization.

In conclusion, we believe a complex of Rap1, Ral-GDS, and Bax translocates to the mitochondria during apoptosis and interacts with RalA at the mitochondria, leading to Bax insertion into the outer mitochondrial membrane. We are further investigating a wider role for these small G binding proteins in apoptosis.

POSTER 11

Exploring the role of EBV-specific CD4⁺ T cells in the pathogenesis of multiple sclerosis

NANCY EDWARDS, Duke University School of Medicine

Preceptor: Roland Martin, M.D., Cellular Immunology Section, Neuroimmunology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland

■ Multiple sclerosis (MS) is believed to be an autoimmune disease in which T cells recognize and destroy components of the axonal myelin sheath in the central nervous system (CNS). Epidemiological studies demonstrate that viral infections often precede MS exacerbations, and it is thought that viral epitopes that share structural or sequence homology to myelin epitopes may thereby activate myelin-specific T cells (i.e., molecular mimicry). One of the leading viral candidates proposed to play a role in MS pathogenesis is Epstein-Barr virus (EBV). The purpose of this study is to ascertain if there are EBV-specific CD4⁺ T cells in the CNS compartment of patients with MS; if so, can these viral-specific T cells cross-recognize myelin antigens.

CD4⁺ T cells recognize peptides bound to MHC class II molecules on the surface of antigen presenting cells. To insure MHC class II processing and presentation of any possible EBV epitope, we generated an EBV-invariant chain (Ii) fusion library. Coding regions encompassing the entire EBV genome were amplified by PCR and ligated into a vector containing an Ii DNA fragment. The EBV-Ii fusion library was then transfected into HEK-293 cells genetically engineered to express MHC class II. When translated, the Ii portion associates with MHC class II molecules, thereby enhancing the presentation of the EBV portion through the MHC class II pathway.

Using this EBV-Ii fusion library, we can then screen CD4⁺ T cell clones (TCC) isolated from the cerebrospinal fluid of MS patients for recognition of any possible EBV epitope. TCC responsive to EBV will then be analyzed for cross-recognition of many possible myelin antigens using a myelin-Ii fusion library. In general, this approach allows us to ascertain T cell receptor specificities in both an unbiased and biologically relevant way (versus traditional peptide scans where MHC class II processing/presentation is bypassed).

POSTER 12

Systems-based gene expression analysis highlights the role of NFκB as a mediator of hepatic fibrosis

ELDAD ELNEKAVE,* Tufts University School of Medicine

Preceptor: Thomas A. Wynn, Ph.D., Laboratory of Parasitic Diseases, National Institute for Allergy and Infectious Disease, National Institutes of Health, Bethesda, Maryland

■ By simultaneously assessing the expression of thousands of individual genes, high throughput techniques such as gene microarray promise to capture a detailed molecular snapshot of disease processes. The resulting data are notoriously cumbersome; a major challenge is to identify relevant genetic pathways from within a complex biological data nexus. To this end, we adopted a systems-based analysis of gene expression in a murine model of *Schistosoma mansoni*-induced hepatic fibrosis.

Pearson coefficients of three k-means derived gene clusters were used to define an *in vivo* system of hepatic fibrogenesis. One cluster was closely correlated with independent clinical measures of fibrosis. An additional cluster appeared to inhibit, whereas the third appeared to promote, fibrogenesis.

The reduction of gene sets into smaller functional clusters lends itself to an interpretation of key pathways driving their expression. By manipulating these key pathways using gene knock-out animals, we could predictably and dramatically alter not only the pathology but also the gene expression in each gene cluster within the system. The juxtaposition of IL-13-deficient mice, which exhibit resistance to fibrosis, versus a profoundly fibrogenic triple knockout strain, permitted a biologically amplified view of fibrogenic gene expression changes.

This approach allowed us to discover a number of gene programs and pathways integrally linked to liver fibrosis. We observed that collagen deposition and matrix remodeling were buttressed by pathways of cell proliferation, PDGF, and VEGF activity, as well as resistance to apoptosis. The molecular portrait of fibrosis was thus surprisingly akin to that of cancer. NFκB has been shown as a mediator of inflammation-induced cancer, so we next utilized two NFκB targeting interventions, the proteasome inhibitor Velcade, and a peptide IκK antagonist, to demonstrate the *in vitro* and *in vivo* benefit of NFκB inhibition upon fibrotic pathology.

*Advanced Scholar

POSTER 13

Hereditary spastic paraplegia and spartin: what is the link?

PARVIN FATHEDDIN, Duke University School of Medicine

Preceptor: Craig Blackstone, M.D., Ph.D., Cellular Neurology Unit, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland

■ Hereditary spastic paraplegias (HSPs) are a family of inherited disorders characterized primarily by bilateral, symmetric lower extremity spasticity. Pathology predominantly involves upper motor neurons with progressive degeneration of the longest axons, beginning distally. In Troyer syndrome, an autosomal recessive HSP, a point mutation in the spartin gene results in truncation and probably loss-of-function. Nothing is known, however, about the normal function of this 666-amino acid (aa) protein. Here we have identified a possible modification of spartin by ubiquitin that may provide insight into spartin's function.

Immunoprecipitation of HeLa cells co-transfected with myc-tagged spartin and a HA-tagged form of ubiquitin unable to form poly-ubiquitin chains was performed using myc-beads. HA immunoreactivity was detected at ~100 kD on denaturing gels, corresponding to myc-spartin (90 kD) plus one HA-ubiquitin moiety. The same band was observed when HA-ubiquitin capable of chain formation was used. Repeating the experiment with various segments of spartin showed that a 1-504 aa segment was ubiquitinated, while the 1-208 segment was not. Thus, spartin is mono-ubiquitinated and the modification seems to occur in the 208-504 aa region.

Since mono-ubiquitination has been described to be involved in endocytosis and spartin also harbors a microtubule interacting and trafficking (MIT) domain, it seems very likely that spartin is involved in endocytic processes. A spartin-ubiquitin fusion protein has been developed to test the effects of mono-ubiquitination on both ligand-dependent and constitutive endocytosis. We have expressed this protein and are currently investigating its effects on localization and function. These studies will provide insights not only into the normal function of spartin but also into the molecular pathogenesis of the Troyer syndrome.

POSTER 14

Comprehensive DNA copy number analysis of breast cancer cell line genomes by microarray comparative genomic hybridization

PAULINE FUNCHAIN, The Ohio State University College of Medicine and Public Health

Preceptor: Paul S. Meltzer, M.D., Ph.D., Cancer Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland

■ Altering DNA copy number is an important mechanism by which a cancer cell gains altered expression of tumor suppressors or promoters. Comparative genomic hybridization (CGH) assesses copy number in small, discrete segments by quantifying relative amounts of experimental genomic DNA versus normal diploid genomic DNA. Performing CGH with microarray technology allows sampling of smaller regions and mapping of genomic alterations at finer resolutions. Because genomic instability, frequent rearrangements, and manifold mutational events derange cancer genomes on both large and small scales, we hypothesized that visualizing breast cancer genomes at finer resolutions by array-based CGH would provide new insights into the genetic events that trigger and progress breast cancer.

We created a comprehensive genomic profile of copy number alterations in breast cancer by surveying the genomes of 51 breast cancer cell lines using array-based CGH. With an average gap of 0.1 Mb between oligos, our high-density arrays map genomic alterations at extremely fine resolutions unachieved in previous CGH arrays, allowing detection of previously undiscovered regions of copy number change. Statistical analysis revealed an overall pattern of genomic loss in gene-poor areas and gain in gene-rich areas across all cell lines. Significant copy number alterations found by array-based CGH include homozygous deletions in known tumor suppressor genes and amplifications of areas containing known proto-oncogenes, as well as numerous previously homozygous deletions and several candidate copy number polymorphisms. Confirmatory PCRs for select homozygous deletions and QPCRs for select regions of low copy deletion and amplification validate and closely correlate with copy numbers derived from our microarray data. We conclude that high-density oligo arrays provide a rich and reliable data mine from which new insights into cancer pathogenesis and diagnosis may be reaped.

POSTER 15

Sublethal doses of chemotherapy and irradiation to human squamous cell carcinoma of the head and neck modulate phenotype resulting in enhanced killing by CTL**ALEXANDER GELBARD**, Tulane University School of Medicine

Preceptor: James W. Hodge, Ph.D., Laboratory of Tumor Immunology and Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

■ The combination of systemic multiagent chemotherapy and tumor irradiation is standard of care for squamous cell carcinomas of the head and neck (H&NSCC). It has recently been recognized that non-cytolytic doses of radiation may alter the phenotype of tumor cells, thus making them more susceptible to T cell-mediated immune attack. Studies have similarly demonstrated a modulation of phenotype and enhanced T cell-mediated killing following sublethal doses of chemotherapy.

Our project sought to elucidate the effect of chemotherapy along with radiation on T cell-mediated killing of H&NSCC. Eight H&NSCC cell lines with distinct biological features were utilized for this study. Following *in vitro* administration of non-lytic doses of cisplatin and 5-Fluorouracil for 24 h, these cells were irradiated (10 Gy). Seventy-two hours post-irradiation, surface molecules were subsequently monitored by flow cytometry. The expression of the tumor antigen CEA was found to be increased, while the tumor antigen MUC-1 remained unchanged. Furthermore, immunomodulatory molecules ICAM-1, MHC class I, Fas, and B7-H1 demonstrated altered patterns of cell surface expression. To ascertain the relationship of these phenotypic changes to sensitivity to immune-mediated killing, the HLA-A2-positive H&NSCC lines were again subjected to multiagent chemotherapy for 24 hrs, followed by radiation. Tumor cells were then exposed to CEA-specific HLA-A2 restricted CD8⁺ CTL and lysis compared with their non-treated counterparts. Significantly, either modality alone did not improve cell lysis over control treated cells. However, when the two modalities were combined, a substantial enhancement of T cell-mediated killing was observed.

Overall, the preliminary results of this study suggest that in at least some human H&NSCC, T cell-mediated killing can be enhanced by the combination of multiagent chemotherapy and radiation. These studies thus form the rational basis for trials of immunotherapy concomitant with the current standard of care of H&NSCC.

POSTER 16

Low-dose nitrite ameliorates myocardial ischemia/reperfusion injury and reduces infarct size in a canine model**FELIX M. GONZALEZ**, University of Medicine and Dentistry of New Jersey Robert Wood Johnson Medical School

Preceptor: Andrew E. Arai, M.D., Laboratory of Cardiac Energetics, Vascular Therapeutics Section, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

■ Conversion of nitrite to nitric oxide under acidic and hypoxic conditions occurs independent of nitric oxide synthase activity and appears facilitated by deoxyhemoglobin. We hypothesized that nitrite would reduce myocardial infarct size *in vivo* in a large animal model via conversion to nitric oxide in ischemic at-risk tissue. An open chest canine model of acute myocardial infarction was used to compare the effectiveness of reducing infarct size with intravenous nitrite infusion (n=5) compared with placebo (n=3). The left anterior descending coronary artery was occluded for two hours followed by six hours of reperfusion. Nitrite infusion aimed to achieve circulating blood concentrations of 3–7 $\mu\text{mole/L}$ was initiated an hour into occlusion and stopped after reperfusion. Infarct size and area at risk were assessed by *in vivo* MRI and *ex vivo* by histopathology with fluorescent microspheres and triphenyltetrazolium chloride (TTC). Prior to nitrite infusion, endogenous arterial and venous nitrite levels averaged $0.62 \pm 0.17 \mu\text{mole/L}$ and $0.28 \pm 0.02 \mu\text{mole/L}$, respectively, consistent with steady-state nitrite consumption by the heart during normal physiological blood flow. At the end of the 60-minute nitrite infusion, arterial and venous nitrite levels averaged $8.60 \pm 1.26 \mu\text{mole/L}$ and $7.57 \pm 1.03 \mu\text{mole/L}$, demonstrating an increased nitrite consumption following ischemia. Nitrite levels approached pretreatment levels around 90 minutes after stopping the infusion. Although only preliminary analysis has been completed and is based on qualitative assessment of the histopathology, the infarcts visually encompassed >67% of the area at risk in control hearts (n=3) and <25% of the area at risk in nitrite-treated animals (n=5). These preliminary studies suggest that the simple anion nitrite, delivered at doses only about 10-fold higher than endogenous levels, substantially modulates ischemia/reperfusion injury and reduces infarct size in dogs. We are performing additional experiments and are evaluating lower doses of nitrite.

POSTER 17

Differential effects of full-length and truncated forms of CAIR-1/BAG-3 on cell proliferation and migration and migration-associated gene expression

ELIZABETH A. GUANCIAL, Harvard Medical School

Preceptor: Elise C. Kohn, M.D., Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

■ CAIR-1/BAG-3, a member of the Bcl-2-associated athanogene (BAG) family of proteins, is over-expressed in multiple forms of human cancer and has been shown to increase cellular proliferation and survival. It associates with the ATPase domain of the stress response chaperones Hsc70/Hsp 70 through its BAG domain, and it also has a proline-rich domain (PXXP) that allows for SH3 binding. Previous studies we conducted with an *in vivo* murine model demonstrated that MDA435 human breast cancer xenografts from cells stably over-expressing full-length CAIR-1/ BAG-3 (FL) grew faster than tumor cells expressing truncated forms of the protein lacking either the PXXP (dPXXP) or BAG (dBAG) domains. We hypothesized that phenotypic differences between the full-length and truncated mutants of CAIR-1/ BAG-3 conferred an advantage for FL for complex behaviors such as migration, proliferation, and survival.

Boyden chamber studies demonstrated that dPXXP had the greatest ability to translocate through collagen IV-coated filters in response to a chemoattractant, with overall number of translocated cells nearly double that of dBAG, FL, and NEO control cells. Matrigel invasion assays showed that FL cells had the greatest ability to invade through Matrigel in response to serum followed by dBAG, NEO, and dPXXP cells. FL cells, followed by Neo, demonstrated the greatest ability to form significantly larger and more numerous colonies in soft agar than dPXXP and dBAG. This findings are currently being investigated further with a liquid-overlay spheroid formation assay. Genetic analysis of cDNA microarray expression profiles of full-length and truncated forms of CAIR-1/BAG-3, with a focus on genes involved in migration and proliferation will be presented.

These studies demonstrate that the full-length and truncated forms of CAIR1/BAG-3 exhibit phenotypic differences between them, thus indicating that the PXXP and BAG domains play pivotal roles in regulating cellular processes such as proliferation, survival, and migration.

POSTER 18

Genetic association of BDNF, DISC1, and the serotonin transporter gene with brain structure in schizophrenic patients

KATHERINE B. HOBBS, University of North Carolina at Chapel Hill School of Medicine

Preceptor: Daniel R. Weinberger, M.D., Clinical Brain Disorders Branch, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland

■ Voxel based morphology (VBM) is an automated method of statistically analyzing brain anatomy. In this study, we are investigating the association between genotype and brain structure using an optimized VBM procedure. The genetic polymorphisms used in our analysis have been associated with schizophrenia or with aspects of human brain development and function in population studies.

One hundred seventy-six Caucasian schizophrenic patients on psychotropic medication were included in this study. We investigated the effect of the following genes on brain anatomy: brain derived neurotrophic factor (BDNF), disrupted in schizophrenia I (DISC1), and the serotonin transporter gene (SERT). Previous studies in healthy populations have shown an association of BDNF and DISC1 with hippocampal volume and function during cognitive tasks. There is an association of SERT with amygdalar and perigenual cingulate structure and function in normal human subjects.

Magnetic resonance images were analyzed by an optimized VBM procedure as described by Good et al. (2001) using the program statistical parametric mapping 2 (SPM2). Statistical analysis was performed according to the general linear model using SPM2. Prior to performing VBM, images were corrected for intensity inhomogeneities, and a subset of these corrected images was used to create a customized template.

Preliminary data indicate an association of DISC1 genotype with hippocampal, prefrontal cortex, and superior temporal gyral volume in schizophrenic patients. There is also an effect of BDNF genotype on hippocampal, prefrontal cortex, and thalamic volume and an association of SERT genotype with amygdalar volume.

In this study of the association between genes and brain anatomy in schizophrenic patients, we have found an effect of BDNF, DISC1, and SERT on areas of the brain previously associated with schizophrenia. Continued work for this study will include further statistical analysis and the precise identification of brain regions affected by genotype.

POSTER 19

The Reelin-Dab 1 signaling pathway: a role in postnatal development of the cerebellum**LERON CELESTE JACKSON**, Wake Forest University School of Medicine

Preceptor: Brian W. Howell, Ph.D., Neuronal Migration Disorders Unit, Neurogenetics Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland

■ The development of the cerebellum from a simple outgrowth of the neural tube to a complex adult structure involves a highly organized series of neuronal migrations. The Reelin-Dab 1 signaling pathway is thought to modulate the radial migration of neurons in the CNS. It is known that Dab1 is required for embryonic migration of Purkinje cells. Given that cerebellar development continues well into the early postnatal period and that Purkinje cells undergo a second wave of migration, it is possible that Dab1 is necessary for the migration of Purkinje cells after birth. A conditional gene inactivation system in a transgenic mouse model provides a suitable model to study the role of Dab 1 in postnatal development. This system allows the Dab 1 gene to be inactivated after the prenatal phase of Purkinje cell migration is completed. Studies in these mice indicate that postnatal activity of the Dab 1 gene is necessary for proper positioning of Purkinje cell soma in the cerebellar cortex. The migration pattern of Purkinje cells in mice with a postnatally inactivated Dab 1 gene has not been fully characterized.

To explore the migration patterns, cerebellar slice cultures were imaged using live cell imaging techniques. The method of cerebellar slice culture is a powerful tool for the study of cell migration because the organotypic architecture is maintained within slice cultures after several days *in vitro*.

I have developed a strategy to fluorescently label and identify Purkinje cells in living cultures. I will use this system to analyze the rate and characteristics of Purkinje cell migration by time-lapse microscopy. I will test the effects of the post-natal disruption of the Dab1 gene. These analyses should enable us to determine the requirements for Reelin-Dab1 signaling in the post-natal period.

POSTER 20

Attention-deficit/hyperactivity disorder and behavioral comorbidities in a genetic isolate: linkage between traits and to loci at 4q13, 13q14, 18q21, and 19p13**MAHIM JAIN**, Indiana University School of Medicine

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

■ Attention-deficit/hyperactivity disorder (ADHD, [MIM 143465]) is the most common behavioral disorder of childhood. Segregation and epidemiological analyses both suggest genetic factors play a role in the pathogenesis of ADHD. It has been previously reported that conduct disorder (CD), oppositional defiant disorder (ODD), and alcohol and nicotine abuse/dependence are highly associated with ADHD. We applied segregation analyses and determined that Mendelian models could not be rejected for each comorbidity. Specifically ADHD, ODD, and alcohol abuse demonstrate an autosomal dominant inheritance with reduced penetrance and CD and nicotine dependence demonstrate a codominant mode of inheritance.

We examined linkage between traits in order to demonstrate cosegregation of disorders within families. The most significant linkage was between ADHD and ODD, which has an overall two-point parametric LOD = 14.2. ADHD was also linked to CD with a LOD = 5.3, and to alcohol abuse/dependence with LOD = 1.2. We applied model-based and model-free linkage analyses, as well as the pedigree disequilibrium test (PDT) to the results of a genomewide scan of extended families with ADHD and behavioral comorbidities. Clustering of suggestive linkage results were demonstrated at D4s2367 (multipoint parametric LOD = 2.1 for CD, two-point parametric LOD = 2.1 for CD and 2.8 for ADHD and two-point allele sharing LOD = 3.3 for ADHD, CD, and alcohol abuse), D13s788 (two-point allele sharing LOD = 5.5 for ODD and 3.6 for ADHD), D18s851 (two-point allele sharing LOD = 4.1 for nicotine dependence, 3.9 for alcohol abuse, 3.7 for CD, and 3.2 for ADHD), and D19s586 (two-point allele sharing LOD = 7.8 for ODD, 4.3 for CD, 2 for ADHD). These results suggest common genetic etiologies for ADHD and behavioral comorbidities.

POSTER 21

Proteomic and functional differences in mitochondria of different tissues

D. THOR JOHNSON, Indiana University School of Medicine

Preceptor: Robert Balaban, Ph.D., Laboratory of Cardiac Energetics, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

■ Mitochondria are partially self-replicating organelles with 2 to 10 copies of a 15,569 bp genome that codes for 13 proteins, 22 tRNAs, and 2 rRNAs. The vast majority of proteins that are resident to the mitochondria are nuclear encoded and subsequently imported into the mitochondria in a tissue-specific distribution. However, little information on the relative distribution of proteins are available from mitochondria residing in different tissues. The purpose of this study was to compare and contrast the protein content of porcine heart and liver mitochondria. The mitochondria protein content was examined using the 2D gel electrophoresis using the DIGE Cydye system with protein identification relying on mass spectroscopy. The DIGE system works by labeling the two different protein samples on the same gel with different colored (green and red) stains that do not differentially interfere with the proteins migration in the gel. Differences in protein content can then be quantitated, via color and position, by eye, or using the Amersham Decyder Cydye quantitation algorithm. For the current study we primarily relied on a novel algorithm developed in our lab for normalizing concentrations between gels. The initial gels (n=#) were loaded with the same amount of liver and heart mitochondria protein. Somewhat surprising was the dramatic difference in protein expression levels in these two systems with very few proteins being present in similar levels. The proteins involved in oxidative phosphorylation (site1–site 5) were several fold higher in heart mitochondria while several synthetic and degradative pathways such as urea cycle and alcohol dehydrogenase system dominated the liver protein distribution. Interestingly, there was almost a complete absence of proteins in similar levels. The protein distributions can be used to reconstruct the relative metabolic capacities and pathway maps which reveal dramatically different functional capacities of the mitochondria in these two organ systems.

POSTER 22

Does the interval between Papanicolaou tests influence the quality of cytology?

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Preceptor: Mark Schiffman, M.D., Ph.D., Hormonal and Reproductive Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

■ It is commonly believed that the sensitivity of Papanicolaou (Pap) tests decreases with a short interval between cytology samplings. There is limited evidence to support this belief.

For 5055 women in the Atypical Squamous Cells of Undetermined Significance (ASCUS) - Low Grade Squamous Intraepithelial Lesion (LSIL) Triage Study (ALTS), we defined the Pap interval as the number of days between the referral ASCUS or LSIL Pap smear (“first cytology”) and the enrollment liquid-based (“repeat”) cytology. We investigated the influence of Pap interval on repeat cytology by looking at percentages of abnormal findings, cellularity, and test sensitivity among women diagnosed with histologic cervical intraepithelial neoplasia grade 3 (CIN3, precancer) during the two-year course of ALTS. Additionally, because human papillomavirus (HPV) DNA adjunct testing is now performed, we evaluated HPV viral load, assayed using residual liquid cytology specimens, among women with CIN3.

The Pap interval (mean 61.3, SD 34) was 8–30 days in 763 women, 31–60 days in 2317 women, 61–90 days in 1090 women, 91–120 days in 491 women, and 121–184 days in 394 women. Repeat cytologic interpretations of unsatisfactory, ASCUS, and high-grade squamous intraepithelial lesion (HSIL) did not vary among the Pap interval groups. However, low-grade cytologic regression occurred with increasing Pap interval: negative cytology increased from 28.3% (8–30 days) to 41.6% (121–184 days; $p < 0.0001$) while LSIL cytology decreased ($p = 0.002$). Approximate cellularity of the samples was slightly better in the 8–30 days group ($p = 0.04$). Among women with CIN3, the repeat test sensitivity at a threshold of \geq ASCUS and the HPV DNA viral load did not vary by Pap interval ($p = 0.80$ and $p = 0.36$ respectively).

We conclude that a short Pap interval (15–120 days) does not significantly affect the quality of a liquid-based repeat cytology, nor the HPV viral load tested from a residual liquid-based specimen.

*Advanced Scholar

POSTER 23

Induction of a graft-versus-host-like skin disease by intradermal injection of CD8⁺ T-cell receptor (OT-1) transgenic T cells into keratin 14-ovalbumin-expressing transgenic mice**BRIAN S. KIM**, University of Washington School of MedicinePreceptor: **Stephen I. Katz, M.D., Ph.D.**, Dermatology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

■ We have reported that mice (No. 6m) that express membrane-associated chicken ovalbumin (mOVA) under a keratin 14 (K14) promoter develop a graft-versus-host-like disease (GVHD) and may die by day 21 following intravenous transfer (IVT) of CD8⁺ OVA-specific T-cell receptor (V α 2/V β 5) transgenic (Tg) T cells (OT-1 cells) from OT-1 x RAG^{-/-} mice. As well, following IVT of OT-1 cells, mice (No. 15s) that express a high level of soluble ovalbumin (sOVA) in epithelia develop GVHD and die by day 5, while other K14-OVA Tg strains (No. 3m – mOVA, No. 5s and No. 17s – sOVA) and a double Tg strain (No. 6m x OT-1) do not develop GVHD. To determine why IVT does not induce GVHD in these mice, we have developed a model that involves intradermal (ID) injection of OT-1 cells into the ears of all types of K14-OVA Tg mice.

Although most mice failed to develop GVHD after IVT, all mice developed local (skin) GVHD by day 7 and No. 15s mice developed systemic GVHD and died by day 7 after ID injection of OT-1 cells. Injection of OT-1 cells into control C57BL/6 (syngeneic) and BALB/c (allogeneic) mice did not cause local GVHD. Furthermore, injection of control CD8⁺ T cells from C57BL/6 mice into No. 6m mice also did not cause GVHD. The skin-draining lymph nodes of the ears demonstrated an increase of OT-1 cells in No. 3m, No. 5s, No. 6m, and No. 17s mice (1.5–1.9% V α 2/V β 5⁺) relative to control C57BL/6 mice (0.7% V α 2/V β 5⁺). The lymph node T cells showed upregulation of some activation markers (CD62L^{hi}, CD44^{hi}, CD25^{lo}, CD69^{med}).

We have demonstrated that all of the K14-OVA Tg mice are susceptible to localized GVHD-like skin disease by ID injection of OT-1 cells that do not appear to exclusively rely on the regional lymph node for acquisition of effector function. Thus, this ID injection model provides a new paradigm to assess 1) the pathophysiology of a localized GVHD and 2) therapies that may modulate cell-mediated autoimmune reactions.

POSTER 24

Differential signaling pathways for low- versus high-dose interleukin-7 in human T cells**THOMAS KRUPICA JR.**, Georgetown University School of MedicinePreceptor: **Crystal L. Mackall, M.D.**, Deputy Branch Chief, Pediatric Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

■ Interleukin-7 (IL-7) is required for lymphopoiesis and homeostatic expansion of T cells. We have postulated that IL-7 is a T cell homeostatic regulator due to its accumulation in lymphopenia. Such a model implicates important dose response effects of IL-7 on T cells. Normal circulating levels of IL-7 are less than 10 pg/ml with physiologic elevations to 100 pg/ml in lymphopenia. Pharmacologic doses, which generate 100–1000 pg/ml levels in mice and primates, dramatically increase circulating and lymphoid tissue T cell numbers, without evident toxicity. Toxic effects, such as widespread lymphocytic tissue infiltration and organ toxicity, occur when IL-7 levels approach 10–100 ng/ml. Therefore, *in vivo*, a substantial dose response effect of IL-7 on T cell activation and expansion exists. We sought to characterize *in vitro* IL-7 dose response effects on both naïve and memory human peripheral blood T cells. We observed that both low-dose (0.1 ng/ml) and high-dose (10 ng/ml) IL-7 augmented T cell receptor-mediated proliferation of both naïve and memory T cells in a dose-dependent manner with a higher proportion of cells proliferating in response to higher doses. This proliferation was dependent on phosphatidylinositol-3-kinase (PI3K) signaling through the mammalian target of rapamycin (mTOR), and it was inhibited with either PI3K inhibitors or rapamycin. Unlike IL-7's proliferative effects, up-regulation of Bcl-2 and increased expression of CXCR4 only occurred at high dose (10 ng/ml) IL-7 in both naïve and memory T cells. These effects were all-or-none, with all cells in culture up-regulating Bcl-2 and CXCR4 at the same dose of IL-7, suggesting that IL-7 receptor occupancy was not the primary regulator of these effects. Further, these high dose effects were not abrogated with either PI3K or mTOR inhibitors. We therefore postulate that high-dose IL-7 induces a PI3K and mTOR independent pathway, resulting in up-regulation of Bcl-2, increase in CXCR4, and perhaps other biologic effects.

POSTER 25

Nonsense-mediated decay gene expression analysis in diffuse large B-cell lymphoma

JOHN LEE, Dartmouth Medical School

Preceptor: Louis Staudt, M.D., Ph.D., Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

■ The inactivation of tumor suppressor genes and the activation of oncogenes signify critical genetic events in the genesis and progression of human cancer. The identification of tumor suppressor genes has traditionally been informed by mapping chromosomal deletions and loss of heterozygosity in tumors, but this process is challenging. An emerging strategy of combining inhibition of nonsense-mediated RNA decay with gene expression analysis in cancer cell lines and tumor samples is an innovative approach to the detection of potential tumor suppressor genes harboring inactivating nonsense mutations. Adjunct information, including insight into the biological function of candidate genes and comparative genomic hybridization data indicating genetic loss, facilitates the screen for tumor suppressor genes. Thus nonsense-mediated decay gene expression analysis may reveal critical mechanisms of lymphomagenesis in diffuse large B-cell lymphoma (DLBCL), the most common sub-type of non-Hodgkin's lymphoma.

We validated this method initially in the previously studied *DUI45* and *PC3* prostate cancer cells that bear known nonsense mutations. The analysis was extended to six germinal centre B-like (*OCI-Ly7*, *OCI-Ly19*, *BJAB*, *HT*, *SUDHL-4*, *SUDHL-6*) and two activated B-like (*OCI-Ly3*, *OCI-Ly10*) DLBCL cell lines using the Lymphochip, a specialized cDNA microarray. Genomic or cDNA sequencing was conducted on prospective genes on the basis of transcript enrichment and known or inferred biological function. In addition to the preceding system, we are developing a stable system for the inducible expression of short hairpin RNAs (shRNA) targeting two essential mediators of nonsense-mediated mRNA decay, *RENT1* and *RENT2*, to circumvent a confounding stress response arising from pharmacologic inhibition of nonsense-mediated RNA decay. We will assess the ability of this RNA interference strategy to block nonsense-mediated RNA decay and minimize global effects on gene expression.

Nonsense-mediated decay gene expression analysis is a promising technology as a genomic screen for candidate tumor suppressor genes in cancer. Additional refinements will enhance the efficacy of this analysis as our knowledge of nonsense-mediated decay improves.

POSTER 26

Characterization of POTE, a prostate and germ-cell specific protein expressed in several breast cancers

YOOMI LEE, Columbia University College of Physicians and Surgeons

Preceptor: Ira Pastan, M.D., Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

■ An analysis of prostate EST databases has identified a novel gene, termed POTE, expressed in prostate, ovary, testis, and placenta but absent from other normal adult tissues. Though its function is unknown, the presence of ten paralogs with 90–98% sequence identity indicates selective pressure to maintain the gene. POTE contains five to seven ankyrin repeats and a spectrin domain at its C-terminus, suggesting interaction with the cytoskeleton. Preliminary studies have localized POTE to the plasma membrane.

POTE is expressed in many cancer types in addition to prostate cancer. To investigate POTE's role in breast cancer, we studied POTE expression by RT-PCR of RNA from breast cancer cell lines. POTE transcripts were not detected in normal breast tissue or in a normal breast epithelial cell line, MCF-10A. Three of four breast cancer cell lines studied (MCF-7, HTB-30, and ZR-75-1) expressed POTE, and the paralogs found in cancer cells were mostly POTE-2 α and POTE-2 γ . MCF-10A cells transformed by the oncogenes ErbB-2 and Ras also expressed POTE-2 α . We conclude that POTE is up-regulated during tumorigenesis in many but not all breast cancers. To study the effects of POTE expression, we transfected POTE-2 γ into MCF-10A by lentivirus, and will analyze changes in gene expression by cDNA microarray.

Expression in germ cells and a range of cancers is characteristic of a class of immunogenic proteins known as cancer-testis antigens. In keeping with the possibility that POTE represents a novel member of this class, we will utilize ELISA to measure the immune response to POTE in sera of patients with breast cancer. POTE is a protein with promise in immune-based therapies for cancer because it is a cancer-specific target molecule, expressed only in nonessential adult tissues. A better understanding of its expression patterns, regulation, and role in cancer cells is essential to exploring its therapeutic potential.

POSTER 27

Heparan sulfate modulates fibroblast growth factor function during submandibular gland branching morphogenesis**KAREN M. LIKAR**, University of Michigan School of Dentistry**Preceptor:** Matthew P. Hoffman, Ph.D., Matrix and Morphogenesis Unit, Craniofacial Developmental Biology and Regeneration Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland

■ Fibroblast growth factors (FGFs) are a family of heparin-binding growth factors that regulate branching morphogenesis of salivary glands. FGF signaling plays a key role in branching morphogenesis by regulating the gene expression and the activity of ECM proteins and their receptors. FGF binding to FGF receptors requires heparan sulfate. In vivo the biological activity of heparan sulfate is regulated by heparanase, an endoglycosidase that degrades heparan sulfate. Chemical inhibitors of heparanase and function blocking anti-heparanase serum inhibit salivary gland branching, suggesting heparan sulfate turnover is required for FGF function during branching morphogenesis. Exogenous heparin or heparan sulfate inhibit branching in SMG organ culture in a dose-dependant manner. Heparin oligosaccharides with defined sulfation patterns also inhibit branching and show that both 2-O-sulfation and 6-O-sulfation are required for branching. We have also shown that exogenous FGF7 and FGF10 induce distinct morphologies when added to epithelial rudiments, and have begun to define the heparan sulfate requirements for the FGF-induced morphology and the effects on epithelial cell proliferation. FGF10-induced duct elongation is modified by exogenous heparin fragments containing 2-O and 6-O-sulfation, suggesting heparan sulfate increases the receptor binding of FGF10, and broadly increases proliferation. Taken together, our data suggest defined sulfation patterns within heparan sulfate regulate FGF function during branching morphogenesis.

POSTER 28

Plexin D1 signals to guide endothelial cells**YULIYA LINHARES**, University of North Carolina at Chapel Hill School of Medicine**Preceptor:** J. Silvio Gutkind, Ph.D., Oral and Pharyngeal Cancer Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland

■ The endothelial cell surface receptor Plexin D1 and its ligand Semaphorin 3E are required for proper patterning of vascular network and cardiac morphogenesis during development. Knowledge of the signaling pathway by which PlexinD1 acts will aid our understanding of its function in vasculogenesis and angiogenesis, which are two essential processes for solid tumor growth. Here we utilized Trk-PlexinD1 chimeric receptors to identify Plexin D1 downstream targets and to demonstrate that Plexin D1 does not require a coreceptor for signaling. We also treated endothelial cells and highly transfectable model cell lines overexpressing Plexin D1 with Semaphorin 3E to investigate how endogenous and ectopically expressed Plexin D1 signals to control processes underlying angiogenesis, such as cytoskeletal rearrangement, cell migration, and tubulogenesis. Furthermore, we investigated the function of C-terminal PDZ-binding domain of Plexin D1 and found that it binds a scaffolding protein known as GIPC. We propose that the Plexin D1 PDZ binding domain is necessary for proper plexin processing. These studies elucidate the nature of the mechanisms by which Plexin D1 signals to guide endothelial cells, thereby controlling angiogenesis.

POSTER 29

Analysis of mammalian SIRT proteins during adipocyte differentiation

STANLEY LIU, University of California, San Francisco, School of Dentistry

Preceptor: J. Carl Barrett, Ph.D., Laboratory of Biosystems and Cancer, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

■ Calorie restriction (CR) is the only experimental manipulation that extends the lifespan of a number of organisms, including yeast, worms, flies, rodents, and potentially humans. CR has also shown to reduce the incidence of age-related disorders (diabetes, cancer, and cardiovascular disorders) in mammals. Recent data supports the hypothesis that CR results in a highly conserved stress-inducible defense response. Underlying this stress response is a family of deacetylase enzymes, the "Sirtuins," named after the yeast silent information regulator, SIR2 longevity protein. Additional copies of the *SIR2* gene extend lifespan of lower organisms, including yeast, worms, and flies by mimicking CR. Mammals have seven homologs of the *SIR2* gene (*SIRT1-7*).

Mammalian aging is regulated in part by fat storage. White adipose tissue (WAT) affords mammals an opportunity to sense diet and send appropriate signals to coordinate aging in all organs. In a cell model of white adipocytes, the 3T3-L1 cells, overexpression of *SIRT1* reduces adipogenesis and triglyceride accumulation in the lipid droplets of cells. This was associated with a repression of peroxisome proliferators-activated receptors gamma (*PPARγ*) transactivation by *SIRT1*.

While *SIRT1* has received the most attention, cellular localization work in our lab shows that *SIRT6* and *SIRT7* are in fact more similar to the yeast *SIR2* in terms of association with the heterochromatin and the nucleolus, respectively. When comparing liver tissues among mice of CR, normal, and overfed diets, the CR group exhibited a decrease in *SIRT6* mRNA expression. When 3T3-L1 preadipocytes are differentiated into mature adipocytes, *SIRT6* mRNA expression is also decreased. We are currently developing a line of stable *SIRT6* overexpressing 3T3-L1 cells via a retroviral transfection system. As there are no current reports on the function of *SIRT6*, our evaluation of its involvement in adipocyte differentiation will provide an additional link to the mediation of longevity via CR.

POSTER 30

Activated Rheb regulates growth and cell migration through TOR and DGAP1/IQGAP mediated pathways

AMIT R. MAJITHIA, New York University School of Medicine

Preceptor: Alan R. Kimmel, Ph.D., Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland

■ TOR (target of rapamycin) kinase complexes monitor signals from hormones, nutrients, and energy levels to effect growth, proliferation, and cytoskeletal rearrangements. Rheb is a ras-like GTPase that, when GTP-bound, activates TOR to increase protein synthesis. Rheb over-expression causes oncogenic transformation while inactivation results in cell-cycle arrest. Rheb is in turn negatively regulated by the GTPase activating protein (GAP) complex TSC1/TSC2. Disruption of TSC1 or TSC2 causes the multi-organ tumor syndrome tuberous sclerosis marked by excess cell proliferation and aberrant migration. This presumably occurs through unregulated activation of Rheb and enhanced TOR signaling. However, the molecular events connecting Rheb to TOR remain unresolved, as do relationships to cytoskeletal regulation and cell migration.

Our current work expands the role of Rheb in cell growth, identifies a novel Rheb effector, and links Rheb to cytoskeletal regulation. We constructed and expressed constitutively active (RhebCA) and inactive (RhebDN) forms of Rheb in *Dictyostelium*. *Dictyostelium* exhibits distinct cellular growth and migratory phases making it ideal to dissect growth parameters from cytoskeletal control. Paradoxically, RhebCA expression, which activates TOR, causes severe growth suppression that is ameliorated by the TOR inhibitor rapamycin. These data indicate that frank activation of TOR is as deleterious to cell survival as its inactivation. We further show that RhebCA inhibits multiple actin-mediated processes, including phagocytosis and cell migration. Utilizing a yeast two-hybrid system, we identify a novel interaction between Rheb and DGAP1, a homolog of mammalian IQGAPs that are involved in cell migration, adhesion, and cytokinesis. DGAP1, like all IQGAPs, contains GAP-homology domains, but lacks essential catalytic residues. We localize the site of interaction with Rheb to a conserved C-terminal domain. Loss of DGAP1 rescues Rheb-mediated cytoskeletal defects. Taken together, our data suggest that DGAP1/IQGAP signals downstream of activated Rheb and may be universally important in cytoskeletal regulation and cell migration.

POSTER 31

Erythropoietin and hypoxic effects on the erythropoietin receptor in HL-1 cardiomyocytes

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Preceptor: Constance T. Noguchi, Ph.D., Molecular and Cell Biology Section, Chief, Molecular Medicine Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland

■ Erythropoietin interacts with the erythropoietin receptor (EpoR) to prevent apoptosis in erythroid progenitor cells and to stimulate their proliferation and differentiation into mature erythrocytes. Until recently, the focus of erythropoietin has been to treat anemia due to conditions such as chronic renal failure, zidovudine treatment for HIV, and anemia due to chemotherapy. In the past decade there has been a growing interest in the biological importance of erythropoietin beyond erythropoiesis. The presence of EpoR in non-hematopoietic tissues and the secretion of erythropoietin by tissues other than the kidney suggest that the protective role of erythropoietin goes beyond erythropoiesis.

Studies have demonstrated the protective effects of erythropoietin in events of brain ischemia, cardiac ischemia, and reperfusion injury. Erythropoietin administered before and during myocardial ischemia-reperfusion reduces cell apoptosis in the area at risk and results in a significant improvement in cardiac physiology. To date, no studies have shown the effects of erythropoietin on cardiac cells under hypoxic conditions at the level of the erythropoietin receptor. To investigate the effects of erythropoietin and hypoxia on EpoR expression in cardiac tissue, we made use of the cardiac muscle cell line, designated HL-1, from the AT-1 mouse atrial cardiomyocyte tumor lineage. We quantified transcript levels of EpoR in HL-1 cells treated with and without erythropoietin and investigated the changes that occur under various oxygen tensions. The change of expression of potential downstream proteins involved in the anti-apoptotic effects of erythropoietin on cardiomyocytes is also being investigated. With these studies we hope to expand the understanding of the cardio-protective effects of Erythropoietin.

POSTER 32

Activation of AMP-activated protein kinase (AMPK) by phosphatidylinositol ether lipid analogues (PIAs)

REGAN MEMMOTT, University of Arizona College of Medicine

Preceptor: Phillip A. Dennis, M.D., Ph.D., Signal Transduction Section, Cancer Therapeutics Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

■ Phosphatidylinositol ether lipid analogues (PIAs) were originally designed to inhibit a novel target in cancer, the serine/threonine kinase Akt. Screening of 25 PIAs in cancer cell lines identified five PIAs that selectively inhibit Akt and preferentially induce apoptosis in cancer cell lines with high levels of constitutively active Akt. To better characterize the specificity of these compounds, active PIAs were screened against other purified kinases in high-throughput kinase assays. In these assays, PIAs were shown to directly activate the metabolic regulator, AMPK, by ~40%. To confirm activation of AMPK in intact cells, we used phospho-specific and native antibodies in immunoblotting experiments in a panel of lung cancer cells that vary in mutational status of LKB1, a tumor suppressor that normally activates AMPK. An active PIA (PIA5) increased phosphorylation of AMPK within minutes, with similar kinetics and concentration-dependence as that required to inhibit Akt and activate p38 α (another biologic activity of PIAs). Activation of AMPK by PIAs occurred in lung cancer cells regardless of LKB1 status. PIA-induced phosphorylation of AMPK increased kinase activity of AMPK, because phosphorylation of one of its downstream targets, acetyl CoA carboxylase, was increased in response to PIA treatment. Activation of AMPK by PIA5 was independent of the other known biologic activities of PIAs (inhibition of Akt and activation of p38 α), because small molecule inhibitors of the Akt pathway or p38 α did not affect activation of AMPK by PIA5. Collectively, these studies have identified activation of AMPK as another biologic activity of PIAs, which occurs independently of LKB1. The mechanism of direct activation of AMPK by PIAs and the cellular consequences of PIA-induced AMPK activation are under current investigation.

POSTER 33

Transvenous access to the pericardial space: a novel approach to epicardial lead implantation for cardiac resynchronization therapy**STEVEN R. MICKELSEN**, University of New Mexico School of Medicine

Preceptor: Eliot McVeigh, Ph.D., Laboratory of Cardiac Energetics, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

■ **Background:** Percutaneous access to the pericardial space may be useful for a number of therapeutic modalities. One such example is the potential to place LV pacing leads for resynchronization therapy when an appropriate site is not available by a conventional transvenous approach. We have developed an alternative catheter-based trans-venous method to access the pericardial space and demonstrated the ability to negotiate pacing leads into this region.

Methods: In eight pigs, a standard transseptal sheath and needle was introduced into the right atrium (RA) from the jugular vein under fluoroscopic guidance. The PS was accessed through controlled puncture of the terminal anterior SVC (n=7) or right atrial appendage (n=1). A guide wire was advanced through the transseptal sheath, which was then removed leaving the wire in PS. The guidewire was used to direct both passive and active fixation pacing leads into the PS using standard over the wire techniques. Pacing was attempted and lead position was documented. Animals were sacrificed at 2 and 6 weeks.

Results: All animals survived the procedure. Pericardial effusion was hemodynamically significant in only 4 animals. At necropsy, lead exit sites appeared to heal without complication at 2- and 6-week follow-up. Significant pericardial fluid accumulation was not observed in 4/6 animals at late follow-up. Moderate fibrinous deposition was observed in animals, which had exhibited significant per-procedural pericardial effusion (n=2).

Conclusions: Access to the PS via a transvenous approach is feasible. Pacing leads can be negotiated into this region. Further procedural and device development focused on reducing the risk of procedural pericardial effusion and pacing lead apposition to the epicardial surface are required.

POSTER 34

Intestinal epithelial cell-dendritic cell interactions in the induction of immunity to type I reovirus**CARMEN RUZICA MIKACENIC**, University of Washington School of Medicine

Preceptor: Brian L. Kelsall, M.D., Laboratory of Molecular Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

■ Intestinal epithelial cells constitute a first line of defense against orally acquired pathogens by formation of a physical barrier and production of chemokines and cytokines that can attract and activate immune cells. Reovirus strain type 1 Lang (T1L) is a double-stranded RNA virus that infects epithelial cells in murine Peyer's patches (PP) and induces strong local sterilizing immunity. We previously demonstrated that subepithelial PP dendritic cells (DC) acquire T1L antigen from infected apoptotic epithelial cells, and that PP DCs from infected mice drive T cell activation *ex vivo*. We have developed an *in vitro* model that allows us to study the direct interaction of T1L with intestinal epithelial cells and the subsequent effects this interaction has on dendritic cells (DCs) that direct adaptive immunity. Exposure of a murine intestinal epithelial cell line (CMT-93) to purified T1L resulted in active viral infection determined by immunofluorescent staining for both structural and non-structural viral proteins. *In vivo*, T1L is activated by gastrointestinal enzymes before infecting the PP. Mimicking this process, T1L was digested with chymotrypsin to create intermediate subviral particles (ISVPs). Infection of CMT-93 cells with T1L ISVPs resulted in increased levels of viral replication, when compared to intact T1L, and induction of apoptosis, as determined by expression of active-caspase-3. DCs could possibly be activated directly by T1L, T1L ISVPs, apoptotic bodies of infected cells, or epithelial cell-produced cytokines. Bone marrow-derived dendritic cells (BMDCs) could not be infected with either T1L or T1L ISVPs. However, exposure of BMDCs to supernatants containing apoptotic bodies from infected CMT-93 cells resulted in DC maturation. Furthermore, in preliminary experiments, T1L ISVP-infected CMT-93 cells secreted IL-6, but not TNF- α , IL12-p40, or IFN- α . Future experiments using this novel system will help identify epithelial cell factors important for activating DCs for the induction of immunity to mucosal viral infection.

POSTER 35

Actions of interleukin-15 on natural killer and CD8⁺ memory T cells *in vivo*

HIRAL PATEL, University of Medicine and Dentistry of New Jersey, New Jersey Medical School

Preceptor: Thomas A. Waldmann, M.D., Metabolism Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

■ Interleukin 15 (IL-15) is a potent cytokine that is involved in innate and adaptive immunity through the development and survival of natural killer (NK) and CD8⁺ memory T cells. IL-15 also inhibits IL-2-mediated activation-induced cell death and enables the maintenance of long-lasting immune responses, and thus may be helpful in cancer and vaccine therapy. Although IL-15 is critical for response to foreign pathogens, the uncontrolled expression of IL-15 may support the survival of autoreactive T cells leading to the development of autoimmune diseases. Uncontrolled IL-15 action has been implicated in the pathogenesis of rheumatoid arthritis, celiac disease, and T cell leukemias. Blocking the action of IL-15 should be evaluated in the treatment of such disorders.

The current study examined the effects of modulation of IL-15 action in wild-type C57/BL6 mice. TMβ1 is the antibody to mouse IL-2/IL-15 receptor β chain and inhibits the actions of IL-15 *in vitro*. Administration of TMβ1 (50 μg) caused a 90% decrease in circulating NK1.1⁺ cells by day 3 and a 70% decrease in circulating CD8⁺ memory phenotype, CD8⁺CD44^{hi}, cells by day 10. Conversely, administration of exogenous IL-15 (5 μg/day, 15 doses total) leads to a greater than twofold increase in CD8⁺CD44^{hi} by day 15. This increase was sustained for 2 weeks following administration of the last dose.

These data suggest potential for IL-15-directed immunotherapy. TMβ1 blocks the actions of IL-15 and leads to a dramatic decrease in IL-15-dependent cells, suggesting that blockade of IL-15 action may be useful in the treatment of autoimmune diseases mediated by such cells. Exogenous administration of IL-15 leads to sustained expansion of CD8⁺ memory T cells and provides support for its use in anti-tumor therapy and as a vaccine adjuvant.

POSTER 36

Functional detection of tumor epithelial cell growth via *in vivo* multispectral optical imaging

JADE QUIJANO, University of California, Los Angeles, David Geffen School of Medicine

Preceptor: King C. Li, M.D., Molecular Imaging Laboratory, Department of Diagnostic Radiology, Clinical Center; National Cancer Institute/SAIC-Frederick, National Institutes of Health, Bethesda, Maryland

■ Monitoring tumor growth and regression plays a significant role in anti-tumor therapy development. Current methods used to determine tumor size *in vivo* include caliper measurement, x-ray, magnetic resonance imaging, computed tomography, and ultrasonography. Although useful for monitoring tumor size, these methods are unable to serially track tumor cell growth and viability. Recent studies have shown a direct correlation between luciferase expression and MRI tumor volumes. Here, we hypothesize that a correlation also exists between enhanced green fluorescence protein (EGFP) expression and MRI tumor volumes.

A novel *in vivo* optical imaging device that allows for multispectral imaging and analysis of fluorophores in the visible and near-infrared region was acquired and optimized. These experiments were the first to evaluate this device in a pre-clinical setting. Mice bearing transgenic M21-EGFP tumors were imaged by optical and MRI every 3–4 days from day 0 to 35 following tumor inoculation. A method to quantify tumor signal intensity has been developed. Fluorescence microscopy was used to correlate tumor signal intensity with gene expression, cell proliferation, and necrosis.

We have found that a direct correlation exists between tumor signal intensity and volume until tumors reach a volume of 900 mm³. We attribute an initial sharp decrease and subsequent rise in tumor signal intensity during the first week after tumor implantation to the death of a majority of injected cells followed by growth of a subpopulation of surviving cells. We attribute all other discrepancies found between tumor signal intensity and volume primarily to cell viability and gene expression in addition to the limited depth of penetration of visible light and saturation effects. These studies suggest that this *in vivo* optical imaging device can be used to accurately monitor changes in tumor epithelial cell viability as opposed to other methods which cannot discriminate between viable and nonviable cells.

POSTER 37

CD4-dependent and -independent enhancement of CD8 T cell proliferation and effector function induced by CTLA-4 blockade**ANJANA RANGANATHAN**, University of Pittsburgh School of Medicine

Preceptor: Nicholas P. Restifo, M.D., Surgery Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

■ Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) is an immunomodulatory molecule which maintains peripheral T cell tolerance by regulating activation and proliferation of T lymphocytes. Absence of the CTLA-4 molecule, as seen in CTLA-4^{-/-} mice, has been shown to result in profound autoimmunity and lymphoproliferative disease. Elucidation of the specific T cell subtype(s) through which CTLA-4 modulates the immune response may then be utilized to enhance anti-tumor immunotherapies by potentially breaking tolerance to self-antigens expressed on tumor cells. By crossing a CTLA-4^{-/-} mouse with a self/tumor-reactive CD8⁺ T cell receptor transgenic mouse (pmel-1), we demonstrated very high levels of CD4⁺ non-transgenic T cells and increased CD8⁺ T cell reactivity to the self-antigen hgp100. The CD8⁺ effector cells from Pmel-1⁺/CTLA-4^{-/-} mice had an enhanced state of activation as evident by high expression of CD44 and low expression of CD62L; additionally, these mice developed profound autoimmune vitiligo at 3 weeks of age. When the CD4⁺ T cell population was eliminated by crossing the Pmel-1⁺/CTLA-4^{-/-} mouse onto a Rag1^{-/-} background, we observed the complete abrogation of CD8⁺ T cell activation and autoimmune manifestations; Pmel-1/CTLA-4^{-/-}/Rag1^{-/-} CD8⁺ T cells retained a completely naïve phenotype, suggesting that CTLA-4 blockade results in the activation of CD8⁺ T cells indirectly through CD4⁺ T cell mediated mechanisms. However, both *in vivo* and *in vitro* studies evaluating the mechanisms of action of CTLA-4 blockade using a CTLA-4 blocking antibody additionally demonstrated a direct effect on the CD8⁺ T cell population. These data suggested that CTLA-4 contributes to the maintenance of peripheral tolerance both through CD4⁺ and CD8⁺ T cell subsets.

POSTER 38

Isolation of a population of neurogenic, platelet-derived growth factor-responsive progenitors from the embryonic cortex**RAJESH RAO**, Yale University School of Medicine

Preceptor: Ronald McKay, Ph.D., Laboratory of Molecular Biology, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland

■ In the developing CNS, progenitors emerge from the ventricular zone to generate neurons, oligodendrocytes, and astrocytes. These progenitors—called stem cells because they clonally generate the three neural cell types *in vitro*—can be isolated from the fetal cortex and propagated. Ventricular zone progenitors express fibroblast growth factor (FGF) receptors; therefore, FGF2, a specific FGF isoform, is commonly employed as a primary mitogen for isolation and expansion of CNS stem cells. Cells expressing platelet-derived growth factor receptor-alpha (PDGFR α), thought to represent non-neurogenic, oligodendrocyte-astrocyte precursors, originate in discrete dorsal and ventral ventricular domains and subsequently disperse uniformly throughout the neural tube.

Surprisingly, we have found that *in vitro* addition of PDGF-AA, a PDGFR α -specific ligand, to primary embryonic mouse cortex enables isolation and expansion of neurogenic progenitors. These progenitors express nestin, a protein associated with CNS stem cells, and in the presence of PDGF-AA, can be serially passaged. When PDGF-AA is withdrawn, the progenitors differentiate into all three neural lineages, but at different proportions relative to stem cells maintained in FGF2, including neurons (50–60% vs. 20–30%), astrocytes (30–40% vs. 60–70%) and oligodendrocytes (10%). Addition of bone morphogenic protein-2 to these progenitors results in a shift toward an astrocytic fate (80%). Unlike FGF2-responsive stem cells, PDGF-responsive progenitors extend long processes, do not proliferate in colonies or cluster, and do not require exogenous insulin for their isolation, survival, or expansion.

This newly isolated population of cortical progenitors generates higher proportions of neurons when compared to other CNS progenitors widely used for study and transplantation. The unique proportion of cell fates, morphology, and growth properties characteristic of PDGF-responsive progenitors makes them an attractive tool by which to dissect fate commitment, neuronal differentiation, and progenitor cell metabolism. Finally, they may represent a novel source of neurogenic cells for transplantation in CNS disorders.

POSTER 39

Histone acetylation at the survival motor neuron gene: identifying a potential target for spinal muscular atrophy therapeutics**MELISSA L. RUSSO**, Georgetown University School of Medicine

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

■ Spinal muscular atrophy (SMA) is caused by deficiency of the survival motor neuron (SMN) protein. One strategy for treating SMA is to increase *SMN* gene transcription. Histone deacetylase (HDAC) inhibitors have been previously shown to activate the *SMN* promoter and increase SMN transcript and protein levels *in vitro*, and this has led to early clinical trials with these drugs in SMA patients. Nonetheless, the role of histone acetylation in directly regulating *SMN* gene expression has not been explored.

Chromatin immunoprecipitation (ChIP) was used to examine the levels of acetylated H3 and H4 histones and specific histone deacetylases associated with the human and mouse *SMN* gene in cultured cells and tissues isolated from mice during the course of development. The *SMN* gene has a distinct pattern of histone acetylation that is largely conserved between different tissues and species. Regions of the *SMN* gene surrounding or immediately upstream of the transcriptional start site showed relatively high levels of histone acetylation, whereas regions further upstream showed 2- to 6-fold lower levels. After histone deacetylase inhibitor treatment, acetylated histone levels increased, particularly in upstream regions, correlating with a two-fold increase in promoter activity. In mouse brain and liver tissues during development, histone acetylation levels decreased and associated HDAC 1 and HDAC2 levels increased, correlating with a 40–60% decrease in SMN transcript and protein levels. The increased binding of HDAC1 and HDAC2 to *SMN* during development is probably not due to increased amounts of protein; preliminary results indicate that protein levels of HDAC1 and HDAC2 remain unchanged or decreased from embryonic to adult stages.

These data provide evidence that histone acetylation modulates *SMN* gene expression and that pharmacological manipulation of this epigenetic determinant is feasible. HDAC2 in particular may be a therapeutic target in SMA.

POSTER 40

TRAIL-expressing adenovirus gene therapy combined with cisplatin results in supra-additive cytotoxicity in thoracic carcinoma cells**SUSAN SHAMIMI-NOORI**, Georgetown University School of Medicine

Preceptor: Dao M. Nguyen, M.D., Section of Thoracic Oncology, Surgery Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

■ **Background:** Cancer cells frequently exhibit resistance to the cytotoxic effect of TRAIL (tumor-necrosis-factor-related-apoptosis-inducing-ligand). Pretreatment of TRAIL-resistant cells with cisplatin (CDDP) sensitizes them to TRAIL. Moreover, CDDP has been shown to enhance adenoviral transgene expression in cancer cells. This study evaluates the ability of CDDP to enhance the expression and the cytotoxicity of AdVgTRAIL in cultured thoracic cancer cell lines and to elucidate the underlying mechanism responsible for this effect.

Methods: Cultured thoracic cancer cells (H322, H513, TE2, TE12) were infected with AdVgTRAIL or AdV-CMV-GFP control vector with or without CDDP. GFP-TRAIL transgene expression was determined by flow cytometry and quantitated by ELISA. Treatment-mediated cytotoxicity and apoptosis were measured by MTT assays and PE-conjugated Annexin-V. Caspase 8 and 9 activities were determined by colorimetric assays.

Results: There was a linear correlation between GFP fluorescence and TRAIL expression following AdVgTRAIL infection. CDDP pretreatment enhanced GFP-TRAIL expression by 1.7- to 5-fold. While cultured cancer cells were refractory to the cytotoxic effect of AdVgTRAIL, pretreatment with CDDP resulted in a dose-dependent enhancement of AdVgTRAIL-mediated cytotoxicity. Flow cytometric analysis of GFP (transgene expression) as well as Annexin V-PE fluorescence showed that apoptosis following AdVgTRAIL or CDDP + AdVgTRAIL treatment involves a paracrine mechanism. When normalized for the levels of GFP-TRAIL expression, CDDP-treated cells were significantly more susceptible to AdVgTRAIL than untreated cells. Either selective caspase 9 inhibitor or over-expression of Bcl-2 abrogated CDDP/AdVgTRAIL-mediated cytotoxicity.

Conclusion: CDDP profoundly potentiates *in vitro* anti-tumor effects of AdVgTRAIL. CDDP-mediated sensitization of cancer cells to adenovirally delivered TRAIL is predominantly responsible for the synergistic effect. This process is dependent on recruitment of the mitochondria-regulated apoptosis inducing cascade (type II pathway).

POSTER 41

Electrospun three-dimensional polycaprolactone nanofibers: candidate scaffold for skeletal muscle tissue engineering**RABIE M. SHANTI**, Harvard School of Dental Medicine

Preceptors: Rocky S. Tuan, Ph.D., and Wan-Ju Li, Cartilage Biology and Orthopaedics Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, Maryland

■ The generation of skeletal muscle tissue using tissue engineering holds promise for treating muscle loss from traumatic injury, tumor ablation, vascular insult, or degenerative muscle disease. Only a few alternatives exist for restoring damaged muscle tissues. For skeletal muscle tissue engineering to succeed, an appropriate autologous cell source needs to interact with a biocompatible and biodegradable scaffold that is capable of mimicking the structure and functions of the natural cellular environment, the extracellular matrix.

Previous studies have shown that electrospun, three-dimensional nanofibrous structures share morphological similarities to collagen fibrils, and are capable of promoting favorable biological responses from seeded cells (Li and Tuan, *JBMR*, 2003). Human bone marrow, in addition to a host of other tissues, has been shown to contain multipotential mesenchymal stem cells (hMSCs) capable of differentiating into a variety of connective tissue lineages when provided with the appropriate culture environment and inductive agents. Specifically, hMSCs have been seeded onto pre-fabricated nanofibrous scaffolds and induced along chondrogenic, adipogenic, and osteogenic lineages using specific induction media. However, to date no report has successfully demonstrated the differentiation of bone-marrow derived hMSCs into myoblasts on any three-dimensional substrate.

In this study, we have fabricated a three-dimensional nanofibrous scaffold made of a synthetic biodegradable polymer, poly (ϵ -caprolactone) (PCL), and examined its ability to support and maintain the myogenic differentiation of bone marrow-derived hMSCs *in vitro*. Preliminary results demonstrate the feasibility of promoting the differentiation of these cells into myoblasts, as evidenced by crystal violet histological staining of cells with a myoblastic morphology. Also, immunofluorescence staining shows the expression of the myogenic marker myogenin in differentiated hMSCs. Further analyses of the myogenic differentiation of hMSCs include scanning electron microscopy, cell proliferation assay, reverse transcriptase-polymerase chain reaction, and immunofluorescence for additional skeletal muscle markers.

POSTER 42

Telomere lengths in tumor infiltrating lymphocytes used for adoptive cell transfer immunotherapy**XINGLEI SHEN**,* University of Pittsburgh School of Medicine

Preceptor: Richard Hodes, M.D., Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

■ The recently reported use of adoptive cell transfer immunotherapy for the treatment of patients with metastatic melanoma depends on the ability to expand *ex vivo* tumor infiltrating lymphocytes (TILs) greater than a thousand fold, to 10^{10} cells. In normal human lymphocytes, both *in vitro* and *in vivo*, telomeres gradually decrease in length with repeated stimulation and proliferation, resulting in a state of replicative senescence. Recently, it has been shown that expanded TILs are oligoclonal populations, and that the presence of at least one persistent TIL clonotype after transfer *in vivo* correlates with clinical response. We hypothesized that telomere length of expanded TILs after *in vitro* expansion may affect clinical response by allowing for greater *in vivo* proliferation and persistence in TILs with longer telomeres. We measured the telomere length of expanded TILs in seven patients with positive clinical response and five non-responders. We observed that telomeres in the TIL of responders were significantly longer than those of non-responders (79 vs. 61 arbitrary fluorescence units, $p < 0.03$) thus establishing a strong correlation of telomere length to clinical outcome. Further preliminary analysis of the TIL from responders using two-color flow-FISH has suggested that within the total TIL population, the specific clonotypes (as determined by TCR V beta staining) that persist following adoptive transfer possess longer telomeres than those that fail to persist. We are currently measuring telomere length of persistent TIL clones after *in vivo* transfer, as well as the level of telomerase activity expressed in TIL populations, to determine factors which may compensate for telomere loss and permit persistence of clinically effective tumor-specific T cells. Understanding the role of telomeres in the function of TILs may help in designing strategies that will improve the success of adoptive immunotherapy for cancer.

*Advanced Scholar

POSTER 43

Pulsed-high intensity focused ultrasound enhanced thrombolysis: from *in vitro* mechanisms to *in vivo* evaluation in a novel thrombosis model**MICHAEL J. STONE**, University of Virginia School of Medicine

Preceptor: Bradford J. Wood, M.D., Diagnostic Radiology Department, Clinical Center, National Institutes of Health, Bethesda, Maryland

■ Thromboembolic disease is a prevalent disorder producing considerable morbidity and mortality. Ultrasound has been studied as an adjunctive treatment with thrombolytic drugs for thrombolysis as well as an independent treatment method in various models. Previously evaluated mechanisms of ultrasound thrombolysis have included mechanical disruption, thermal elevation and acoustic cavitation, which all carry unwanted risks. Pulsed-high intensity focused ultrasound (HIFU) has been used in our lab to increase *in vivo* delivery of various materials without thermal or cavitation effects. In a recent study, these same exposures were used to significantly enhance tissue plasminogen activator (t-PA) mediated thrombolysis *in vitro*.

In this study, a novel thrombosis model was developed in New Zealand White rabbits in order to evaluate the potential of using pulsed-HIFU to enhance t-PA mediated thrombolysis *in vivo*. Vascular clamps were used to externally occlude the marginal ear vein, followed by the injection of thrombin. The clamps were left in place during clot formation. Various clot lengths, clamping time lengths and thrombin concentrations were employed to optimize clot stability. Clot formation and dissolution were monitored non-invasively by infrared imaging, and will be validated with angiography and high-resolution diagnostic ultrasound.

To augment the clinical potential of pulsed-HIFU enhanced thrombolysis, the exact mechanism for producing these effects must be elucidated. Previous pulsed-HIFU displacement simulations demonstrated a linear increase in axial displacement in typical soft biological tissues with increased total acoustic power. Our hypothesis is that these repetitive tissue displacements cause structural changes in the clots that allow increased t-PA penetration and fibrin binding. In order to assess this hypothesis, clots were created *in vitro* and treated with pulsed-HIFU for observing these structural changes with scanning electron microscopy. Anticipated increases in t-PA binding due to these structural changes are also being investigated using fluorescently labeled monoclonal antibodies to t-PA.

POSTER 44

FKBP-8 is a potential mediator of the pro-survival effects of Notch-1 in glioma cells**TILAK K. SUNDARESAN**, University of Medicine and Dentistry of New Jersey Robert Wood Johnson Medical School

Preceptor: Howard A. Fine, M.D., Neuro-Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

■ We have previously demonstrated that knock-down of Notch-1 by RNA interference causes apoptosis in glioma cell lines. To better understand the mediators of this effect, we performed cDNA microarray of glioma cell lines transfected with Notch-1 siRNA. One of the genes showing a large decrease in expression with Notch-1 knockdown was FKBP8 (also called FKBP38), an immunophilin with strong similarities to the rapamycin-binding FKBP12. FKBP8 has been shown to have important anti-apoptotic functions, including transport of Bcl-2 and Bcl-xL to mitochondria and inhibition of calcineurin. A dramatic drop in FKBP8 protein with Notch-1 siRNA transfection of glioma cells was confirmed by western blot. To explore the importance of FKBP8 in glioma cell survival, its expression was knocked down by transfection with an FKBP8 siRNA. Immunoblotting indicated the efficiency of this siRNA. A marked decrease in glioma cell survival was noted upon FKBP8 knockdown, as shown by alamarBlue assay. Interestingly, the effects of FKBP8 knockdown were not all anti-survival, as immunoblotting indicated increased activation of the mTOR protein. These data indicate for the first time that Notch-1 is a key regulator of FKBP8 and that FKBP8 is important in glioma cell survival. They suggest FKBP8 as a potential new target in the therapy of gliomas.

POSTER 45

Recombinant human parainfluenza virus type I C protein mutants demonstrate increased sensitivity to interferon- β **WILLIAM C. VAN CLEVE**, University of Chicago, Pritzker School of Medicine

Preceptor: Brian R. Murphy, M.D., Laboratory of Infectious Diseases, Respiratory Viruses Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

■ Human parainfluenza virus type 1 (HPIV1) causes severe lower respiratory tract disease in infants and young children and, when grouped with HPIV2 and HPIV3, accounts for 18% of hospitalizations for pediatric respiratory disease. Live-attenuated vaccines for HPIV1/2/3 are in development. The HPIV1 P/C gene encodes both the viral phosphoprotein as well as 4 additional proteins, collectively termed C proteins, in a separate reading frame. The HPIV1 C proteins are believed to function as interferon (IFN) antagonists. In Sendai virus, the murine homolog of HPIV1, the C proteins have been shown to inhibit the production of type 1 IFN and to interfere with the signaling of IFN through its receptor. We are evaluating the IFN-antagonizing properties of both recombinant wild-type HPIV1 (rHPIV1wt) and a set of recombinant HPIV1 viruses containing mutations in the C proteins (C^{R84G} , C^{F170S} , $C^{\Delta 170}$, and $C^{\Delta 10-15}$) that are attenuated in small mammals and are under evaluation for inclusion in a live-attenuated vaccine virus. In Vero cells, a simian line that does not produce IFN but maintains a functional IFN response, both rHPIV1wt and rHPIV1 C protein mutants are sensitive to pre-treatment with IFN- β , exhibiting almost complete inhibition of single cycle replication at doses ≥ 100 IU/mL. At an IFN- β concentration of 10 IU/mL, the rHPIV1 C mutants are more sensitive than rHPIV1wt, with rHPIV1 C^{R84G} being most sensitive. The mechanisms underlying the sensitivity of the C protein mutants to IFN and their relative ability to induce IFN production are under study. These findings suggest that the attenuation phenotype of our HPIV1 C protein mutants is due in part to their increased sensitivity to IFN, perhaps to their increased ability to induce IFN, or to a combination of these mechanisms.

POSTER 46

Characterization of macrophage subpopulations in atherosclerotic disease**STEPHEN W. WALDO**, University of California, San Diego, School of Medicine

Preceptor: Howard S. Kruth, M.D., Experimental Atherosclerosis Section, Cardiovascular Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland

■ The monocyte-derived macrophage plays a pivotal role in vascular lipid deposition and the subsequent progression of atherosclerotic disease. Research has demonstrated that monocytes will differentiate into unique macrophage subpopulations in response to the cytokine microenvironment. The present study sought to evaluate the pro-atherosclerotic potential of divergent macrophage subpopulations *in vitro*. Human monocytes were differentiated for seven days in the presence of two prominent macrophage development cytokines: macrophage colony stimulating factor (M-CSF) or granulocyte-macrophage colony stimulating factor (GM-CSF). The resulting monocyte-derived macrophages exhibited unique morphologies. Immunocytochemical staining confirmed the upregulation of pro-inflammatory antigen expression (CD14, CD16) in the M-CSF dependent macrophages without parallel expression in the GM-CSF dependent subpopulation. A quantitative analysis of cytokine secretion profiles established at least a 10,000 arbitrary unit increase in IL-1 β , IL-6, IL-8, and TNF- α production of the M-CSF dependent cells when compared to the GM-CSF dependent macrophages. Chemical assays demonstrated the significant accumulation of unmodified low-density lipoprotein derived-cholesterol in the M-CSF dependent macrophages in comparison to the GM-CSF dependent cells. These experiments suggest that human monocytes can be differentiated into phenotypically distinct macrophage subpopulations when exposed to alternative cytokine environments. The unique macrophage subpopulation differentiated with M-CSF exhibits proinflammatory antigen expression, cytokine secretion, and cholesterol accumulation. Further experimentation should evaluate the role of this pro-inflammatory macrophage subpopulation in human atherosclerotic disease.

POSTER 47

Self-organization of salivary gland epithelium during branching morphogenesis

CINDY HSIN-YAO WEI, Washington University School of Medicine

Preceptor: Kenneth M. Yamada, M.D., Ph.D., Developmental Mechanisms Section, Craniofacial Developmental Biology and Regeneration Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland

■ The mouse submandibular gland epithelium (SMGE) undergoes branching morphogenesis during its transition from a single bud to the fully branched adult structure. Although some molecules required for branching have been identified, little is known about how branching is initiated and if it occurs at predetermined sites. To begin to investigate this question, we evaluated the ability of SMGE fragments to undergo branching morphogenesis. We found that pieces as small as one-fourth of a bud retained the ability to branch and develop into glandular structures. We then treated whole SMGE with trypsin-EDTA to obtain single cells, and found that dissociated cells could aggregate and self-organize into gland-like structures that also

retained the ability to branch. To examine the cellular arrangement and cell adhesion molecules present in these aggregates, we performed immunofluorescence staining for adherens junction proteins and compared the expression patterns in aggregates and intact embryonic day 12 (E12) and E17 SMG. Notably, E-cadherin and β -catenin were uniformly expressed along cell borders in the aggregates and E12 SMGE, but became localized to the lateral borders just below the apical surface by E17. This suggested that mature adherens junctions are not present in early SMGE, which may contribute to the high degree of tissue plasticity required for branching to occur. We next examined the effect of an inhibitory anti-E-cadherin antibody on the ability of whole SMGE to branch or dissociated SMGE cells to form aggregates. The antibody significantly inhibited both branching and aggregation, and caused the glands or aggregates to develop a flattened morphology. Together, these data suggest that regulation of E-cadherin is important for SMGE branching morphogenesis and structural integrity, and that scrambling the organization of SMGE cells by dissociation and reaggregation still permits branching. These findings provide insights into self-assembly and branching that may facilitate future salivary gland regeneration strategies.

WEDNESDAY
ROOM A

8:00 A.M.

The influence of regulatory T cells on gender- and age-associated susceptibility to experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis

EYTAN MOSHE STEIN, Northwestern University Feinberg School of Medicine

Mentor: Stephen D. Miller, Ph.D., Congressman John E. Porter Professor; Director, Interdepartmental Immunobiology Center, Department of Microbiology-Immunology, Northwestern University Feinberg School of Medicine, Chicago, Illinois

■ Regulatory T cells (T_{reg}) suppress the proliferative response of auto reactive T cells that escape clonal deletion and activation-induced T cell anergy. Relapsing-remitting experimental autoimmune encephalomyelitis (R-EAE), a mouse model of multiple sclerosis (MS), is an inducible CD4⁺-mediated demyelinating disease of the central nervous system (CNS) that causes a relapsing-remitting paralysis in susceptible mouse strains. Like MS, R-EAE predominantly affects females and the young. We hypothesized that this gender and age bias is influenced by the precursor number and function of T_{reg} s in naïve mice, or by enhanced T_{reg} activation, trafficking to the CNS, or suppression of effector CD4⁺ T cells in males and old mice induced to develop R-EAE.

Using a monoclonal antibody to CD25, we depleted T_{reg} s from naïve male and female mice, induced R-EAE, and followed disease severity. Male mice depleted of T_{reg} s exhibited more severe paralysis than depleted female mice, implying that male T_{reg} s exert tighter control over R-EAE severity than female T_{reg} s. Using flow cytometry and quantitative PCR, we looked at the precursor frequency, phenotype, and function of T_{reg} s in the lymph nodes and spleen of naïve male and female SJL mice *ex vivo*. The percentage of T_{reg} s, expression of surface molecules important in T_{reg} function, mRNA levels of Foxp3, a transcription factor necessary for T_{reg} development and function, and suppressor capacity of naïve male and female T_{reg} s were all equivalent. This suggests that gender differences in T_{reg} activation, trafficking, and/or function develop after disease induction. We are assessing these differences using T cell proliferation, cytokine secretion, and CNS immunohistochemistry assays. In addition, we are investigating T_{reg} function in young and old SJL mice with R-EAE. The outcome of these studies will have important implications for understanding T_{reg} function, and gender and age differences in the pathogenesis of MS.

8:15 A.M.

Development of non-myeloablative hematopoietic cell transplantation protocols for the treatment of the murine model of the autoimmune disease multiple sclerosis

MARY-ELIZABETH ANSELMO MUCHMORE, Stanford University School of Medicine

Mentor: Judith A. Shizuru, M.D., Ph.D., Associate Professor, Department of Medicine, Stanford University School of Medicine, Stanford, California

■ The use of hematopoietic cell transplantation (HCT) for treatment of autoimmune disease (AD) was initially incidental to treatment of a co-morbid hematopoietic condition. Now the potential exists for HCT to be used as a curative therapy to induce long-lasting remissions in AD. The overall goal is to develop pre-clinical protocols using non-myeloablative, allogeneic HCT and to assess the effect of each treatment strategy on engraftment and autoreactive T cell populations. In the experimental allergic encephalomyelitis (EAE) model used, C57BL/6 mice, an EAE-susceptible strain, are immunized with a peptide derived from myelin oligodendrocyte glycoprotein, thereby inducing disease mimicking multiple sclerosis (MS). Two principal non-myeloablative regimens have been studied: one of total lymphocyte irradiation (TLI) combined with three doses of anti-thymocyte globulin (ATG), and another of cyclophosphamide, ATG, and low-dose total body irradiation (TBI). Congenic transplants, between strains differing only in a single allele, roughly correspond to autologous transplants in humans; the effects are compared to allogeneic transplants, which use strains that are matched only at the major histocompatibility complex or are completely mismatched. In each regimen, the engraftment observed after transplanting purified hematopoietic stem cells (HSC) is compared to that of whole bone marrow (WBM).

Using the TLI/ATG regimen for allogeneic HSC and WBM transplant, mixed chimerism between donor and host cells has been observed in all hematopoietic lineages. The levels of engraftment are dose-dependent. Durable mixed chimerism has also been observed with the cyclophosphamide, ATG, and TBI regimen. Now that high levels of engraftment have been consistently achieved, the next step will be to pre-condition and transplant EAE-affected mice.

Provided toxicity can be minimized and regimens can be optimized, HCT with autologous or allogeneic stem cells has the potential to become a viable treatment for an AD such as severe, refractory MS.



E.M. STEIN



M-E.A. MUCHMORE

8:30 A.M.

The effects of immunomodulatory therapy on FOXP3+ regulatory T cells in autoimmune diabetes

JASON Y. ADAMS, University of California, San Francisco, School of Medicine

Mentors: Jeffrey A. Bluestone, Ph.D., Professor; Director, Diabetes Center, Departments of Medicine and of Pathology, and Qizhi Tang, Ph.D., Assistant Adjunct Professor, Department of Pathology, University of California, San Francisco, San Francisco, California

■ Accumulating evidence suggests that defects in peripheral tolerance are an essential underlying cause of autoimmunity. In the non-obese diabetic mouse model of type 1 diabetes, immune regulation is mediated in part by a small subset of T lymphocytes known as regulatory T cells (Tregs) that are thought to be critical to maintaining peripheral self-tolerance. Tregs are enriched amongst CD4+CD25+ T cells; however, these markers are not unique to Tregs. Attempts to characterize Tregs by other cell surface markers have thus far not identified a Treg-specific phenotype thereby limiting studies of Treg biology. More recently, a novel member of the forkhead/winged-helix family of transcriptional regulators known as FOXP3 was found to be expressed preferentially in Tregs and when mutated, resulted in loss of Tregs and multi-organ autoimmunity in mice and humans. Furthermore, over-expression of FOXP3 in CD4+CD25- cells induced regulatory function. Thus, at present, FOXP3 is the most specific marker of Treg lineage commitment. To date, the majority of FOXP3 analysis has been on whole populations, raising several issues regarding the distribution of FOXP3+ cells within the immune system and other tissues, the response of Tregs to antigen exposure *in vivo* and the mechanism of suppression. Thus, we sought to develop polyclonal and monoclonal antibodies targeting FOXP3 for detailed immunohistochemical and flow-cytometric analyses of FOXP3 at the single cell level. Our data suggest that the distribution and number of FOXP3-expressing cells change upon antigen encounter in autoimmunity and that immunomodulatory therapy further alters these parameters. In addition, we found that a significant number of CD4+CD25- T cells express FOXP3 *in vivo* which may explain the regulatory activity of this subset reported in certain disease settings. Studies currently ongoing to assess the role of Tregs in disease progression and in response to immunomodulatory therapeutics will be discussed.

8:45 A.M.

T cell homing to metastatic melanoma: immune evasion by tumor vessel dysregulation

KARLA NICOLE MUÑOZ, Harvard Medical School

Mentors: Thomas S. Kupper, M.D., Thomas B. Fitzpatrick Professor of Dermatology; Chairman, Department of Dermatology, Brigham and Women's Hospital; Director, Harvard Skin Disease Research Center, and Robert C. Fuhlbrigge, M.D., Ph.D., Assistant Professor of Pediatrics, Department of Rheumatology, Children's Hospital Boston, Department of Dermatology, Brigham and Women's Hospital; Associate Director, Harvard Skin Disease Research Center, Harvard Medical School, Boston, Massachusetts

■ Despite increased public awareness, the incidence of melanoma continues to rise and the prognosis for metastatic disease remains bleak. Anti-tumor vaccine strategies have yielded limited success, despite evidence for successful stimulation of host anti-tumor T cells. Effective anti-tumor immunity requires efficient recruitment of T cells to sites of tumor. The observed lack of benefit may, therefore, indicate a block in T cell homing. This study characterizes the homing receptors on circulating and tumor infiltrating T cells, as well as the counter-receptors on tumor vasculature in patients with melanoma. Tissue (n=56) and blood (n=26) samples were collected over a two-year period from 48 patients undergoing surgery for malignant melanoma. Patients had received various therapies and were not all on one protocol. Of the tissues received, 42/56 contained metastatic melanoma and 14/56 showed normal tissue without evident melanoma. Homing receptor expression on peripheral blood T cells from melanoma patients was not different from controls. In contrast, immunohistochemical staining of many metastatic tissues (including lymph node, lung, and skin) showed little or no E- or P-selectin expression on CD31+ tumor vessels, despite increased expression on vessels surrounding the tumor. ICAM-1 staining also appeared to be decreased on tumor vessels. Interestingly, CD3+ staining demonstrated that 17/27 (63%) samples tested had few T cells within melanoma tumor, while numerous T cells could be seen in the surrounding tissue. These results suggest that metastatic tumors that down regulate endothelial adhesion receptors, or that encourage growth of vessels lacking such receptors, can avoid the immune response and maintain a selective growth advantage. The mechanisms restricting adhesion receptor expression in tumor vessels are unknown, but methods to enhance T cell recruitment should be evaluated and incorporated into the design of anti-melanoma treatment strategies. Ongoing studies include identification and analysis of the subset of MART-1 tetramer-reactive CD8+ T cells.



J.Y. ADAMS



K.N. MUÑOZ

WEDNESDAY
ROOM A

9:00 A.M.

Inhibition of HLA-DM: defining the active site and mechanism of HLA-DO

JAMES JOSEPH HARDING, Albert Einstein College of Medicine of Yeshiva University

Mentor: Elizabeth D. Mellins, M.D., Associate Professor, Department of Pediatrics, Stanford University Medical Center, Stanford, California



JJ. HARDING

■ Peptide presentation by the major histocompatibility complex (MHC) class II glycoproteins is a critical step in T-cell selection and homeostasis as well as T-cell activation. Activation of mature T-cells is essential for protective immune responses against foreign pathogens and also plays a role in both regulatory and auto-aggressive responses to self-proteins. Information regarding this pathway has the potential to impact health-related goals, including enhancement of responses against pathogens or tumors and the reduction of unwanted responses. The observation that distinct class II glycoproteins confer susceptibility to rheumatoid arthritis, insulin-dependent diabetes mellitus, and multiple sclerosis also suggests that a detailed molecular understanding of peptide selection in the class II pathway will clarify the pathogenesis of these disorders. Several crucial accessory molecules are necessary for proper peptide loading. Class II invariant chain peptide (CLIP) prevents premature peptide loading by occupying the class II binding cleft. HLA-DM catalyzes the removal of CLIP from the class II and “edits” the peptide repertoire. Finally, HLA-DO inhibits DM activity. The exact inhibitory mechanism of HLA-DO is unknown; the crystal structure of HLA-DO, alone or in complex with DM, has not been solved; and the kinetics of DM/DO binding are poorly understood.

To map the DM/DO binding face, we have utilized mutagenesis to create 28 rationally designed DM mutants expressed in EBV-transformed B-cell lines expressing fully functional DO. Fluorescence activated cell sorting (FACS) will be used to screen these mutants for low surface CLIP/DR ratios, a consequence of active DM that inadequately associates with HLA-DO. Co-immunoprecipitation of the proteins of interest will also provide a crude estimate of binding affinity. To robustly assess the kinetics of DM/DO binding, a method based on surface plasmon resonance will be established to determine affinity measurements for the wild-type DM/DO complex.



J. PEACOCK

9:15 A.M.

BCAP serves an immunoregulatory role in macrophage TNF-alpha production in response to LPS stimulation

JAMES PEACOCK, Vanderbilt University School of Medicine

Mentor: Steven Greenberg, M.D., Associate Professor, Department of Medicine, Columbia University, New York, New York

■ Excessive production of TNF-alpha is seen in many inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease. Anti-TNF-alpha drugs are currently on the market to manage such diseases. B cell adaptor protein (BCAP) is a recently identified tyrosine kinase substrate that connects the B cell receptor for antigen (BCR) to phosphoinositide 3-kinase (PI3K) activation. BCAP has been shown to be predominantly expressed in B cells and macrophages by Northern blot analysis. While BCAP plays a pivotal immunoregulatory role in B cell development and humoral response, BCAP-deficient mice show only a slight decrease in macrophage numbers in the spleen. It is perhaps for this reason that the potential role of BCAP in macrophages has remained largely unexplored.

Here we report that BCAP serves an immunoregulatory role in macrophage TNF-alpha production. BCAP null thio-elicited macrophages, pre-activated with interferon-gamma, secrete less TNF-alpha upon LPS stimulation than heterozygous BCAP macrophages. In addition, LPS stimulation increases BCAP expression in macrophages as well as induces recruitment of BCAP to glycolipid-enriched microdomains (GEMs). Furthermore, BCAP null thio-elicited macrophages show a reduced basal level of AKT activation compared to their heterozygous counterparts.

Interestingly, BCAP null macrophages retain their ability to undergo phagocytosis, demonstrated by their ability to ingest antibody-coated sheep red blood cells. Also, production of other cytokines does not appear to be significantly affected by the absence of BCAP. If BCAP's primary role is to modulate TNF-alpha secretion in macrophages, then the potential for knocking out BCAP while retaining other macrophage functions may open new possibilities in the treatment of inflammatory diseases.

9:30 A.M.

Identification of autoantigens in autoimmune uveitis by subtractive phage display**ANIL VEDULA**, Yale University School of Medicine

Mentors: Wei Li, Ph.D., Assistant Professor, and M. Elizabeth Fini, Ph.D., Professor and Scientific Director, Department of Ophthalmology, University of Miami, Miami, Florida

■ Identification of disease-relevant autoantigens using antibodies from the sera of uveitis patients is important for our understanding of disease mechanisms but presents a formidable challenge due to antibody heterogeneity. A novel system of subtractive phage display was recently developed to overcome this challenge. Tribbles homolog 2 (TRB2) was identified by this system as a candidate autoantigen. Herein, we characterize TRB2 expression in uveitis-susceptible tissue and further optimize the system for autoantigen identification by competitive immunoblotting.

To verify TRB2 expression in the eye, recombinant TRB2 was expressed as a glutathione-S-transferase fusion protein and purified. Anti-TRB2 antibody was affinity purified using immobilized TRB2. Western blot analysis with purified TRB2 indicated that TRB2 was recognized by uveitic patient IgG, but not by control human IgG. TRB2 expression was detected in the ciliary body by immunohistochemistry using affinity purified TRB2-specific antibody.

To optimize the phage display system, we report the development of competitive immunoblotting. In this new approach, all phage clones binding to common antibodies were enriched and used to block disease-irrelevant antibodies, facilitating the detection and isolation of patient-specific phage clones. Of 88 phage clones isolated by competitive immunoblotting, 17 have been verified by dot blotting. These clones are under further investigation for their roles in uveitis.

In conclusion, we have successfully verified TRB2 expression in uveitis-susceptible ocular tissue, suggesting that phage display can identify disease-relevant autoantigens using patient polyclonal antibodies. Furthermore, we have demonstrated that competitive immunoblotting can improve the accuracy of isolating patient-specific autoantigens. Identification of autoantigens by this phage display system will advance our understanding of disease mechanisms and facilitate disease diagnosis.

10:30 A.M.

Role of virulence pathways in *Candida albicans***EMILY DAWN EADS**, Duke University School of Medicine

Mentor: Joseph Heitman, M.D., Ph.D., Investigator, Howard Hughes Medical Institute; Director, Center for Microbial Pathogenesis; Director, Duke University Program in Genetics and Genomics; James B. Duke Professor, Departments of Molecular Genetics and Microbiology, of Pharmacology and Cancer Biology, and of Medicine, Duke University, Durham, North Carolina

■ The human fungal pathogen *Candida albicans* causes a spectrum of disease ranging from mucocutaneous to disseminated candidiasis. In *C. albicans*, the yeast-to-hyphal transition and signaling via the serine/threonine phosphatase calcineurin are important for virulence. Previous studies demonstrated that the cAMP pathway promotes hyphal formation and morphogenesis on solid media via the putative G protein-coupled receptor Gpr1 and the G α subunit Gpa2 and that germ tube formation is stimulated by glucose. We hypothesized that germ tube formation would be stimulated via the putative glucose receptor Gpr1; however, we found that glucose-induced germ tube formation is not dependent on Gpr1 or Gpa2. In light of recent studies that describe Gpr1 as a putative amino acid sensor, we are now investigating whether methionine acts via the Gpr1 receptor to promote filamentation via the cAMP pathway.

We have also investigated downstream targets of calcineurin, which is required for survival in serum and virulence. Disruption of calcineurin via deletion or inhibition by calcineurin inhibitors increase *C. albicans* sensitivity to membrane perturbation by azoles. Previous work in our laboratory demonstrated that the transcription factor Crz1, a putative target of calcineurin, plays a partial role in azole tolerance. To identify other effectors in the calcineurin pathway, we have screened a homozygous deletion library and have identified candidates that mimic the calcineurin mutant phenotype and display hypersensitivity to azoles. We have also investigated whether the drug synergy observed between calcineurin inhibitors and ergosterol biosynthesis inhibitors is conserved in other pathogenic fungi such as the dermatophyte *Trichophyton rubrum*. Preliminary data suggest that calcineurin inhibitors display antifungal activity in *T. rubrum*. Both the cAMP pathway and the calcineurin pathway are involved in modulating *C. albicans* sensitivity to azoles; therefore, understanding their contributions to virulence and drug tolerance will provide critical information for the development of new antifungal drugs.



A. VEDULA



E.D. EADS

WEDNESDAY
ROOM A

10:45 A.M.

CD4⁺ T cell and anti-basal ganglia antibody characterization in response to streptococcal virulence factor immunization

KYLE ALLEN WILLIAMS,* University of Minnesota Medical School—Twin Cities

Mentor: Patrick M. Schlievert, Ph.D., Professor, Department of Microbiology, University of Minnesota Medical School—Twin Cities, Minneapolis, Minnesota

■ Sydenham's chorea (SC) is a sequela of group A β -hemolytic *Streptococcus pyogenes* (GABHS) infection and is recognized as a major manifestation of acute rheumatic fever (ARF). Multiple studies have isolated auto-reactive anti-basal ganglia antibodies from blood in patients with SC, and these antibodies have been shown to cross react with streptococcal M proteins. Additional virulence factors, bacterial superantigens (SAGs), are a class of immunostimulatory exotoxins produced by rheumatogenic GABHS strains. SAGs undermine immune function by binding the class II major histocompatibility complex (MHC II) molecules on antigen-presenting cells (APC) and variable- β (V β) regions on the T cell receptor (TCR). We hypothesize that rheumatogenic strains of GABHS encode autoimmune-inducing epitopes and SAGs capable of stimulating T cells which bind these epitopes.

We assessed CD4⁺ T cell response to the M protein and the superantigens SpeC, SpeL, and SpeM cloned from a GABHS strain which produces high rates of SC. Cloned M18 M protein was injected subcutaneously (50 μ g + adjuvant) into PL/J mice (n=5). Experimental groups received M18 subcutaneously and 10 η g of superantigens (SpeC) (n=5). Groups were sacrificed at 2, 6, and 8 weeks. Splenocytes were used in a T cell proliferation assay with overlapping, artificially generated M protein peptide fragments.

Our data indicate a highly significant (p<0.001) down-regulation of T cell reactivity to the variable region of the M protein in all mice injected with superantigens at 2, 6, and 8 weeks. Homogenized mouse striatum (15 μ g/lane) was used for Western blotting. Membranes were incubated in pooled serum (1:100 dilution) from each experimental group and goat-anti mouse IgG (1:1000). Serum from mice immunized with SpeC demonstrated reactivity to two striatal antigens approximately 70 and 50 kD in size. The data support our hypothesis that superantigens significantly the CD4⁺ T cell antigen response to M protein and may induce autoimmune antibody production.

*Second-Year Medical Fellow



K.A. WILLIAMS



J.B. WINGARD

11:00 A.M.

Dendritic cells transduced with an HIV-derived vector encoding Gag-Pol are able to induce a potent *in vivo* CD4 T cell response

JEREMY BLAINE WINGARD, Duke University School of Medicine

Mentor: Drew Weissman, M.D., Ph.D., Assistant Professor, Department of Medicine, Division of Infectious Diseases, University of Pennsylvania, Philadelphia, Pennsylvania

■ Dendritic cells (DC) are the most potent antigen presenting cells in the body. Recent research has invoked direct DC participation in the preparation of anti-tumor vaccines. Our aim is to develop an *ex vivo* antigen delivery strategy that stimulates specific and potent T cell responses through DC antigen presentation. We used an HIV-derived vector that encodes enhanced green fluorescent protein (EGFP) and Gag-Pol, but no envelope (VRX418). While EGFP expression is used to follow transduction of DC, mice injected with transduced DC should develop an immune response against both EGFP and Gag-Pol. In addition, Gag-Pol encodes reverse transcriptase, meaning this vector could replicate if its RNA is taken up by another DC. The use of this vaccine approach would therefore lead to continuous reloading of DC with antigen and potentially induce a maximal immune response after a single delivery.

First, we proved the ability of VRX418 to transduce human and mouse DC. Second, we showed that new DC added to transduced DC are able to take up amplified amounts of vector *in vitro*. EGFP expression from new DC added to VRX418 transduced DC was tenfold higher than EGFP expression from new DC added to DC transduced with VRX494, a vector expressing EGFP but not Gag-Pol. Finally, two mice displayed powerful CD4 T cell responses to the Gag antigen at four and six weeks after a single vaccination with VRX418 transduced DC. Intracellular cytokine assays for IFN- γ production showed that 15–22% of CD4 T cells isolated from the spleens of vaccinated mice responded specifically to the Gag antigen.

11:15 A.M.

T-bet controls CD8⁺T cell-mediated inflammation in the heart

VIVIANY RODRIGUES TAQUETI, Harvard Medical School

Mentor: Andrew H. Lichtman, M.D., Ph.D., Associate Professor of Pathology, Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts

■ The differentiation of naïve CD8⁺T cells into cytolytic T lymphocytes (CTLs) is critical for protective immune responses against viruses, tumors, and other intracellular pathogens. Yet, improperly controlled CTL responses may cause tissue injury that is harmful to the host. This likely occurs in idiopathic myocarditis, an autoimmune disease characterized by T cell infiltrate to the heart. Our study uses a new transgenic mouse model of CD8⁺T cell-mediated myocarditis to investigate the regulation and traffic of pathogenic T cells in this disease. Ovalbumin-specific T cell receptor transgenic cells (OT-1) are adoptively transferred into mice (CMy-mOva) engineered to express ovalbumin exclusively in cardiac myocytes. CTL-mediated myocarditis and heart failure ensue in a CTL dose-dependent fashion.

T-bet, a member of the T-box family of transcription factors, is critical for driving differentiation of CD4⁺T cells into the Th1 helper subset. Although T-bet is also expressed in CD8⁺T cells, its role in the regulation of CTLs is poorly understood.

Here, we demonstrate that T-bet^{-/-} CTLs are significantly impaired in their ability to mediate lethal myocarditis. CMy-mOva mice receiving T-bet^{-/-} OT-1 effectors show a dramatic survival benefit (compared to those receiving wild-type OT-1 effectors), correlating with minimal myocardial necrosis and lymphocytic infiltrate. Paradoxically, *in vitro* analysis of T-bet^{-/-} cells reveals hyperproliferation in response to antigenic stimulation, with increased expression of activation markers. Surprisingly, functional assays and gene expression data indicate these cells can respond to IL-12 and produce cytokines consistent with a pro-inflammatory Tc1 phenotype. Diminished pathogenicity of T-bet-deficient CTLs *in vivo* results, rather, from their impaired migratory capabilities, reduced cytolytic activity, and increased susceptibility to negative regulation.

Thus, we conclude that T-bet is required for a program of gene activation promoting multiple aspects of the pathogenic CD8⁺T cell response. These results may aid in developing novel treatments against autoimmune disease and transplantation rejection.

11:30 A.M.

A novel approach to tolerance induction in cynomolgus monkeys

BIDHAN BIHARI DAS, Yale University School of Medicine

Mentor: Joren C. Madsen, M.D., D.Phil., Head, Cardiothoracic Transplantation Laboratory; Surgical Director, Cardiac Transplant Service, Massachusetts General Hospital; Associate Professor, Department of Surgery, Harvard Medical School, Boston, Massachusetts

■ Despite advances in immunosuppression, long-term transplant success rates remain unsatisfactory, owing particularly to chronic allograft vasculopathy. It seems necessary to better characterize exceptional therapies from rodent models and extend them to preclinical models in order to deductively and sequentially evaluate the efficacy of a tolerance regimen.

A tolerizing regimen using an agonist IL-2/Fc fusion protein to enhance activation-induced cell death of activated T cells, an antagonist IL-15/Fc fusion protein to block proliferative and anti-apoptotic IL-15 signals, and rapamycin to block the early expansion of alloreactive T cells has proven itself an exceptional regimen in rodent models. Our aim was to evaluate *in vitro* functionality of humanized versions of the proteins in the preclinical cynomolgus monkey model by assessing the effects of the components on different populations of T cells. With no formal characterization of the T regulatory cell and T memory cell population in the cynomolgus monkey system, effects were assessed through mixed lymphocyte reaction (MLR) assays, cytokine profiling, and binding assays.

The data have illustrated that in primary cynomolgus MLR and enzyme-linked immunospot (ELISPOT) cultures the agonist IL-2/Fc is able to increase alloreactivity, and the mIL-15/Fc can decrease alloreactivity, but through a dose-response/kinetics study, the fusion proteins seem to bind effectively only at concentrations that are much higher than previously understood.

It is our conclusion that producing robust, tolerizing effects through these humanized components in the cynomolgus monkey would require a higher expected dose per given response, but they are able to positively bind and demonstrate expected *in vitro* effects.



V.R. TAQUETI



B.B. DAS

WEDNESDAY
ROOM A

11:45 A.M.

Macrophage depletion suppresses cardiac allograft vasculopathy**WILLIAM HENRY KITCHENS JR.**, Harvard Medical School

Mentors: Joren C. Madsen, M.D., D.Phil., Associate Professor, Department of Cardiac Surgery, and Paul S. Russell, M.D., John Homans Distinguished Professor of Surgery, Department of Transplant Surgery, Massachusetts General Hospital, Boston, Massachusetts

■ While chronic rejection encumbers all types of organ transplants, it is particularly virulent in cardiac allografts, where it manifests as diffuse and rapidly progressive arteriosclerosis known as cardiac allograft vasculopathy (CAV). CAV is the leading cause of mortality in cardiac allograft recipients after the first post-transplant year. Although T and B cells are implicated in its pathogenesis, the identity of the end-effectors that fuel CAV development is ill-defined. Because of their abundant presence in CAV lesions and their capacity to produce growth factors implicated in neointimal cell proliferation, macrophages are leading candidates to serve as these end-effectors.

To assess their role in CAV formation, macrophages were depleted in a murine heterotopic cardiac transplant system known to develop fulminant CAV lesions. C57BL/6 hearts were transplanted into (C57BL/6 × BALB/c)F1 hybrid recipients, which then received an anti-macrophage therapy for 30 days before euthanasia. Attempts to deplete macrophages with clodronate-encapsulated liposomes were abandoned because they provoked allograft thrombosis. A new regimen of intraperitoneal carrageenan and aspirin anticoagulation enabled the successful depletion of macrophages in these transplant recipients with minimal adventitious effects upon T, B, or NK cells as confirmed by flow cytometry and NK cytotoxicity assays. CAV development was prevented in 70% of carrageenan-treated recipients, a significant contrast with the universal CAV development occurring in control transplant recipients treated with aspirin alone. The suppression of CAV in carrageenan-treated recipients correlated with the degree of macrophage depletion achieved. Inhibition of macrophage phagocytosis alone with gadolinium chloride failed to prevent CAV in transplant recipients, suggesting that macrophages primarily contribute to CAV through cytokine and growth factor production. Extension of these results in a full MHC-mismatch transplant system is in progress.

These findings offer a new target for immunosuppression and raise the possibility that anti-macrophage strategies in humans may improve the long-term outcome of transplanted organs.



W.H. KITCHENS JR.



K. EASH

Noon

The role of mutations in the CXCR4 gene in the pathogenesis of WHIM (warts, hypogammaglobulinemia, infections, myelokathexis) syndrome**KYLE EASH**, Washington University School of Medicine

Mentor: Daniel C. Link, M.D., Associate Professor, Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri

■ WHIM (warts, hypogammaglobulinemia, infections, myelokathexis) syndrome is a rare disorder characterized by chronic neutropenia despite normal to increased numbers of mature neutrophils in the bone marrow (myelokathexis). It is associated with heterozygous, gain-of-function mutations of the CXCR4 gene. Recent evidence has shown that CXCR4 signaling induced by its ligand, stromal derived factor-1 (SDF-1), may play a key role in the trafficking of neutrophils from the bone marrow. Together, these observations suggest the hypothesis that the CXCR4 mutations found in WHIM syndrome induce neutropenia through abnormal neutrophil retention in the bone marrow.

To test this hypothesis, we generated oncoretroviral vectors containing either wild-type (WT) CXCR4 or a mutant CXCR4 (R334X) representative of those found in patients with WHIM syndrome. The retroviral vectors also contained an internal ribosomal entry sequence coupled to the enhanced green fluorescent protein (EGFP) to track transduced cells. BAF3 cells transduced with WT or R334X CXCR4 retrovirus exhibited a high level of surface CXCR4 expression and increased calcium flux upon stimulation with SDF-1, with the greatest calcium flux observed in cells transduced with CXCR4 R334X. To directly assess the effect of the R334X CXCR4 mutant on neutrophil trafficking *in vivo*, primary murine hematopoietic cells were retrovirally transduced and transplanted into irradiated syngeneic recipients. Following hematopoietic reconstitution (5–6 weeks post-transplantation) the number of transduced (EGFP+) cells in the blood and bone marrow was assessed by flow cytometry. Surprisingly, a similar percentage of GFP-positive neutrophils and B-lymphocytes was observed in mice reconstituted with cells transduced with retroviral vector alone, WT CXCR4 or R334X CXCR4. However, preliminary analysis suggests that neutrophils transduced with R334X CXCR4 may be preferentially retained in the bone marrow.

Though additional mice need to be analyzed, these data suggest that expression of R334X CXCR4 in neutrophils results in their abnormal retention in the bone marrow.

8:00 A.M.

Survival of new neurons in the dentate gyrus after trauma to the developing brain**MATTHEW BRYAN POTTS**, University of California, San Francisco, School of Medicine

Mentors: Linda J. Noble, Ph.D., Professor, and John R. Fike, Ph.D., Professor, Department of Neurosurgery, University of California, San Francisco, San Francisco, California

■ Traumatic brain injury (TBI) is the leading cause of disability in children and is associated with significant cognitive deficits. We have developed a murine model of injury to the developing brain that mimics features of human pediatric TBI. We have previously shown that cognitive deficits are not apparent within the first weeks after injury but rather develop during maturation of the brain. We hypothesize that impaired neurogenesis contributes to this cognitive decline after injury. To begin to test this hypothesis, newly formed, mature neurons within the granular cell layer (GCL) of the dentate gyrus, a neuronal population associated with cognitive function, were examined after TBI at postnatal day 21. 5-Bromo-2'-deoxyuridine (BrdU) was used to label dividing cells after injury or sham surgery. Immunohistochemistry and confocal microscopy were used to identify newly born, mature neurons (NeuN+, BrdU+) within the GCL at 6 weeks and 4 months post injury.

Preliminary results show that the total number of BrdU-positive cells within the ipsilateral GCL is more than double that of age-matched sham controls 4 months post injury. Importantly, there is an increase of greater than 400% in newly born, mature neurons in the ipsilateral GCL compared to age-matched controls. There is also an increase in these neurons within the ipsilateral GCL compared to the contralateral GCL of injured animals 4 months post injury.

We have previously shown a reduction in the number of immature neurons 4.5 months after TBI. Despite this reduction in immature neurons, our current findings suggest a marked increase in the number of newly born, mature neurons. Together, these findings suggest that increased survival of these mature GCL neurons may counteract the reduction in formation of immature neurons. How this marked increase in neurons influences hippocampal structure and function is currently under investigation.

8:15 A.M.

Spatio-temporal patterns of mitral and tufted cell dendritic development in the mouse main olfactory bulb**MASHA RAND**, Yale University School of Medicine

Mentor: Charles A. Greer, Ph.D., Professor, Departments of Neurosurgery and Neurobiology; Co-Director of Interdepartmental Neuroscience Program, Yale University School of Medicine, New Haven, Connecticut

■ Correct targeting and arborization of dendrites are essential for the development of functional neuronal circuits. Prior research focused on axonal development, with little attention given to determinants of dendritic development. The olfactory bulb (OB) is a particularly favorable model for studying dendritic development because of its laminar organization, precocious development, and easy access. OB projection neurons, mitral and tufted (M/T) cells, receive odor information from olfactory sensory neuron (OSN) axons via axodendritic synapses on their apical dendrite; that signal is refined via dendrodendritic synapses on their lateral dendrites. In the adult, each M/T cell apical dendrite targets a single glomerulus, ending in a characteristic glomerular tuft and receiving input from molecularly defined subsets of OSNs. M/T cell lateral dendrites segregate deep to the glomerular layer, in different sublaminae of the external plexiform layer. Dendritic maturation occurs well into postnatal development, suggesting involvement of afferent activity.

Using DiI fills, we have shown that mitral cells progress from an undifferentiated, broadly spread dendritic arbor in the embryo to the single apical dendrite and lateral dendrites characteristic of the adult. The postnatal transition from non-specific to specific targeting of a glomerulus by the apical dendrite is consistent with the notion that functional activity, or specificity of synapse formation, may play a role. To further our analyses of M/T cell dendritic development, we also examine the expression of the dendritic growth cone specific protein, CDA1. Analysis of CDA1 expression patterns in the different lamina at embryonic day 17, postnatal days 0 and 8 suggests that dendritic development occurs radially outward. The data further suggest distinct temporal windows of dendritic development in subpopulations of M/T cells. CDA1 expression decreases significantly by postnatal day 8 and is indistinguishable from background in the adult. Thus, both lines of data show evidence of significant postnatal dendritic remodeling.

WEDNESDAY
ROOM B

M.B. POTTS



M. RAND

WEDNESDAY
ROOM B

8:30 A.M.

Evidence for the role of PSD-95 in dopamine receptor signaling and behavioral plasticity**MARK HOWARD NEELY**, Duke University School of Medicine

Mentor: Marc G. Caron, Ph.D., James B. Duke Professor, Department of Cell Biology, Duke University, Durham, North Carolina



M.H. NEELY

■ Behavioral sensitization is the phenomenon in which repeated drug exposure results in augmentation of the response to that drug. This sensitization likely reflects alterations in synaptic plasticity. Work in our lab implicates a role for the post-synaptic density scaffolding protein PSD-95 in the regulation of dopamine synapse plasticity. Chronic cocaine treatment in mice reduces striatal levels of PSD-95 mRNA and protein 50% relative to acute administration. Additionally, PSD-95-null mice show behavioral characteristics of sensitization after a single psychostimulant administration. These results demonstrate PSD-95 involvement in dopamine receptor signaling. The present work employs behavioral and molecular techniques to elucidate the mechanism whereby PSD-95 modulates dopamine receptor signaling.

Physiological actions of dopamine are mediated by D₁-like and D₂-like classes of G protein-coupled receptors. To determine the specific signaling pathway affected by changes in PSD-95, we assessed the effects of selective dopamine agonists on locomotor behavior. PSD-95-null and wild-type mice were treated with the dopamine D₁ agonist SKF81297, D₂/D₃ agonist quinpirole, or both agonists simultaneously. SKF81297 administration significantly augmented locomotor activity in PSD-95-null relative to wild-type mice, while quinpirole administration alone failed to alter locomotor activity. Co-administration of quinpirole with SKF81297 not only reversed the hyperlocomotor effect of the D₁ agonist, but also reduced locomotor activity in PSD-95-null relative to wild-type mice. PSD-95 and dopamine receptor interactions were evaluated using co-immunoprecipitation experiments. HEK-293 cells transfected with D₁ or D₂ receptors in the presence of PSD-95, reveal that D₁, but not D₂, receptors associate with PSD-95.

Reduced PSD-95 levels alter receptor responsiveness to both D₁ and D₂ agonists and that PSD-95 directly interacts with D₁ receptors. Disruption of this interaction in PSD-95-null mice may underlie the chronic psychostimulant-induced changes in dopamine receptor responsiveness and behavioral plasticity. Moreover, these observations suggest that PSD-95 may influence physiological functions regulated by dopamine, including learning, locomotion, and affect.



P.B. SERGOT

8:45 A.M.

Characterizing the potential for neurogenesis in the cortex of adult mammals after stroke**PAULINA BARBARA SERGOT**, Columbia University College of Physicians and Surgeons

Mentors: James E. Goldman, M.D., Ph.D., Professor, Department of Pathology, and E. Sander Connolly Jr., M.D., Associate Professor, Department of Neurosurgery, Columbia University College of Physicians and Surgeons, New York City, New York

■ Stroke is the third leading cause of death and the leading cause of serious long-term disability in the United States. The discovery of neurogenesis in the adult mammalian brain has suggested the possibility of neuronal replacement as a new therapeutic option. In the context of acute stroke, this approach could avoid the time constraints faced by neuroprotective agents, which have very narrow therapeutic windows. Currently, the manipulation of neuronal precursors (NPs) as a therapeutic strategy remains largely unexplored, mainly because the potential for neurogenesis in the adult mammalian cortex remains unclear.

There may be two sources of NPs with the potential to repair cortical infarction: endogenous cells and ones that migrate from the subventricular zone (SVZ). Furthermore, the addition of growth factors may amplify this response. To explore the degree of cortical neurogenesis in our rodent model of middle cerebral artery occlusion/reperfusion, we used markers for DNA replication (BrdU) and mature neurons (NeuN). To determine the source of regenerated neurons, we specifically labeled either the SVZ or the ischemic penumbra of infarcted animals using a GFP-producing retrovirus, which is only incorporated into the DNA of dividing cells. We then followed the migration and fates of these cells, with or without infusion of growth factors.

Preliminary data show little to no co-localization of BrdU and NeuN up to 14 days post-ischemia, indicating a lack of cortical neuronal regeneration in the short term. However, we have seen some migration of cells originating from the SVZ into the adjacent ischemic striatum, white matter, and cortex, but none were regenerated neurons. Whether or not any of these migrating cells or ones already present in the cortex will become neurons, possibly with the addition of growth factors, will be elucidated in future studies that allow animals to survive longer periods of times.

9:00 A.M.

Selective isolation and gene expression analysis of sprouting neurons after focal cortical stroke**DIANA KATSMAN**, University of California, Irvine, College of Medicine

Mentor: Stanley Thomas Carmichael, M.D., Ph.D., Assistant Professor, Department of Neurology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California

■ Stroke induces axonal sprouting within pericortex, which plays a role in recovery. Post-stroke axonal sprouting means that neurons must elaborate a growth program: form a growth cone, extend an axon, and establish new synapses. Defining the molecular growth-associated program underlying post-stroke axonal sprouting has been difficult because sprouting neurons sit within a region that also contains many non-sprouting neurons. We used a stroke model with precisely localized areas of damage in the barrel field cortex of the rat to selectively label and isolate individual sprouting neurons from peri-infarct cortex. To selectively label sprouting neurons, the retrograde tracers cholera toxin subunit-B (CTb), coupled to Alexa-488 and -594 fluorophores, were sequentially microinjected into several barrels within peri-infarct area. CTb-488 was injected at the time of stroke. After 7 or 21 days, initiation and maintenance phases in the axonal sprouting response, CTb-594 was injected exactly within CTb-488 injection. The first injection labels all neurons projecting to the injection site at the time of stroke, the second labels neurons newly projecting to this site. Neurons labeled only with the second tracer have thus sprouted into the injection site after stroke. High-resolution digitized maps show that CTb-594-labeled neurons are found in high numbers post-stroke, while minimal in control animals. Newly sprouting neurons were collected by laser capture microdissection and total RNA isolated. In ongoing experiments, total RNA will be amplified, and the pattern of gene expression in sprouting neurons vs. non-sprouting neurons from the same animal determined with oligonucleotide microarray analysis. This approach allows selective gene expression analysis of axonal sprouting post-stroke, leading to the identification of a regeneration transcriptome for neurons in the adult CNS.

9:15 A.M.

12-Lipoxygenase and matrix metalloproteinase-9: potential therapeutic targets for stroke**SOPHIA WANG**, Mount Sinai School of Medicine

Mentor: Eng H. Lo, Ph.D., Associate Professor, Departments of Radiology and Neurology, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts

■ Stroke is the third leading cause of death in the United States. Tissue plasminogen activator (tPA), its only FDA-approved therapy, restores blood flow but may also cause cerebral hemorrhage. Neuroprotection against cell death and tPA-induced hemorrhage would vastly improve stroke treatment. Here, we investigated the involvement of 12-lipoxygenase (12-LOX), an enzyme in the arachidonic acid cascade which mediates cell death from oxidative stress, and matrix metalloproteinase (MMP-9), an extracellular protease which specifically degrades matrix surrounding blood vessels and has been associated with hemorrhage.

12-LOX mediates oxidative stress in neuronal cells, possibly by damaging the mitochondria, whose proteins are eventually degraded by the proteasome. Here, we test the hypothesis that a similar pathway exists in human endothelial cells (HBECs), which would contribute to blood-brain barrier breakdown and further compromise blood flow. Inhibition of 12-LOX (baicalein and AA-861) but not of caspases (zVAD-fmk) or the proteasome (epoxomicin) protected HBECs against hydrogen peroxide-induced toxicity. Thus, 12-LOX may play a role in the oxidative toxicity of HBECs.

A relationship between hemorrhage from tPA and cerebral amyloid angiopathy (CAA), a feature of Alzheimer's disease (AD), has been observed. MMP-9 levels are increased both in tPA-induced hemorrhage and in vasculature of CAA mice. Thus MMP-9 inhibition may ameliorate hemorrhage due to tPA or CAA.

Statins are associated with a lower incidence of stroke and AD and decrease MMP-9 levels in macrophages. Here, we test the hypothesis that in astrocytes, which help regulate cerebral blood flow, statins reduce tPA or beta amyloid-induced MMP-9 levels via their known vasculoprotective inhibition of Rho kinase.

Interleukin-1 beta (a cytokine increased in stroke and AD), tPA, and beta amyloid increased MMP-9 production. Pretreatment with either simvastatin or Y-27632, a Rho kinase inhibitor, reduced this response. Therefore, statins may improve neurovascular dysfunction by preventing MMP matrix degradation via Rho kinase inhibition.

WEDNESDAY
ROOM B

D. KATSMAN



S. WANG

9:30 A.M.

Intracerebrally administered anti-amyloid- β antibodies clear amyloid- β deposition in a transgenic murine model of Alzheimer's amyloidosis**LEWIS ZHIYUAN LENG**, University of Pennsylvania School of Medicine

Mentor: Virginia M.-Y. Lee, Ph.D., The John H. Ware III Professor of Alzheimer's Research, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

■ A key characteristic of Alzheimer's disease is the deposition of amyloid- β ($A\beta$) plaques in the brain. Both active and passive immunization have been shown to decrease $A\beta$ deposition and improve cognitive function in transgenic murine models of Alzheimer's disease. However, in clinical trials active immunization caused aseptic meningoencephalitis in a subset of patients. Passive immunotherapy is now being pursued as a safer alternative. In the current study, a novel $A\beta$ -specific monoclonal mouse antibody, NAB61, was injected into the hippocampus of mice doubly heterozygous for a human $A\beta$ precursor protein (APP) gene that harbors the Swedish familial Alzheimer's disease (FAD) mutation and the presenilin-1 FAD knock-in mutation, P246L.

Peripheral administration of NAB61 was previously found to improve cognition but not affect $A\beta$ deposition. Transgenic mice intracerebrally treated with NAB61 showed a reduction in $A\beta$ deposition by immunohistochemistry compared to mice treated with non-specific mouse IgG. This effect was first apparent at 3 days post-injection and more pronounced after 7 days. In a subset of NAB61-treated mice vasocentric lymphocytic infiltrates were observed in regions in close proximity to the injection track. This finding was consistent with a previous case of meningoencephalitis in a mouse peripherally treated with NAB61.

NAB61 administered peripherally is not able to clear $A\beta$ deposits. However, direct inoculation into the hippocampus does lead to rapid clearing of deposits. A significant inflammatory response that was observed in a subset of NAB61-treated mice highlights the need for caution in possible future human trials of passive amyloid immunotherapy.



L.Z. LENG



K.A. HUTCHESON

10:30 A.M.

Gene transfer to tissue engineered blood vessels**KELLEY AMBER HUTCHESON**, Duke University School of Medicine

Mentor: Jeffrey H. Lawson, M.D., Ph.D., Assistant Professor, Departments of Surgery and of Pathology, Duke University Medical Center, Durham, North Carolina

■ **Introduction:** Thrombosis remains the major cause of autologous and tissue engineered vascular graft failure in coronary and peripheral circulations. Adult endothelial cells (EC) appear to have limited potential for lining vascular conduits. To improve the biocompatibility of vascular grafts, we have isolated a new population of porcine endothelial progenitor cells (EPC) from bone marrow. The phenotypes of both EC and EPC were enhanced by genetic over-expression of natural anticoagulant protein to improve the function of bioengineered vascular grafts.

Methods/Results: Human thrombomodulin (hTM) was over-expressed for its known anticoagulant and anti-inflammatory properties. Over-expression of hTM in adult and progenitor EC via several transfection methods resulted in varied levels of increased hTM expression verified by quantitative Western blotting. Lipofectin transfection resulted in 9- and 25-fold increased hTM expression in EPC and HMEC compared to 2-fold increased hTM expression in HUVEC. Adenoviral infection of HMEC and EPC resulted in 100-fold and 65-fold increased hTM expression, respectively. The change in anticoagulant function of these EC as measured by an activation of protein C assay showed adenovirus infected HMEC produced 5 times more functional TM activity than either untransfected or control cells. Finally, vascular cells were seeded onto PTFE and decellularized vascular grafts where HMEC and EPC resulted in almost complete surface coverage where HUVEC showed little engraftment. The synthesis of this work, which includes seeding hTM over-expressing EC onto bioengineered blood vessels to enhance their anticoagulant function, is ongoing.

Conclusions: Anticoagulant genetic manipulation of EC to enhance the biologic compatibility of vascular grafts holds promise to reduce graft failure rates. Transfected cells can be used to line vascular grafts with the EPC subtype, which could be autologously harvested from each patient, providing maximal graft coverage. Studies of long-term TM expression using adeno-associated viral vectors to produce a durable anticoagulant phenotype are under way.

10:45 A.M.

Notch signaling mediates the control of arterial identity by fluid mechanical forces

JOHANNES R. KRATZ, Harvard Medical School

Mentor: Guillermo Garcia-Cardena, Ph.D., Assistant Professor, Department of Pathology Harvard Medical School, Boston, Massachusetts

Recent studies of embryonic vascular development have suggested that genetic factors determine the establishment of arterial and venous identity in the developing cardiovascular system. Clinical experience suggests, however, that vessel identity can also be modulated by epigenetic factors. For example, when a surgeon performs a bypass graft, the venous conduit undergoes anatomical arterialization in response to its new biomechanical environment, suggesting that environmental factors may be important determinants of vessel identity.

Here, we have examined the effect of different hemodynamic environments on arteriovenous identity of cultured human venous endothelial cells using a novel *in vitro* flow device. We demonstrate that venous endothelial cells express arterial identity markers (ephrin-B2, connexin 40, and neuropilin 1) when exposed to a biomechanical stimulus that simulates the shear stress component of arterial circulation. To gain mechanistic insight into this process, we have used cDNA microarray-based screening to unveil the orchestrated upregulation of several members of the Notch pathway (NOTCH1, NOTCH4, DLL4, JAG1, JAG2, HEY1, and HEY2), a signaling axis previously implicated in the acquisition of arterial identity during development. We show that arterial flow can activate this signaling pathway as demonstrated by the localization of the Notch intracellular domain to the nucleus in venous endothelial cells exposed to arterial flow. In addition, using a well-characterized Notch pathway small molecule inhibitor, we demonstrate a decrease in the expression of arterial markers in venous endothelial cells exposed to arterial flow, suggesting that the Notch pathway is a critical mediator of flow-induced arterial marker expression.

Together, these studies demonstrate that hemodynamic forces can act as extrinsic modifiers of endothelial cell identity via the Notch signaling pathway, and may suggest new therapeutic strategies for diseases in which dysregulation of vessel identity may lead to serious pathologies, including vein graft failure and vascular malformations.

11:00 A.M.

The role of CX₃CR1 in the arterial response to injury

SARITA ULHAS PATIL, Duke University School of Medicine

Mentor: Dhavalkumar D. Patel, M.D., Ph.D., Director, Thurston Arthritis Research Center; Professor, Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

CX₃CR1, a receptor for the chemokine fractalkine, is expressed on monocytes, vascular smooth muscle cells (VSMC), and platelets, which are critical in vascular inflammatory pathologies, such as atherosclerosis. This study investigates whether CX₃CR1 is involved in the inflammatory repair process after arterial injury, and the mechanisms by which it may play a role. Femoral arteries of CX₃CR1-deficient (CX₃CR1^{-/-}) and wild type (WT) mice were injured by the passage of an angioplasty guidewire. After 1, 5, 14, and 28 days, mice were euthanized and perfused with 4% paraformaldehyde and their tissues were harvested for immunohistochemistry.

CX₃CR1 deficiency resulted in protection from the inflammatory response to arterial injury following guidewire-induced injury. The incidence of arterial injury was decreased in CX₃CR1^{-/-} mice at 5, 14, and 28 days after injury (38.1% vs. 86.4% for WT, p=0.001). At day 5, the injured arteries of WT mice had a marked monocyte infiltration into the intima compared to CX₃CR1^{-/-} arteries (13.6±11.5 vs. 0.0±0.0, p=0.006). At day 28, the intimal area was decreased in CX₃CR1^{-/-} mice by 62% compared to WT mice (p=0.11), with a concomitant decrease in the numbers of intimal VSMC (87.1%, p=0.055). There was no difference in platelet recruitment to the sites of femoral artery injury in CX₃CR1^{-/-} vs. WT animals at day 1 (22446±4839 vs. 22349±4536, p=0.48).

In conclusion, CX₃CR1 deficiency results in protection from arterial injury as a result of deficient monocyte migration but not platelet adhesion. CX₃CR1 is required for monocyte migration to sites of arterial injury. Further studies will be needed to examine the role of CX₃CR1 on VSMC.



J.R. KRATZ



S.U. PATIL

WEDNESDAY
ROOM B

11:15 A.M.

STRO-1 positive human mesenchymal stem cells delivered in a fibrin scaffold enhance myocardial neovascularization and cardiogenesis after acute ischemia

ALLAN WILEY TULLOCH JR., Columbia University College of Physicians and Surgeons

Mentor: Silviu Itescu, M.D., Associate Professor; Director, Transplant Immunology, Department of Surgery, Columbia University, College of Physicians and Surgeons, New York, New York

■ The monoclonal antibody STRO-1 has been demonstrated to select a population of stromal precursors from adult bone marrow mononuclear cells that is devoid of accessory cells while specifically including detectable fibroblast colony-forming units. These stromal precursors have the ability to differentiate into a number of different tissue types, including fat, bone, and cartilage. However, the ability of these cells to differentiate into other tissues is not well defined. In the current study, I examined the benefits of STRO-1 positive mesenchymal cells injected into rat myocardium 48 hours after ligation of the left anterior descending artery. The aim was not only to look for evidence of cell differentiation, but also to assess the best form of cell delivery.

The results indicate that STRO-1 cells clearly benefited the infarcted heart, with increased arteriogenesis in the peri-infarct region as demonstrated by trichrome staining, and immunostaining with smooth muscle actin. However, perhaps more compelling was the observation that delivery of the same cells in a fibrin scaffold best supported cell survival and growth. STRO-1 cells delivered within the scaffold appeared to differentiate into endothelial and smooth muscle cells, forming large arterioles clearly perfused with red blood cells. The infarcts appeared smaller compared to controls, and there were distinct islands and fingers of viable myocardium that appeared to surround these arterioles. They could be found not only in the peri-infarct region, but also scattered throughout the region of the infarct. Given the size and rapidity with which these arterioles formed, they were most likely of human origin.

This report demonstrates an easily selected and expandable population of stromal precursors that can be used to induce myocardial neovascularization and cardiogenesis after acute cardiac ischemia. Furthermore, when delivered in a commercially available fibrin scaffold, this effect is enhanced, which could have important therapeutic ramifications and clinical applications.



A.W.TULLOCH JR.



I. NIKOLIC

11:30

Cardioprotective effects of selective β estrogen receptor agonist

IVANA NIKOLIC, Duke University School of Medicine

Mentors: Charles Steenberg, M.D., Ph.D., Associate Professor, Department of Pathology, Duke University, Durham, North Carolina, and Elizabeth Murphy, Ph.D., Principal Investigator, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

■ Under hypercontractile conditions induced by isoproterenol, hearts isolated from male mice and ovariectomized female mice show enhanced ischemia/reperfusion injury compared to hearts isolated from intact female mice. Studies of transgenic animals, namely β estrogen receptor (ER) and α ER knock out mice, suggest that the estrogen-mediated cardioprotective effects are β ER dependent. The goal of this study was to investigate the cardioprotective potential of the selective β ER agonist. β ER agonist, 17- β estradiol, or vehicle was delivered to ovariectomized female mice. Following a two-week dosing period, the hearts were isolated and reperfused using Langendorff reperfusion system. Just before inducing ischemia, the hearts were exposed to one minute of hypercontractile conditions using 10 nM isoproterenol. Following a 20-minute period of global no flow ischemia, the hearts were reperfused for 40 minutes. A latex balloon inserted into the left ventricle was used to record left ventricular developed pressure (LVDP), maximum/minimum rate of contraction, and heart rate. Recovery of contractile function at the end of the 40-minute reperfusion period was expressed as the percentage of the preischemic rate pressure product (product of the LVDP and heart rate).

The data show that under hypercontractile conditions, the ovariectomized female mice treated with the selective β ER agonist had significantly more functional recovery as measured by the rate pressure product ($18.4 \pm 2.8\%$) compared to the vehicle-treated ovariectomized females ($10.25 \pm 1.3\%$, $p = 0.048$). This finding suggests that selective β ER agonist has a cardioprotective role in ischemia reperfusion injury, opening up possibilities for novel pharmacological interventions in cardiovascular disease.

11:45 A.M.

Characterization of Z-band alternatively spliced PDZ-motif protein in development of ventricular dysfunction

WILLIAM BUCK KYLE, Cornell University John and Sanford I. Weill Medical College and Graduate School of Medical Sciences

Mentors: Jeffrey A. Towbin, M.D., Professor and Chief, and Matteo Vatta, Ph.D., Assistant Professor, Department of Pediatrics (Cardiology), Baylor College of Medicine, Houston, Texas

■ Z-band alternatively spliced PDZ-motif protein (ZASP), as its name implies, is a novel PDZ motif-containing protein that is expressed in the Z-line of cardiac and skeletal muscle. Six polypeptides are known to be generated by ZASP alternative splicing in humans. Both exon arrangement and expression pattern of human ZASP isoforms differ dramatically from Cypher, the ZASP murine homolog. Mutations in ZASP have been found in patients with dilated cardiomyopathy (DCM) and/or left ventricular noncompaction (LVNC). The aim of this project is to characterize ZASP isoform expression in cardiac and skeletal muscle. Reverse transcriptase-polymerase chain reaction (RT-PCR) identified two novel isoforms. In order to determine the role of these splice variants, I cloned them into a mammalian expression vector as a fusion protein with green fluorescent protein (GFP). I used cultured murine HL-1 cardiomyocytes and C2C12 skeletal myoblasts to study both wild-type (WT) and mutant ZASP. We used immunofluorescence to determine cellular localization, Western blotting to evaluate protein expression levels, and coimmunoprecipitation to identify protein binding partners. I hypothesized that mutations in ZASP will alter protein-protein interactions and weaken the structural integrity of the cell, thereby leading to impaired contractile force transmission and ventricular dysfunction in the heart. Since hypertrophy occurs as a compensatory mechanism subsequent to striated muscle stress, we will investigate the effect of both WT and mutant ZASP overexpression on cellular metabolism and hypertrophy. I will analyze cell lysates from ZASP-transfected cells for hypertrophic markers, which would suggest that such pathway has been activated. These studies will serve as the first comprehensive investigation of human ZASP isoform expression in cardiac and skeletal muscle. Further, studies of the WT and mutant ZASP could lead to a better understanding of the mechanisms leading to DCM and/or LVNC in patients.

Noon

Amyloid toxicity in heart failure: characterization of the pathology associated with the R120G missense mutation in α B-crystallin

CHET RIDALL VILLA, University of Cincinnati College of Medicine

Mentor: Jeffrey Robbins, Ph.D., Professor, Department of Pediatrics; Chair, Department of Molecular Cardiovascular Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

■ An R120G missense mutation in the small heat shock protein α B-crystallin causes cataractogenesis and desmin-related cardiomyopathy (DRM). The R120G mutation has been shown to affect both the chaperone and anti-apoptotic functions of α B-crystallin. *In vitro* experiments in cardiomyocytes using adenovirus containing α B-crystallin^{R120G} recapitulate the characteristics of amyloid-based pathologies found in neurodegenerative diseases like Parkinson's and Alzheimer's. Oligomeric amyloid intermediates, which have been hypothesized to represent the toxic species in amyloidoses, have also been found in a variety of cardiomyopathies. Using cardiac-specific expression of α B-crystallin^{R120G}, we show that in addition to gross mitochondria defects, decreases in the activity of respiratory complex I have compromised mitochondrial function and these deficits correlate with decreases in heart function. Initial characterization of α B-crystallin^{R120G} mice has also revealed abnormal serine phosphorylation patterns of α B-crystallin in addition to mitochondrial abnormalities. Phosphorylation has been shown to affect the localization, chaperone, and anti-apoptotic function of α B-crystallin *in vitro*. To investigate the role of phosphorylation *in vivo*, site-directed mutagenesis has been used to create α B-crystallin constructs in which constitutive phosphorylation is mimicked by mutating serine to aspartate. These proteins will be expressed using a cardiac-specific, inducible promoter so that effects of phosphorylation on α B-crystallin function may be described for a number of processes. Finally, we describe the effects of amyloid load on cell toxicity in cell culture, animal and human LVAD tissue samples. We show that amyloid oligomer load correlates with cell toxicity, while aggregate size is inversely proportional to cell toxicity. This agrees with data from other studies suggesting that soluble, oligomeric amyloid intermediates are responsible for cell injury in amyloidoses.



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