Official Program

VANDERBILT
School of Medicine

EMORY
UNIVERSITY
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MEDICINE

APSA
American Physician Scientists Association

SEMSS 2012

Southeastern Medical Science Symposium

November 10th – 11th Nashville, TN

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Welcome to SEMSS 2012!

Welcome to the third annual Southeastern Medical Scientist Symposium, hosted by the Vanderbilt University, University of Alabama at Birmingham, and Emory University Medical Scientist Training Programs in partnership with the American Physician Scientist Association. The SEMSS brings MD/PhD students together from schools around the southeastern United States to encourage a collaborative and interdisciplinary educational environment within the region, while fostering the continuation of institutional ties.

The theme of this year's conference is *personalized medicine*. More and more, physicians and scientists are discovering the necessity of tailoring treatments to individuals. For even within the bounds of a single diagnosis the response of a disease to our efforts to cure it depends largely on the DNA of the patients themselves. As music functions as an expression of the soul, our DNA serves as an expression of the cell itself; the future of medical science lies not only in understanding that expression, but also integrating it into the myriad of specialties and treatment protocols in the most effective way possible. Breakout sessions, keynote speakers, and student presentations have been specially selected to explore the progress and promise that personalized medicine holds, learn about ongoing research in participating institutions, and provide opportunities to meet and reconnect with future colleagues. On behalf of the student organizers, thank you for your support and WELCOME TO SEMSS 2012!

Vanderbilt
Scott McCall (Exec)
Matthew Surdel (Exec)
Merla Hubler (Programming)
Nathaniel Bloodworth (PR)

UAB
Jennifer Hadley (Exec)
Zach Dobbin (Programming)
Mika Guzman Karlsson (PR)
Travis Hull (APSA Rep)
Jarrod Meadows (Committee)
Elizabeth Ma (Committee)

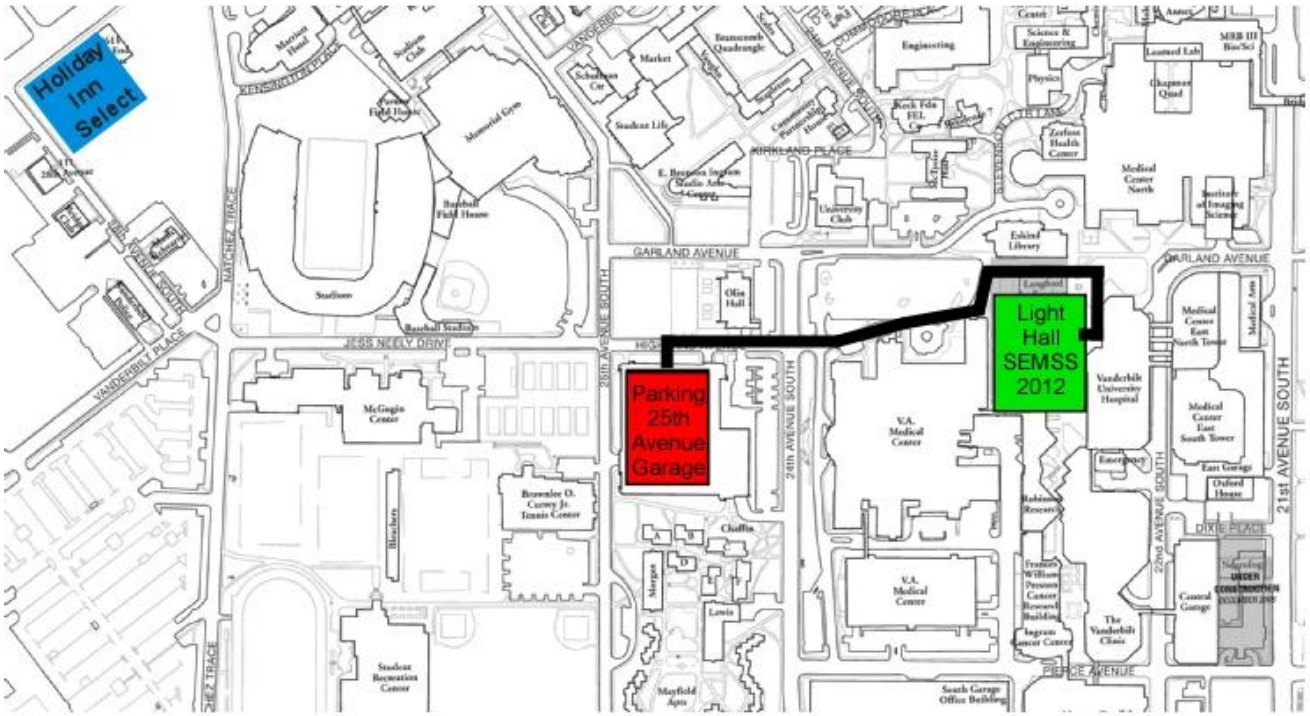
Emory
Ana Monteiro (Exec)
Matt Kudelka (Programming)
Neal Laxpati (Treasurer)
Lizz Iffrig (Treasurer
Dwight Chambers (PR)

Directions for SEMSS 2012

Directions to the Holiday Inn: From I-40, take Exit 209 (Broadway). Go southwest on Broadway, take a slight right onto West End Avenue. Travel about 1 mile and the Holiday Inn will be on your left.

Directions to Parking: From the Holiday Inn take 28th Avenue toward Vanderbilt Campus. It will change to Jess Neely Drive. The 25th Avenue Garage will be on your right at teh corner of 25th and Jess Neely Drive. Please park on the roof.

Directions from Parking to Light Hall: After exiting the parking ramp on foot, please proceed toward the VA Medical Center via Highland Avenue. Walk around the north side of the VA and you will see Light Hall, the location of the SEMSS 2012



Holiday Inn at Vanderbilt
2613 West End Avenue
Nashville, TN 37203
(615) 327-4707

25th Avenue Garage
1400 25th Avenue South
Nashville, TN 37203

Light Hall, Vanderbilt Medical Center
2215 Garland Avenue
Nashville, TN 37203

SEMSS 2012

Southeastern Medical Science Symposium
November 10th-11th,
Vanderbilt University Medical Center

Saturday, November 10th

Event	Time	Location
Registration	12:00pm-1:00pm	North Lobby Light Hall
Intro Remarks	1:00pm-1:30pm	
Keynote 1 Dr. Kenneth Brigham	1:30pm-2:30pm	Light Hall 208
Breakout Session 1	2:45pm-3:45pm	4th floor rooms Light Hall
Breakout Session 2	4:00pm-5:00pm	
Poster Session 1	5:15pm-6:30pm	North Lobby Light Hall
Dinner Served	6:15pm-7:00pm	Langford Lobby
Keynote 2 Dr. Mia Levy	7:15pm-8:30pm	Light Hall 208

Sunday, November 11th

Event	Time	Location
Breakfast	7:45am-8:00am	Outside Light Hall 208
Keynote 3 Dr. Chris Willey	8:00am-9:00am	Light Hall 208
Breakout Session 3	9:15am-10:15am	4th floor rooms Light Hall
Poster Session 2	10:15am-11:15am	North Lobby Light Hall
Lunch and Student Speaker sessions	11:30am -12:30pm	4th floor rooms Light Hall

SEMSS 2012
Southeastern Medical Science Symposium

Breakout Schedule

Saturday, November 10th

	Room 407	Room 411	Room 415	Room 419
Breakout Session 1 **2:45pm - 3:45pm**	Substance and Curb Appeal: Research/Medical Experience before Matriculation Student Panel	Why we do what we do: Specific examples of an elusive goal. Gregory Barnes, MD PhD Dan Roden, MD	Grant Writing Success for the Physician-Scientist in Training Caroline Lai Robin Lorenz, MD PhD Kim Petrie, PhD	Technology Advancing Medicine Josh Denny, MD Pietro Valdastri, PhD
Breakout Session 2 **4:00pm - 5:00pm**	Director Panel for MSTP Admissions Emory, UAB, and Vanderbilt Directors	Communicating research with public / journalism Melissa McPheeters, PhD, MPH David Salisbury	So you want to be a physician and a scientist: Uncommon specialty choices Tyler Barrett, MD Brian Donahue, MD PhD	Intellectual Property+ Academia + Industry= Technology Transfer: Making ideas reality Hassan Naqvi, PhD

Sunday, November 11th

Breakout Session 3 **9:15am - 10:15am**		What is the deal with all the –omics? David Tabb, PhD Chris Willey, MD PhD	I Am Legend: Welcome to the age of personalized viral therapies Kevin Cassady, MD Justin Roth, PhD	History of Personalized Medicine Arleen Tuchman, PhD
Student Speaker Session **11:30am - 12:30pm**	Lunch Pick-Up	Kira Newman-Emory Melissa Musser- Vanderbilt Jacquelyn Zimmerman- UAB	Kristie Aamodt- Vanderbilt Catherine H. Poholek- UAB Vineet Tiruvadi- Emory	Brian Warmus- UAB Quaovi Sodji- Georgia Health Sciences University Frances Cheng – Vanderbilt

Keynote Speakers

Mia A. Levy, MD, PhD

Assistant Professor of Biomedical Informatics

Assistant Professor of Medicine

Cancer Clinical Informatics Officer, Vanderbilt Ingram Cancer Center

Dr. Mia A. Levy is the Director of Cancer Clinical Informatics for the Vanderbilt-Ingram Cancer Center and an Assistant Professor of Biomedical Informatics and Medicine. Dr. Levy's research interests include biomedical informatics methods to support the continuum of cancer care and cancer research. She is the informatics lead for the Vanderbilt Personalized Cancer Medicine Initiative (PCMI) working to integrate tumor genetics biomarkers into the electronic health record in computable form and provide decision support for standard of care and clinical trial eligibility based on those predictive biomarkers.

Kenneth L. Brigham, MD

Professor Emeritus

Former Associate Vice President and Director of the Predictive Health Institute

As director of the Emory/Georgia Tech Predictive Health Institute and its Center for Health Discovery and Well Being, Dr. Brigham is guiding an institution wide effort to focus a spectrum of essential disciplines on human health with the goal of creating and validating a new paradigm of health and healing for the 21st century. Dr. Brigham is a professor of medicine in the Emory University School of Medicine and director of the Center for Translational Research of the Lung. He is internationally recognized for his pioneering work in the pathogenesis of lung injury, gene therapy technology, and the application of gene therapy to a spectrum of inherited and acquired lung disorders. He received his medical degree from Vanderbilt University and trained in internal medicine at Johns Hopkins University and in pulmonary research at the University of California at San Francisco. He was on the faculty of Vanderbilt University from 1973 until 2002, when he joined Emory University School of Medicine.

Keynote Speakers

Chris Willey, MD

Assistant Professor of Radiation Oncology

Dr. Willey is an Assistant Professor in the department of Radiation Oncology at the University of Alabama, Birmingham. His research is focused on the identification of new molecular targets to make radiation treatment for cancer more effective, reducing radiation-induced damaged. Dr. Willey completed his medical training and PhD at the Medical University of South Carolina, and a residency in Radiation Oncology at Vanderbilt University.

Breakout Speakers

David Roberston, MD Vanderbilt University

Professor of Medicine
Professor of Neurology
Elton Yates Professorship in Autonomic Disorders
Professor of Pharmacology

Dr. David Robertson received his M.D. from Vanderbilt and medicine residency training at Hopkins. As Elton Yates professor of Medicine, Pharmacology and Neurology, he has ongoing interest in identifying the mechanisms of neural regulation of heart rate and blood pressure in normal individuals, as well as in patients with autonomic cardiovascular, psychiatric or neurological disorders.

Gregory Barnes, MD, PhD Vanderbilt University

Assistant Professor of Neurology

Dr. Barnes earned his medical degree and Ph.D. from the University of Kentucky , and received his Pediatrics training at Washington University/St. Louis Children's Hospital. His major clinical interests are medical and surgical approaches to pediatric epilepsy, neonatal neurology, and neurogenetics.

Pietro Valdastri, PhD Vanderbilt University

Assistant Professor of Mechanical Engineering

Professor Valdastri completed his degree in Bioengineering at Scuola Superiore Sant'Anna. His research interests are focused on designing and creating mechatronic and self-contained devices to be used inside specific districts of the human body to detect and cure diseases in a non-invasive way.

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Breakout Speakers

Dan Roden, MD Vanderbilt University

Assistant Vice Chancellor for Personalized Medicine
Professor of Medicine
William Stokes Chair in Experimental Therapeutics
Professor of Pharmacology

Dan Roden was born and raised in Montreal, and received his medical degree and training in Internal Medicine from McGill University. He then went to Vanderbilt where he trained in Clinical Pharmacology and Cardiology, and has been a faculty member here since. His initial career focus – that he has maintained – was studies of the clinical, genetic, cellular, and molecular basis of arrhythmia susceptibility and variability responses to arrhythmia therapies, and he is widely-recognized for his expertise in drug-induced arrhythmias. Over the last 10 years, he has led Vanderbilt's broader efforts in pharmacogenomics discovery and implementation. Dr. Roden is Principal Investigator for the Vanderbilt sites of the NIH's Pharmacogenomics Research Network and the Electronic Medical Records and Genomics Network. He directs the Vanderbilt DNA databank BioVU, and is one of the key leaders overseeing the PREDICT project that is preemptively embedding pharmacogenomic variant data into the Vanderbilt EMR.

Kim Petrie, PhD Vanderbilt University

BRET Director of Career Development
Office of Biomedical Research Education and Training VUMC

Dr. Petrie completed her graduate training at Vanderbilt University investigating the involvement of central dopamine systems in neuropsychiatric disorders. She is currently Director of Career Development in the Biomedical Research Education and Training office at the Vanderbilt University Medical Center.

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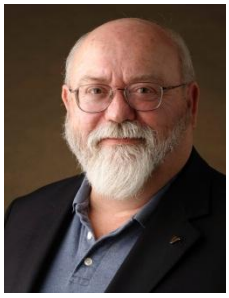
Breakout Speakers



David Salisbury Vanderbilt University

Vanderbilt News, Senior Research Writer

David Salisbury is responsible for promoting the work of scientists within the College of Arts and Science and the School of Engineering. He is also one of the primary authors of the Research News @ Vanderbilt blog. He received a bachelor's degree in physics from the University of Washington and learned how to write about science for the general public while working for *The Christian Science Monitor*. He has received a number of awards for his reporting, including the National Association of Science Writer's Science in Society award. After leaving the *Monitor*, he wrote about scientific research at the University of California, Santa Barbara and Stanford University before moving to Vanderbilt. He is the author of a book on using calculators for personal finance and has written freelance articles for a wide range of publications, including *MIT Technology Review*, *Popular Science*, *New York Times*, and *National Wildlife*.

Brian Donahue, MD, PhD Vanderbilt University

Associate Professor
Division of Pediatric Cardiac Anesthesiology

Dr. Donahue graduated from the University of Dayton and went on to receive his MD and PhD degrees from the Emory University School of Medicine. He then did a residency in Anesthesiology at Mayo. His research focuses on the genetic variability of the coagulation system and its impact on clinical outcomes as well and the role of Tissue factor during cardiopulmonary bypass. He is actively involved in teaching and mentoring medical students at Vanderbilt in both the lab and the classroom.

Breakout Speakers

Tyler Barrett, MD Vanderbilt University
Assistant Professor of Emergency Medicine

Dr. Barrett attended Vanderbilt University School of Medicine and completed a transitional year internship at Harbor-UCLA Medical Center followed by residency in Emergency Medicine at UCLA/Olive View-UCLA. He completed a Masters of Science in Clinical Investigation in 2010. He studies the risk stratification of ED patients with atrial fibrillation. He has numerous other research and practice interests including the personalized treatment of atrial fibrillation, pharmacogenomics, trauma resuscitation, measuring efficacy of common ED therapies, and the investigation of clinical decision instruments in the emergency department. Dr. Barrett was awarded the 2010-2011 Young Investigator Award by the Emergency Medicine Foundation. Dr. Barrett also serves as editor for the *Annals of Emergency Medicine* Journal Club section.

Melissa McPheeters, PhD, MPH Vanderbilt University
Research Associate Professor
Institute for Medicine and Public Health
Director, Vanderbilt Evidence-based Practice Center

Dr. McPheeters is a health services researcher whose work focuses on evidence-based medicine and comparative effectiveness. She is the director of Vanderbilt's Evidence-based Practice Center, where she leads a team of systematic reviewers and health services researchers in conducing comparative effectiveness research on a range of topics.

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Breakout Speakers

Terence S. Dermody, M.D., Vanderbilt University School of Medicine

Dorothy Overall Wells Professor of Pediatrics
Professor of Pathology, Microbiology, and Immunology
Director, Medical Scientist Training Program
Director, Division of Pediatric Infectious Diseases

Professor Dermody earned his M.D. degree at Columbia University and completed his residency at Presbyterian Hospital in New York and infectious diseases training at the Brigham and Womens Hospital in Boston. He completed a research fellowship in Microbiology and Molecular Genetics at Harvard and joined the faculty at Vanderbilt in 1990. His research focuses on viral pathogenesis and vaccine development.

Robin G. Lorenz, M.D., Ph.D. University of Alabama at Birmingham

Professor of Pathology
Director, UAB Medical Scientist Training Program

Dr. Lorenz received her B.S. in Biological Sciences from Stanford University. She attended Washington University School of Medicine as a Medical Scientist Training Program Fellow and received her Ph.D. in Immunology and M.D. in 1990. From 1990 to 1994 she was a resident in Laboratory Medicine (Clinical Pathology) at Barnes Hospital. From 1994-2002 she was an Assistant Professor in the Departments of Pathology and Medicine at Washington University. Dr. Lorenz joined the University of Alabama at Birmingham faculty in 2002 in the Departments of Pathology and Microbiology. The National Institutes of Health the Juvenile Diabetes Foundation, and the Crohn's and Colitis Foundation fund her laboratory research investigating the mucosal immune system. She is currently Program Director of the SIBS Undergraduate Research Program and the Short Term Training Program for Medical Students, is the Associate Director of the Mucosal HIV and Immunobiology Center, and is the Director of the UAB MSTP.

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Breakout Speakers

David Tabb, Ph.D. Vanderbilt University
Associate Professor of Biomedical Informatics
Associate Professor of Biochemistry

Dr. Tabb completed his Ph.D. at the University of Washington's Molecular Biotechnology department in 2003, focusing on proteome informatics. His lab creates tools to produce information from biological mass spectrometry data, with an emphasis on protein identification and post-translational modifications. His team has contributed algorithms for three complementary technologies in peptide identification: spectral library matching, sequence tagging, and conventional sequence database searching.

Justin Roth, PhD University of Albama at Birmingham
Instructor-Infectious Disease

Dr. Roth, an early stage investigator, is a new faculty member in the Department of Pediatrics, Division of Infectious Disease. Dr. Roth received his doctorate in the Molecular Virology Program at Case Western Reserve University, where he worked under Dr. Stanton Gerson, utilizing lentiviral vectors to deliver drug resistance genes into hematopoietic stem cells for novel *in vivo* selection strategies. Dr. Roth carried out his postdoctoral training in the lab of Dr. David Curiel. As a postdoctoral fellow, he worked to develop targeting strategies for cell- and virus-based delivery of therapeutics. In this regard, he developed a myeloid cell-binding adenovirus that, to date, has achieved the most robust lung-targeted gene transfer reported in mouse models. Dr. Roth has extensive experience with lentiviral and adenoviral vectors, and he is currently applying these skills towards oncolytic herpes virus (oHSV) development. Specifically, he is working to develop oHSV that express cytokines, engineered to be tumor-specific, for enhanced efficacy of virotherapeutic approaches. Dr. Roth also runs the Vector Production Core, aimed at producing high titer and certified oncolytic HSV stocks for assessment in glioma models

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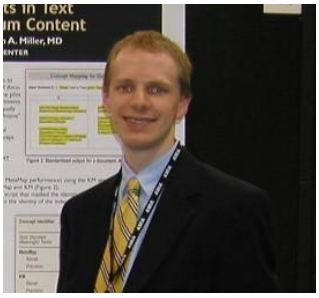
Breakout Speakers



Joshua C Denny, M.D., M.S.

Assistant Professor of Biomedical Informatics
Assistant Professor of Medicine

Josh is currently an assistant professor in the Department of Biomedical Informatics and Medicine. He completed an internal medicine residency as a Tinsley Harrison Scholar at Vanderbilt. His interests in medical informatics began while in medical school with the development of a concept-based curriculum database to improve medical education. Other interests include natural language processing, clinical phenomics to support genome-phenome correlation, just-in-time information delivery, and terminology development. Dr. Denny serves on several local committees and remains active in teaching medical students and clinical roles.

Kevin Cassady, MD University of Albama at Birmingham

Assistant Professor of Pediatrics – Infectious Disease

Dr. Cassady completed his medical degree at the University of Albama at Birmingham and currently works in the area of pediatric infectious disease at UAB. He was involved in the development of a chimeric oncolytic virus encoding foreign genes for anti-tumor therapy.

Arleen Tuchman, PhD Vanderbilt University

Professor of History

Professor Tuchman earned her degree in history of science at the University of Wisconsin-Madison. She is a specialist in the history of science and medicine in the United States and Europe. Her research interests include the cultural history of health and disease, the rise of scientific medicine, and scientific and medical constructions of gender and sexuality.

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Breakout Sessions

Session 1 2:45pm – 3:45pm Light Hall 4th Floor

Why we do what we do: Specific examples of an elusive goal

Gregory Barnes, MD, PhD

Dan Roden, MD

So you are a doctor and scientist, so what? How are these dual degrees used together in real life? This session will demonstrate how research ideas went from a clinical observation to a research question and solution. The intention is to explore direct applications of a dual degree.

Technology Advancing Medicine

Pietro Valdastri, PhD

Josh Denny, MD, MS

From electronic record keeping to Watson, a computer that can diagnose disease, technologies impact medicine in many exciting ways. This breakout session will describe where medical technology is heading and how it will impact the medical field.

Grant Writing Success for the Physician-Scientist in Training

Caroline Lai

Robin Lorenz, MD, PhD

Kim Petrie, PhD

Grant writing is one of the most challenging and important skills to have as a scientist, yet it is often overlooked or underemphasized in graduate pedagogy. In this session, a panelist of NIH grant reviewers, PIs, and students will discuss everything MD/PhD students need to know to successfully apply for grants.

Substance and Curb Appeal: Research/Medical Experience before Matriculation (for Undergraduates)

Student Panel

One of the most daunting processes for undergraduates is identifying and navigating medical and research opportunities. In this session, current MD/PhD students will give an overview of available opportunities, provide advice on pursuing these opportunities, and comment on what types of opportunities best prepare your mind and your résumé for the medical field.

Breakout Sessions

Session 2 **4:00pm – 5:00pm** **Light Hall 4th Floor**

Intellectual Property + Academia + Industry = Technology Transfer: Making Ideas Reality

Hassan Naqvi, PhD

You made a discovery. Congratulations! But what do you do now? The process turning a discovery into a viable patient treatment is a long path. The patent process, the interface of intellectual property with academia, and ultimately generating a business can be a daunting process that most physician-scientists never learn about. This session aims to explore this process as a whole, as well as the part you can play in making these discoveries into reality.

Communicating Research with the Public

Melissa McPheeters, PHD, MPH

David Salisbury

This session will focus on information dissemination to the public. Specifically, it will help students explain their research succinctly and help them to become effective communicators as future P.I.s.

So you want to be a Physician and a Scientist: Uncommon Specialty Choices

Tyler Barrett, MD

Brian Donahue, MD, PhD

So you are training to be a physician-scientist? But what if your clinic interest fall in specialities that are not deemed "MD/PhD Friendly"? This session will feature a panel of physician-scientists who have taken the road less travelled into specialties that are not the expected choice for MD/PhDs. The panel will discuss how they fared in residency interviews, fellowship selection and what their PhD brought to the table. Also, they share why they entered their field of choice and how they are balancing their career.

Breakout Sessions

Session 2 **4:00pm – 5:00pm** **Light Hall 4th Floor**

Director Panel for MSTP Admissions (for Undergraduates)

Terrence Dermody, MD

Robin Lorenz, MD, PhD

Mary Horten, MPH, MA

This session offers undergraduates interested in MD/PhD programs the opportunity to learn about MSTPs at Emory, UAB, and Vanderbilt as well as ask questions on admissions, research, medicine, potential career paths as a physician-scientist, and much, much more. Also, students will have the chance to meet MD/PhD directors from all three institutions.

Breakout Sessions

Session 3 9:15am – 10:15am Light Hall 4th Floor

History of Personalized Medicine

Arleen Tuchman, PhD

Personalized medicine is revolutionizing patient care in many exciting ways. This breakout session will explore the history of personalized medicine – where it came from and how it evolved to become what it is today.

What is the Deal with all the –omics?

Chris Willey, MD, PhD

David Tabb, PhD

Description: You hear it in every paper. You see it on every seminar announcement. But seriously, what is the deal with the new "omics" in research? Have you wondered how kinomics and proteomics differ from genomics? Frequently it is discussed how one "omic" or another will provide the best approach to personalized therapy. In this session you will hear 2 experts fighting for their "omic" to be supreme.

I Am Legend: Welcome to the Age of Personalized Viral Therapies

Dr. Kevin Cassady MD

Dr. Justin Roth, PhD

Have you wondered what you would do if you were alone in New York City just like Will Smith? Do you fear the zombie apocalypse will be born from a viral based therapy? If you answered yes to any of the above questions, come to this session to have your fears put to rest. This panel will discuss the science behind viral based therapies, their utility in personalized medicine and what ethical concerns will need to be addressed when dealing with personalized viral therapies.

Oral Presentations

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Oral Presentations

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NOROVIRUS TRANSMISSION DURING PRODUCE HARVEST AND PACKING: DEVELOPING QUANTITATIVE MICROBIAL RISK ASSESSMENT MODELS

Kira L. S. Newman BA, Justin Remais PhD MS, Juan Leon PhD MPH
Emory University Rollins School of Public Health, Laney Graduate School of Arts and Sciences, and Emory School of Medicine.

Purpose: Noroviruses (NoV) are a major cause of acute gastrointestinal illness worldwide. NoV are highly infectious and can withstand chemical and physical stressors including freezing, drying, low Ph, and commercial disinfectants. Yearly, NoV is associated with 40% of all produce outbreaks. Quantitative microbial risk assessment (QMRA) is a form of probabilistic modeling that simulates the transmission of microbes in the environment to estimate exposure levels. QMRAs elucidate the effect of specific practices on exposure and provide an opportunity for testing interventions before widespread implementation.
Methods: We developed a QMRA model of NoV transmission in farms and packing sheds using a four stage model beginning with simple deterministic modeling in Microsoft Excel and culminating in a complex stoichiometric model with agent and temporal components as well as multiple exposure channels constructed in Oracle Crystal Ball. To enhance the model's data quality, we conducted transfer experiments in the laboratory to estimate NoV transmission to a variety of surfaces and products under a range of conditions.
Results: Based on the deterministic model, the NoV excretion level was the greatest determinant of contamination. Modifiable factors with significant impact included hand washing and reducing the number of times items are handled Packer hand washing reduced contamination by 6.3% when the NoV excretion level. Harvester hand washing led to a 2.5% reduction on ultimate contamination level. When the NoV excretion level was high and produce was handled more than once during packing, the additional handling resulted in an increase of 26 NoV particles/cm^2, nearly twice the ID50.
Discussion/Conclusion: QMRA modeling for NoV highlights modifiable factors that contribute to NoV transmission and allows for prioritization by effect size. The model supports agricultural practice guidelines that concentrate on hand washing and field toilet use and suggests that mechanization may reduce the risk of NoV contamination.

ALTERED ENTERIC NEURAL CREST CELL DIFFERENTIATION IN THE POSTNATAL GANGLIONATED BOWEL OF *Sox10$^{Dom/+}$* HIRSCHSPRUNG MOUSE MUTANTS

Melissa A. Musser[1], Jennifer C. Corpenning[2], E. Michelle Southard-Smith[2,3]
[1]PhD Program in Human Genetics, Center for Human Genetics Research, [2]Department of Genetic Medicine, [3]Department of Cell & Developmental Biology, Vanderbilt University, Nashville, TN

Purpose: Hirschsprung disease (HSCR) is a congenital disorder that is clinically defined as an absence of ganglia in a variable length of distal intestine. In humans, mutations in*SOX10*, *GDNF*, *RET*, *EDNRB*, and *EDN3* have been associated with this disease. The first line of treatment for patients with HSCR is surgical removal of the aganglionic portion of the bowel followed by reanastomosis of ganglionated bowel to the anus. Unfortunately, many patients suffer from post-surgical complications that do not appear iatrogenic, such as enterocolitis and chronic constipation. These observations in patients suggest that HSCR may alter the enteric nervous system (ENS) in the ganglionated, proximal bowel. *In vitro* and *in vivo* studies in the*Sox10$^{Dom/+}$* HSCR mouse model also suggest that neural crest cells (NCC) in this HSCR mutant undergo inappropriate differentiation. We therefore hypothesize that aberrant lineage segregation and differentiation of NCC in the ganglionated intestine contribute to the chronic disease suffered by HSCR patients.
Methods/Results: To test our hypothesis, we have begun fate-mapping NC-derived lineages in postnatal (P17-P20) *Sox10$^{Dom/+}$* mutants. Our studies have identified an ENS cell population that does not express typical neuronal or glial markers and that appears more prevalent in *Sox10$^{Dom/+}$* mutants compared to wild type littermates. Additionally, aberrant expression of glial cell markers and abnormal ENS cell morphology suggest deficits in glial cell differentiation.
Discussion/Conclusion: Efforts are ongoing to evaluate percentages of neuronal subtypes within*Sox10$^{Dom/+}$* enteric ganglia in the proximal intestine. Disruption of neural crest derived lineages in *Sox10$^{Dom/+}$* mutants could readily explain altered gut motility and enterocolitis in HSCR patients.

CANCER CELL PROLIFERATION IS INHIBITED BY SPECIFIC MODULATION FREQUENCIES

Jacquelyn W. Zimmerman[1], Michael J. Pennison[1], Ivan Brezovich[1], Nengjun Yi[1], Celeste T. Yang[1], Ryne Ramaker[1], Devin Absher[2], Richard M. Myers[2], Niels Kuster[3], Frederico P. Costa[4], Alexandre Barbault[5], Boris Pasche[1]

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Purpose: Hepatocellular carcinoma (HCC) incidence in the US is dramatically increasing. Five-year survival remains 3-5%, demonstrating urgent need for additional therapies. Intrabuccal administration of amplitude modulated electromagnetic fields (RF EMF) is a novel, minimally invasive treatment modality. Clinical evidence demonstrates this treatment approach elicits therapeutic responses in cancer patients. *In vitro* we described a phenotype in HCC cells following RF EMF exposure that included proliferative inhibition, modulation of gene expression, and disruption of the mitotic spindle. This phenotype was specific for HCC cells exposed to HCC-specific RF EMF. We hypothesize modulation frequencies affect intracellular calcium release in cancer cells, resulting in our *in vitro* phenotype and *in vivo* efficacy.

Methods: HCC cells were exposed to radiofrequency electromagnetic fields modulated at specific frequencies previously identified in HCC patients. MicroRNA arrays compared exposed and control groups of HCC cells, with validation followed by Western blot. NOD SCID mice received HCC subcutaneous cellular xenografts. Following palpable tumor establishment, mice were exposed to HCC-specific RF EMF, euthanized following excessive tumor burden, and evaluated by immunohistochemistry.

Results: We identified increased levels of miRNAs that target mRNAs used to synthesize proteins important in the PI3K pathway, specifically IP3/DAG signaling and intracellular calcium release. This pathway is frequently disrupted in HCC, making it an excellent candidate for modulation by RF EMF; furthermore, downstream effects include: cell cycle progression, proliferation, inhibition of apoptosis, and cell migration. *In vivo,* normal tissue architecture was preserved, and xenograft tumors were infiltrated with fibrous tissue and also showed decreased proliferation and increased apoptosis. Xenograft tumors in RE EMF treated mice also showed significantly decreased growth rate as compared to controls.

Conclusion: These findings uncover a novel mechanism that controls cancer cell growth at specific modulation frequencies, possibly through modulation of PI3K signaling and downstream release of intracellular calcium.

BONE MARROW-DERIVED HEMATOPOIETIC CELLS ARE RECRUITED TO PANCREATIC ISLETS MODULATED BY VASCULAR ENDOTHELIAL GROWTH FACTOR-A (VEGF-A) SIGNALING AND PROMOTE B CELL REGENERATION

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Pancreatic β cell mass is significantly reduced in diabetes. Because β cell regeneration is limited, new strategies are needed to promote this process. Transiently increasing VEGF-A production in β cells using a doxycycline (Dox)-inducible mouse model (βVEGF-A) increased intra-islet endothelial cell (EC) number and led to β cell loss. Surprisingly, β cell proliferation and islet regeneration follow Dox withdrawal (WD) and require the VEGF-A-modulated islet microenvironment, and not the pancreatic site or circulating factors.

To determine if BMCs are recruited to βVEGF-A islets, we transplanted βVEGF-A mice with GFP^+ bone marrow (BM) then induced VEGF-A overexpression with Dox. GFP^+ BMCs infiltrated islets after 1 wk Dox and remained high 2 wks after Dox WD, when β cell proliferation is at a maximum. Nearly all of these recruited BMCs (RBMCs) expressed the hematopoietic marker CD45 and macrophage marker F4/80 by IHC, and qPCR analysis of βVEGF-A islets isolated after 1 wk Dox showed increased expression of unique M2 macrophage markers and M2 polarization factors.

To determine if $CD45^+$ RBMCs contribute to the β cell proliferative response, we blocked RBMC migration to βVEGF-A islets by partially ablating βVEGF-A BM with 5Gy irradiation immediately prior to Dox treatment. This ablation significantly reduced $CD45^+$ RBMC infiltration into islets, which had no effect on EC expansion or β cell loss, but caused a significant decrease in β cell proliferation 2 wks after Dox WD.

Increased VEGF-A production in β cells leads to the recruitment of $CD45^+$ BMCs, which persist in islets and directly, or with intra-islet ECs, promote β cell proliferation and regeneration. Further characterization of the specific population and phenotype of RBMCs responsible for the β cell proliferative effect will allow us to define the mechanism by which these RBMCs promote β cell proliferation and develop them as a potential therapeutic for diabetes.

THE PATHOGENICITY OF INTERLEUKIN-21 IN INFLAMMATORY BOWEL DISEASE

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Purpose: Th17 CD4 T cells are necessary for protection against pathogens but have also been cast as pathogenic in the context of many autoimmune diseases, including Inflammatory Bowel Disease (IBD). Although several Th17-associated cytokines may act in concert to induce inflammation, IL-21 is a strong candidate for further scrutiny. IL-21 expression is increased in biopsies from patients with ulcerative colitis compared to healthy controls, and Genome Wide Association Studies have shown an association between the locus containing *il2/il21* and IBD. Our aim is to elucidate the role of IL-21 in chronic intestinal inflammation.
Methods: We utilized two well-described mouse models of colitis (the IL-10-deficient spontaneous model and the CD4 T cell-dependent adoptive transfer model) in order to evaluate the requirement and role of IL-21 signaling for disease. In addition, primary cell and organ culture was used to further elucidate the role of IL-21 signaling on T and non-T cell subsets.
Results: We have shown that a large number of IL-21-producing CD4 T cells are present in the intestines of mice with colitis. Further, our data suggests that IL-21 is required for the full induction of IBD. While others have shown *in vitro* that exogenous IL-21 acts to induce IL-17 production by CD4 T cells, our data suggests that IL-21-deficient cells can produce IL-17A and IL-17F to a greater degree than IL-21-competent cells. Our data also disputes the previous finding that IL-21 suppresses the transcription factor Foxp3 as we show that IL-21-deficient T-regulatory cells express equal or less Foxp3 both *in vitro* and *in vivo*. Interestingly, IL-21 receptor expression on CD4 T cells is not essential for colitis, suggesting that IL-21 may act on additional cell types to promote disease induction. We have shown that IL-21 promotes production of IL-17A and IL-22 by non-CD4 T cells in the intestine.
Discussion/Conclusion: Taken together, our data indicate that IL-21 plays an important role in the induction of chronic intestinal inflammation that is independent of IL-17 production and T regulatory cell induction, highlighting a previously unrecognized role for IL-21 in the intestine.

INJURY DYNAMICS WITHIN IN SILICO NEUROGLIAL NETWORKS

Vineet R. Tiruvadi

Purpose: Astrocytes are beginning to be considered active elements in modulating synaptic communication in addition to their passive, metabolic roles. However, many current models of neuronal networks fail to take into account astrocytic input and processing of neuronal signals. We developed a computational model of a hybrid neuron-astrocyte network to explore the mechanics of neuronal injury mediated by astrocytic glutamate and excitotoxic processes.
Methods: We utilized a compound computational model developed with Python and GPGPU libraries to simulate a large-scale neuroglial network. We develop a neuronal network using a modified Izhikevich model and then couple a parallel Ca2+-wave based astrocyte network into the neuronal topology. Injury was modeled as nodal deletion of neurons following a particularly large, excitotoxic glutamate release from astrocytes. Connectivities and model parameters were drawn from the literature and varied to characterize simulation sensitivity.
Results: Our model demonstrates the importance of astrocytes in distributing local signaling and providing a parallel circuit for signal propagation at modulation. In particular, inter-spike intervals demonstrate a dependence on astrocyte presence and strength of interactions between neurons and astrocytes. As lesions are introduced into the neuronal network, we see a change in the inter-spike interval due to the loss of important neuron-neuron and neuron-astrocyte hubs.
Discussion/Conclusion: Computational models of disease states may require the unique influences and dynamics of astrocytic networks to fully account for patterns of neuronal death. Our model provides a platform to develop more accurate models of neuroglial interactions while providing a glimpse into the interesting network dynamics provided by the inclusion of astrocytic elements. We hope to further extend the model to include effective model-building from basic in vitro recordings, account for other astrocytic neurotransmitters and introduce lesions within the astrocytic network.

MUTANT TAU CAUSES NMDAR-MEDIATED INSULOSTRIATAL DYSFUNCTION AND BEHAVIORAL ABNORMALITIES IN A MOUSE MODEL OF FRONTOTEMPORAL DEMENTIA

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Tau mutations cause behavioral variant frontotemporal dementia (FTD), a progressive and lethal disease commonly presenting with repetitive behaviors and personality changes. FTD patients have connectivity dysfunction and atrophy in a network of brain regions called the salience network. In this network, FTD patients have insulostriatal abnormalities with neurodegeneration beginning in insular cortex and repetitive behaviors correlating with ventral striatum dysfunction. Understanding how tau mutations cause insulostriatal abnormalities is critical to determine potential treatment targets. We found that transgenic mice with the FTD-associated V337M human tau mutation (hTauV337M mice) have NMDA receptor (NMDAR) dysfunction selective to insulostriatal regions. hTauV337M mice have decreased NMDAR levels and decreased excitatory transmission in ventral but not dorsal striatum. NMDAR levels are also decreased in insular but not motor cortex. hTauV337M mice have dendritic simplification of Golgi-stained neurons in insular cortex and ventral striatum, but not of neurons in motor cortex or dorsal striatum. These underlying NMDAR-mediated changes in hTauV337M mice manifest as behavioral abnormalities such as age-dependent repetitive grooming, impaired nest building, and impaired marble burying. Treatment with the NMDAR co-agonist D-cycloserine (20 mg/kg, i.p.) decreased repetitive grooming in hTauV337M mice. These data suggest that tau mutations cause NMDAR dysfunction preferentially in neurons of insulostriatal regions to cause behavioral abnormalities in a mouse model of FTD.

SELECTIVE INHIBITION OF HISTONE DEACETYLASE 6 AND 8 BY 3-HYDROXY-PYRIDIN-2-THIONE BASED COMPOUNDS

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Purpose: DNA is subjected to various epigenetic regulations including histone deacetylation mediated by histone deacetylase (HDAC) which results in gene silencing. This mechanism has been exploited by various cancer cell lines to silence key pro-apototic genes such p53 resulting in uncontrolled cell growth. As such, HDAC has been a target for cancer therapy. This has led to the approval of HDAC inhibitors (HDACi) such as SAHA (Vorinostat) and FK-228 (Romidepsin) for cutaneous T-cell lymphoma by the Food and Drug Administration. The prototypical HDACi SAHA, a hydroxamate based pan-inhibitor, has been associated with severe side effects including a potentially fatal QT-prolongation. This project is aimed at replacing the labile hydroxamate (zinc binding group) with novel chemical moieties stable to hydrolysis and devoid of cardiotoxicity.
Methods: Through molecular docking, 3-hydroxypyridin-2-one (3-HP) and 3-hydroxypyridin-2-thione (3-HPT) were selected and incorporated into the pharmacophoric model of HDACi. Various inhibitors were synthesized for biological studies. Using SAMDI mass spectrometry, the inhibitory activity of the compounds against HDAC 1, 6 and 8 isoforms was measured . Western blot was used to confirm the mechanism of action of these HDACi in the prostate cancer cell line LNCaP.
Results: 3-HP based HDACi were inactive against all three HDAC isoforms whereas the 3-HPT based ones displayed various degrees of selectivity against HDAC 6 or 8. These compounds led to tubulin acetylation and apoptosis in various cancer cell lines including Jurkat, DU-145 and LNCaP.
Conclusion: 3-HPT is compatible with HDAC inhibition and can thus replace the hydroxamate used in most HDACi. Furthermore, the isoform selectivity seen with some of the compounds may lead to their use against malignancies overexpressing those specific isoforms resulting in potentially fewer side effects with HDACi therapy.

HINDBRAIN ROOF PLATE CELLS ARE MULTIPOTENT AND SUSCEPTIBLE TO ONCOGENIC TRANSFORMATION BY DEREGULATED SHH SIGNALING

Frances Y. Cheng, Xi Huang, Anuraag Sarangi, Tatiana Ketova, Michael K Cooper, Ying Litingtung and Chin Chiang

Medulloblastoma, the most common pediatric malignant brain tumor, can arise from cerebellar granule neuron precursors (CGNPs) with aberrant Sonic hedgehog (Shh) signaling. However, the molecular mechanism by which Shh pathway-mediated proliferation and transformation of CGNPs occurs is poorly understood. A deeper understanding of the cellular basis of medulloblastoma is crucial for improving early diagnosis and treatment of this devastating disease. To date there have been few distinct subsets of CGNPs identified which can be transformed to initiate medulloblastoma formation. We demonstrate that focal activation of Shh signaling in a distinct subset of CGNPs, specifically derived from hindbrain roof plate cells expressing growth differentiation factor-7 (Gdf7), is sufficient to promote cerebellar tumorigenesis. This is accomplished by utilizing a $Gdf7^{Cre/+}$ line to drive constitutive activation of the Shh pathway activator Smoothened (SmoM2).
$Gdf7^{Cre/+}$;SmoM2 (GM2) mutant mice were observed to display stunted growth, cranial bulging, and impaired motor coordination; all GM2 mice died within three weeks of birth. Analysis of mutant cerebellar architecture revealed severe hyperplasia suggestive of tumor formation. Tumors from both GM2 and established medulloblastoma model $Patched^{LacZ/+}$ mice displayed strong expression of CGNP, neural progenitor, and proliferative markers. In addition, cultured GM2 cerebellar cells expressed multiple stem cell markers and were clonogenic and multipotent. Collectively, these data indicate that targeting constitutively active Shh signaling to the Gdf7-lineage leads to formation of medulloblastoma. Detailed fate-mapping was performed of the Gdf7 lineage which revealed that surprisingly, Gdf7-lineage cells contribute to a small subset of proliferating CGNPs. In addition, Gdf7-lineage cells also contribute to an extensive array of mature cerebellar cell types. The GM2 medulloblastoma mouse model demonstrates how remarkably few cells are sufficient for oncogenic transformation and tumor formation. Thus hindbrain roof plate cells are established as a novel source of diverse neural cell types in the cerebellum that is also susceptible to oncogenic transformation by deregulated Sonic hedgehog signaling.

JAM-A REGULATES EPITHELIAL BARRIER FUNCTION BY ESTABLISHING A RAP2C ACTIVATING COMPLEX

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Intestinal barrier function is regulated by the apical junctional complex (AJC), a structure composed of many transmembrane and scaffold molecules that control the passage of nutrients and solutes across epithelial surfaces. Under pathologic conditions such as inflammation, barrier function is compromised due to disruption of cell-cell contacts. *AJC assembly after barrier disruption is an essential and intrinsic part of homeostasis.* One constituent of the AJC is a transmembrane protein termed Junctional Adhesion Molecule-A (JAM-A), which contains a short cytoplasmic tail that associates with scaffold proteins and two extracellular immunoglobulin-like domains that allow for its homodimerization. Observations from JAM-A deficient epithelial cells and JAM-A knockout animals indicate that JAM-A is an important regulator of epithelial paracellular permeability to small and large molecules, however the mechanism(s) linking JAM-A to barrier function are not understood. From what is known about JAM-A effectors in other pathways and on characterized modulators of barrier function, we have identified candidate proteins that mediate JAM-A regulation of epithelial paracellular permeability. We report that JAM-A associates with the scaffold proteins ZO-2 and Afadin as part of a complex that may play a role in regulating PDZ-GEF1-dependent activation of the small GTPase Rap2c. We have also characterized a novel role for Rap2c as a mediator of barrier function in intestinal epithelial cells. Since ZO-2 and Afadin have known actin-binding domains, and since Rap GTPases have been implicated in regulating actin dynamics, we propose that JAM-A dependent Rap2c activity is responsible for organizing the apical actin-myosin ring known to stabilize the AJC. Such insights on the mechanisms important for barrier function may provide potential therapeutic targets for epithelial restitution after inflammation-triggered barrier defects.

ASSESSMENT OF sEH ACTIVITY BY OXYLIPIN METABOLITES: RELEVANCE TO DIABETES

Sambita Basu

Arachidonic acid derivatives include prostaglandins, leukotrienes, and epoxyeicosantrienoic acids (EETs). EETs are formed by cytochrome P450 (CYP450) enzymes, specifically the CYP2C or CYP2J epoxygenases, and are degraded to biologically inactive dihydroxyeicosatetraenoic acids (DHETs) by epoxide hydrolases. EETs have beneficial effects on glucose metabolism *in vitro* and *in vivo*. Increased concentrations of 5,6-EET induces insulin release by isolated pancreatic islet cells. EETs also prevent hyperglycemia *in vivo* by preserving islet mass through prevention of apoptosis. Blocking EET degradation with a soluble epoxide hydrolase (sEH) inhibitor or sEH knockout increases glucose-stimulated insulin secretion. Therefore, EETs may attenuate the development of diabetes in high-risk individuals (those with an impaired glucose tolerance or with impaired fasting glucose). sEH activity can be assessed by measuring the ratio of EETs to DHETs in plasma. EETs, however, are relatively unstable and found in low amounts in plasma. CYP2C and CYP2J epoxygenases oxidize linoleic acid, yielding 9,10- and 12,13-epoxy-octadecenoic acids (EpOMEs), which sEH also degrades to dihydroxy-octadecenoic acids (DHOMEs). As EpOMEs and DHOMEs circulate in higher levels than EETs and DHETs, plasma EpOME/DHOME ratio has provided a more reliable measure of sEH activity *in vivo*. This study tested the hypothesis that sEH activity is associated with impaired insulin secretory response in humans, using the ratio of EpOME/DHOME in plasma to assess endogenous sEH activity. Development and validation of the assay to quantify EpOME/DHOME in human plasma was the primary focus of this research experience. Optimization of collection and processing conditions for plasma was performed in order to assess sEH activity across studies. Blood was collected into citrate, EDTA, and as serum and underwent solid phase extractions using Waters Oasis HLB 60 mg SPE cartridges. Analysis was conducted by UPLC/MS/MS by the Vanderbilt Eicosanoid Core. Samples were quantified and calibrated with deuterated standards (Cayman) for 9,10 EpOME, 12,13 EpOME, 9,10 DiHOME, 12,13 DiHOME. EpOME degradation into their corresponding DHOME species was noted using previously published methods; modification of the protocol by removal of acidic components in both the solid phase extraction and UPLC/MS/MS methods improved epoxide yield. Ongoing studies include synthesis of EpOME standards, optimization of the extraction protocol, optimization of various collection methods (plasma, serum), and determining the stability of stored plasma (freeze/thaw cycles). sEH inhibitors are presently in development for treatment of hypertension, and may provide additional beneficial metabolic side-effect profiles, including glycemic control. This LC/MS method to quantify the ratio of EpOME/DHOME as a measure of sEH activity will be used to assess sEH activity, and to correlate with glucose tolerance, insulin resistance, and insulin secretion measurements in humans.

ACTIVATION OF THE BRAIN RENIN-ANGIOTENSIN SYSTEM BY TRANSLATIONAL APPROACHES POST-STROKE IS NEUROPROTECTIVE IN A RAT MODEL OF ISCHEMIC STROKE

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Purpose: Toward discovering novel stroke therapies, recent research showed that activation of the newly-discovered angiotensin converting enzyme 2/angiotensin-(1-7)/mas (ACE2/Ang-(1-7)/Mas) pathway, a counter-regulatory axis of the brain renin-angiotensin system, is neuroprotective in ischemic stroke in rats. Efficacy must now be demonstrated using minimally-invasive methods if this therapy is to be translated to the care of human patients. In this study, we assessed the hypothesis that systemic administration of the novel ACE2 activator diminazine aceturate (DIZE) or orally active Ang-(1-7) post ischemic stroke would be neuroprotective.

Methods: Adult male Sprague-Dawley rats underwent sham surgery or ischemic stroke by endothelin-1 induced middle cerebral artery occlusion followed by drug or control H2O administrations at +4, +24, and +48 h after stroke. Rats were randomly divided into 6 groups (n=10-15/set): 1) sham 0.9% saline stroke and intraperitoneal (IP) administrations of H2O; 2) ET-1 stroke and IP administrations of H2O; 3-5) ET-1 stroke and IP administrations of high, medium, and low dose DIZE; 6) ET-1 stroke with oral gavages of cyclic Ang-(1-7), 50 µg/kg. At 24 and 72 h after stroke, rats underwent blinded neurologic assessments. Immediately following the 72 h tests, animals were sacrificed and cerebral infarct volumes assessed by TTC staining. Data are expressed as mean ± SEM with significance inferred at $p<0.05$.

Results: Mean infarct volume was significantly decreased by IP injections of DIZE at high dose (28.51%±4.48, p=0.015) and medium dose (30.44%±3.77, p=0.032) and by gavages of orally active Ang-(1-7) (23.37%±5.24, p=0.004) as compared to control (42.61%±4.04). At 24 h post stroke, neurologic deficits were significantly improved by high dose DIZE (16.47±0.22) versus the control group (15.46±0.40, p=0.016).

Conclusions: Our findings suggest that targeting the ACE2/Ang-(1-7)/Mas axis post stroke can improve function and reduce infarct volume – a significant translational step in brain renin-angiotensin system research.

VARYING RESIDUE NUMBER OF POLYGLUTAMATE DOMAINS FACILITATES DIFFERENTIAL LOADING TO AND RELEASE FROM HYDROXYAPATITE AND ALLOGRAFT BONE

William M. Webb

Bone matrix proteins such as bone sialoprotein (BSP) and osteocalcin contain stretches of negatively-charged amino acids that facilitate their binding to hydroxyapatite (HA), the principle mineral component of bone. Synthetically, heptaglutamate (E7) domains derived from BSP have been employed to facilitate binding and retention of the collagen-mimetic peptide DGEA to HA and allograft bone for the purposes of encouraging osteoblastic differentiation of mesenchymal stem cells. This E7-HA technology could also be exploited to enhance the osteoinductive capacity of HA-coated implants and allograft bone.

Because the negative charge of residues is known to regulate binding of peptide to HA, it was hypothesized that varying the number of glutamate residues attached to a cargo peptide would result in differential binding and adjustable release from HA and allograft bone, paving the way for more adjustable, personalized drug delivery regimens. Furthermore, mixed solutions of different polyglutamate-linked cargo molecules could potentially be combined to facilitate controlled timing and release of a variety of cargo molecules simultaneously, increasing the utility of polyglutamate domains for delivery of a wide range of biomodifiers onto HA-containing materials and allograft bone.

E2, E4, & E7-FITC-tagged peptide solutions were tested for loading to and percent release from both pure HA and allograft bone over time. Fluorometry and qualitative imaging revealed that increasing the number of ‘E’ residues facilitated more rapid binding, experienced greater total loading, and exhibited slower release from the surface of both materials. Solutions of polyglutamate-tagged peptides retained their average propensity to facilitate binding when combined in mixtures; therefore, ratios of E2, E4, and E7-tagged peptides could be adjusted to produce unique, customized release schedules.

These findings suggest a specific but adjustable means for pharmacotherapy delivery to bone as well as for gradual release of bioactive factors from implanted grafts and biomaterials.

DIFFERENTIAL SUSCEPTIBILITY OF OROPHARYNGEAL CARCINOMA TO TARGETED THERAPY BASED ON HUMAN PAPILLOMAVIRUS STATUS

Alice Weaver

In recent years, oropharyngeal cancer has changed from a disease of older men with a history of tobacco and alcohol abuse to a cancer occurring in younger adults as a result of human papillomavirus (HPV). HPV oncoproteins are known to disrupt cell signaling pathways, including DNA repair. We previously reported a synthetic lethal interaction between inhibitors of epidermal growth factor receptor (EGFR) and poly (ADP-ribose) polymerase (PARP), two important proteins in DNA repair, in HPV-negative head and neck cancers. In this study, we investigate the susceptibility of HPV-positive oropharyngeal squamous cell carcinoma (OPSCC) to two systemic agents, cetuximab and veliparib, which target EGFR and PARP, respectively.

Interestingly, our data support a differential response to these agents based on HPV status. *In vitro*, cetuximab/veliparib combination caused the greatest reduction in HPV-negative OPSCC proliferation (62%), compared to veliparib (32%) or cetuximab (30%) alone. In contrast, the same study in HPV-positive OPSCC cells found that veliparib alone caused the greatest reduction in growth (55%), compared to cetuximab/veliparib combination (40%) and cetuximab alone, which surprisingly stimulated growth in these cells. These results were confirmed *in vivo* by biweekly tumor xenograft volume measurements in mice. Mice bearing orthotopic HPV-negative OPSCC tumors exhibited a 20-day growth delay following combination cetuximab/veliparib treatment, compared to 10 days with cetuximab and no delay with veliparib (*$p<0.01$ vs. control). Conversely, veliparib induced a 15-day tumor growth delay alone and in combination with cetuximab (*$p<0.05$ vs. control) in mice bearing HPV-positive OPSCC human tumor explants, compared to no delay with cetuximab.

These results indicate that different therapeutic strategies may be appropriate for OPSCC depending on HPV status. Additional studies are needed to elucidate the mechanism of enhanced cell death in HPV-positive OPSCC, particularly in response to DNA damaging agents.

MECHANISMS OF INFLAMMATORY GENE REGULATION BY ESTROGEN

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Purpose: In premenopausal women, estrogen (E2) acts as a cardioprotectant, retarding atherosclerosis and mitigating the inflammatory response in vascular tissue after damage. In earlier studies, treatment of ovariectomized mice with E2 following vascular injury resulted in significantly reduced injury compared to mice treated with a control. **Our purpose is to determine the molecular mechanisms by which E2 attenuates the inflammatory response to vascular injury by utilizing primary aortic smooth muscle cell (AoSMC) culture**. Identification of the molecules that mediate the effects of E2 will potentially aid in the development of drugs that confer the beneficial anti-inflammatory and vasoprotective effects of estrogen.
Methods: Mouse AoSMC cultures were grown to 95% confluence in 6-well cell culture plates. The cultures were then pretreated with estradiol. To stimulate the inflammatory response, the cells were treated with the cytokine tumor necrosis factor-alpha (TNF-α). Transcripts of the genes CCL2, CXCL9, TNF- α, and VCAM1 (common inflammatory response genes) were then quantified using reverse transcription and qPCR to measure the suppressive effect of E2. The amount of inflammatory gene transcripts correlated with both the length of time the cells were pretreated with E2 and the presence of certain signaling pathways.
Results: Pretreatment with E2 with a minimum time of four hours is required in order for the suppression of the inflammatory response to take place. Mutant cells in which the non-genomic pathway is disrupted failed to suppress inflammatory gene activation in response to TNF-α.
Discussion/Conclusion: Knowing the two mechanisms in which estrogen attenuates vascular damage (rapid nongenomic signaling and a slower genomic signaling pathway), the relatively short amount of pretreatment time suggests to us that the estrogen does not directly antagonize TNF-α, but rather sets up transcripts to suppress the inflammatory response. Loss of this suppression in the mutants suggests that E2 employs a non-genomic signaling pathway.

NETRIN (UNC-6) PROMOTES ACTIN ASSEMBLY TO DRIVE DENDRITE SELF-AVOIDANCE

Elana Feingold-Link

Sensory neurons that detect noxious stimuli exhibit complex arrays of topical dendritic arbors in both vertebrate and invertebrate organisms. In order to comprehensively sample the environment and avoid redundant cues, neurons that detect the same sensory modality often occupy distinct receptive fields in a phenomenon known as "tiling." A similar organizational principle is known as "self-avoidance" when the territories of dendrites of the same neuron avoid overlap. The dendritic processes of *C. elegans'* PVD neurons (L+R), which envelop the whole worm and mediate aversive reactions in response to harsh touch, display both tiling and self-avoidance. Work in the Miller lab has identified mechanisms by which the diffusible axonal guidance protein UNC-6/Netrin is captured at dendritic tips by UNC-40/DCC and presented to UNC-5 on the apposing dendrite to mediate self-avoidance. We propose a model in which the evolutionarily conserved Netrin can pattern a fundamental feature of dendritic architecture.

PREGNANCY- AND LACTATION-RELATED OSTEOPOROSIS: A MODEL FOR IDENTIFYING PREDICTORS OF LATER BONE HEALTH

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Purpose: More than 40 million Americans have or are at high risk for osteoporosis. Though bone mineral density (BMD) decline initiates early, minimal research defines parameters of bone health throughout the life course or addresses lower cost or less invasive methods of BMD assessment. The U. S. Preventive Services Task Force (USPSTF) recommends routine osteoporosis screening for women aged 65 and older, but this reliance on after-the-fact screening contradicts recent initiatives focused on preventive care. Methodological developments for predicting BMD decline are hindered by the long-term nature of osteoporosis, but may be overcome by studying pregnancy- and lactation-related BMD loss.
Methods: A systematic literature review using three databases (MEDLINE, PubMed, and the Cochrane Library) evaluated pregnancy- and lactation-related BMD loss as a model for investigating bone health and identified promising mechanisms for consideration. Inclusion required publication in English between 1992 and 2012. Keywords were combined as follows: (pregnancy OR lactation) AND (bone density OR osteoporosis). Titles and abstracts were scanned for inclusion by the author, who subsequently extracted and coded information from relevant papers.
Results: The review identified more than 2,500 articles, though a significant proportion addressed general women's health rather than the review aim. BMD loss of 3-7% during lactation has been routinely identified, which mimics that observed in age-related osteoporosis. Studies to date primarily utilize dual energy X-ray absorptiometry (DXA), though are often confounded by a lack of pre-conception data. The molecules most commonly studied include vitamin D and calcium, though emerging evidence suggests leptin, osteocalcin, and osteoprotegerin as potential targets.
Discussion/Conclusion: Predictors of bone health are understudied, and pregnancy- and lactation-related osteoporosis represents a relatively unexplored model. Animal models may be ideal for initial studies for three key reasons: to reduce confounding effects, decrease observation time, and clarify the key biological molecules correlated with changes in BMD.

A PATIENT CENTERED MEDICAL HOME PROSPECTIVE BEFORE AND AFTER EVALUATION

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Purpose of Study: In spring of 2011, Internal Medicine 1 (IM1) at Kirklin Clinic changed many aspects of its practice in order to become a certified patient centered medical home (PCMH) based on conditions set forth by the NCQA. The goal of this project was to report differences in outcomes after the first year of PCMH implementation for IM1 patients.
Methods Used: Using baseline (2011) and 1 year preventive care measures, we performed a before and after cohort study comparing the study population (IM1, N≈1000) to a control (IM2/IM3 patients, N≈2000). Measures of comparison (MoC) were generated using VIVA Health claims and billing data. Inclusion/exclusion criteria were set using 2011 Healthcare Effectiveness Data and Information Set (HEDIS) parameters. MoC included colorectal cancer, breast cancer and glaucoma screenings as well as multiple screening measures exclusive to those with diabetes mellitus-II (A1C and LDL-C screening, Nephropathy monitoring, etc.).
Summary of Results: With the exception of colorectal screening, all other measures declined more steeply for patients enrolled in the PCMH group (Image 1). The average pre-post % change for all measures was -2.7% for the PCMH unit and +5.1% for the control groups. Controlling for patient characteristics (i.e., age, gender, comorbidities), the best predictor of post-PCMH diabetes processes was the pre-PCMH diabetes process score.
Conclusions: The effects of a PCMH vary depending on patient population and measures considered. This study showed that, in general, preventive measures were not augmented by PCMH changes to practice. Moreover, compared to controls, the PCMH unit fared worse when looking at % change in the measures considered.

ONCOLYTIC HSV-1 EXPRESSING INTERLEUKIN-15 FOR BRAIN TUMOR THERAPY

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Oncolytic herpes simplex type-1 virus (oHSV) vectors are promising therapeutics for malignant gliomas because of their ability to replicate within and lyse malignant cells. However, gliomas from individual patients differ in their permissiveness to oHSV replication. Engineering oHSV vectors to express immunostimulatory molecules can promote cellular anti-tumor immune responses and improve oHSV efficacy in gliomas resistant to oHSV replication. Our laboratory group recently identified interleukin-15 as a candidate for oHSV expression using microarray analysis. In patients with glioblastoma responding positively to oHSV therapy transcripts for IL-15 and all IL-15 receptors were significantly upregulated, as were transcripts suggesting the presence and cytotoxic activity of cellular effectors responsive to IL-15; $CD8^+$ T and natural killer (NK) cells. Accordingly, we hypothesize production of IL-15 from an oHSV vector will stimulate cellular anti-glioma immune responses to improve oHSV efficacy in gliomas resistant to oHSV replication. To investigate this hypothesis we constructed oHSV vectors expressing murine (m)IL-15 with and without the IL-15 receptor α (IL-15Rα), which is necessary for optimal IL-15 presentation and signaling. The vector dually encoding mIL-15 and mIL-15Rα releases the physiologically relevant mIL-15/IL-15Rα complex (2-3 ng/mL) from infected murine glioma cells independent of vector replication. The mIL-15/IL-15Rα complex stimulates robust proliferation of NK cells as well as NK cell cytotoxicity against syngeneic murine glioma cells. Both vectors are aneurovirulent (LD_{50}> $1x10^7$ plaque forming units, comparable to other oHSV vectors). Dual mIL-15/IL-15Rα expression from oHSV improved survival over saline in preliminary survival studies using an aggressive murine brain tumor model (median survivals: saline – 14 days; mIL-15/IL-15Rα oHSV – 25 days. p = 0.001), whereas mIL-15 expression alone did not (median survival: mIL-15 oHSV – 17 days. p = 0.1128 compared to saline). In vivo studies investigating the efficacy oHSV-produced mIL-15/IL-15Rα in murine models of malignant glioma resistant to oHSV replication are underway.

HEME OXYGENASE-1 EXPRESSION PROTECTS THE MYOCARDIUM FROM CRE-INDUCED SYSTOLIC DYSFUNCTION

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Background: Heme oxygenase-1 (HO-1) is an enzyme that catalyzes the breakdown of pro-oxidant heme into carbon monoxide, biliverdin, and iron. Our laboratory has shown that HO-1 is tissue-protective due to its antiapoptotic, cytoprotective, and immunomodulatory functions. We generated a novel transgenic mouse with cardiac-specific, tamoxifen-inducible HO-1 overexpression to investigate the tissue specific requirement for the cardioprotective function of HO-1. We bred a transgenic mouse containing a floxed β-gal-stop-codon cassette upstream of the *hmox1* gene (**CBA** mice) with a transgenic mouse containing a myosin heavy chain (**MHC**) promoter driving a Cre recombinase gene (**MHC-Cre** mice) to generate cardiac-specific HO-1 overexpressing mice (**MHC-HO-1** mice). The Cre recombinase in these mice is flanked by mutated estrogen receptor ligand-binding domains (MerCreMer), such that nuclear translocation of Cre only occurs after tamoxifen (**TAM**) administration. Although MHC-Cre mice have been widely utilized as a tool in cardiovascular research, it was recently shown that Cre expression in the myocardium causes transient systolic dysfunction.
Hypothesis: We hypothesize that, relative to MHC-Cre single transgenic controls, MHC-HO-1 mice, which over express HO-1 in a cardiac specific manner, are protected from Cre-induced systolic dysfunction. This hypothesis is based on studies showing that HO-1 is cytoprotective to the myocardium because the byproducts of heme degradation exert antiapoptotic, antioxidant, and immunoregulatory functions.
Methods/Results: To test our hypothesis, we injected MHC-HO-1, MHC-Cre, and CBA mice with 40 mg/kg of tamoxifen on two consecutive days, and monitored HO-1 induction, survival, cardiac function by echocardiography, and the process of cardiomyocyte death by apoptosis over time. We compared MHC-HO-1 mice with single transgenic MHC-Cre mice to determine the effect of HO-1 overexpression on Cre-induced toxicity. We also included CBA single transgenic control groups to determine if TAM administration by itself had any effect on the heart. Using Western blot analysis, real-time PCR, and HO activity assays, we determined that maximal HO-1 induction occurred by three days after TAM administration (~60-fold increase in HO-1 mRNA message compared to TAM untreated controls), but decreased thereafter due to Cre-induced toxicity. Interestingly, HO-1 induction provides a significant survival advantage, as mortality occurred in 83% of MHC-Cre mice by day 3 while 85% of MHC-HO-1 mice survived for greater than 7 days. By echocardiography, we observed severe but transient systolic dysfunction in MHC-HO-1 mice, as shown by a decline in ejection fraction from 61.53% at baseline to 38.69% three days after TAM (n=6). In the absence of HO-1 induction, in MHC-Cre mice, ejection fraction declined from 61.2% to 19.2% the day after administration of TAM (n=6) and resulted in subsequent death by day 3. In CBA single transgenic mice treated with TAM, we observed 100% survival and no decline in ejection fraction over time, indicating that TAM itself does not exert functional changes on the myocardium. Histologically, we observed significant diffuse inflammation and cardiomyocyte damage in MHC-Cre cardiac sections by day 2 after TAM. By flow cytometry analysis, we confirmed that this cardiac infiltrate was predominantly neutrophils. In MHC-HO-1, this inflammation and cardiac damage was focal in nature, and the majority of the myocardium was indistinguishable from vehicle treated control animals.
Conclusion: We have shown that Cre expression, but not TAM administration, in the myocardium is cardiotoxic. This Cre-induced cardiac toxicity and systolic dysfunction is partially ameliorated by HO-1 expression.

THE ROLE OF B CELL RECEPTOR SIGNALING IN B CELL MEDIATED IMMUNE REGULATION

Blair Stocks

Background: Islet transplantation represents a clinical solution to Type 1 diabetes (T1D); however the obligatory immunosuppression that follows the operation significantly limits the widespread use of this procedure. Clinically applicable islet transplantation for T1D will therefore depend on restoring immune tolerance in graft recipients.
Objective: While transplantation tolerance is easily achieved in normal strain mice (B6), no protocol has been successful in non-obese diabetic (NOD) mice. We hypothesize that defective B-lymphocyte function in NOD mice may lead to an inability to restore tolerance. Recent evidence suggests that monoclonal antibody to CD45RB restores immune tolerance in a B-lymphocyte dependent manner. In the present study, we examined protein tyrosine kinase (PTK) and phosphate (PTP) gene expression, B-lymphocyte receptor (BCR) signaling, and splenic B-cell distribution in anti-CD45RB treated, tolerance-susceptible B6 and tolerance-resistant NOD mice.
Materials and Methods: Age-matched wild type B6 and NOD mice were injected with saline or an anti-CD45RB mAb (treated) every other day for five days. Mice were sacrificed on day six and spleens were harvested. To assess gene expression, RNA was prepared from purified B cells and subjected to RNA sequencing by Illumina (Vanderbilt GSR Core). To analyze B cell signaling in the "activated" state, unsorted splenocytes and MACS purified B-cells (>95% purity) were exposed to an anti-IgM antibody (stimulated) for 10 minutes. Cytoplasmic pSyk and pPLCg2 levels from the unsorted splenocytes were evaluated via flow cytometry by measuring changes in mean fluorescent intensities. Flow cytometry was used to assess B-cell compartmentalization by the relative expression of CD21 and CD23 among CD19+ splenocytes. Statistical significance was defined as $P<0.05$ as determined by one- and two-way ANOVAs, except in RNA sequence where significance was defined as $p<0.0025$.
Results: Gene expression of PTKs ad PTPs both directly and indirectly involved in BCR signaling differed at baseline between B6 and NOD mice. Furthermore, treating NOD mice with anti-CD45RB significantly increased IgM-stimulated splenocyte pSyk and pPLCγ2 levels versus their littermates not receiving treatment. Characterization of B cell subsets by the standard markers demonstrates that treatment with anti-CD45RB completely eliminated the marginal zone compartment ($CD21^{hi}$, $CD23^{lo}$) in NOD but not B6 mice
Conclusions: Preliminary data suggest that anti-CD45RB mAb treatment may alter splenic B-lymphocyte function in NOD mice by altering PTK and PTP gene expression, increasing Syk and PLCg2 phosphorylation, and redistributing splenic B cell compartmentalization, however further experimentation is required to confirm these initial data. Overall, identification of alterations in BCR signaling may reveal how antigen-specific tolerance is induced, how B lymphocytes function during tolerogenesis, and new pathways to correct fundamental deficits in the biology of the diabetes-prone immune system.

SMALL MOLECULE INHIBITORS OF BACILLUS ANTHRACIS PROTECTIVE ANTIGEN PROTEOLYTIC ACTIVATION AND OLIGOMERIZATION

Alexander Wein

Protective antigen (PA), lethal factor, and edema factor, the protein toxins of Bacillus anthracis, are among its mostimportant virulence factors and play a key role in infection. We performed a virtual ligand screen of a library of 10000 members to identify compounds predicted to bind to PA and prevent its oligomerization. Four of these compounds slowed PA association in a FRET-based oligomerization assay, and two of those protected cells from intoxication at concentrations of 1−10 µM. Exploration of the protective mechanism by Western blot showed decreased SDS-resistant PA oligomer on cells and, surprisingly, decreased amounts of activated PA. In vitro assays showed that one of the inhibitors blocked furin-mediated cleavage of PA, apparently through its binding to the PA substrate. Thus, we have identified inhibitors that can independently block both PA's cleavage by furin and its subsequent oligomerization. Lead optimization on these two backbones may yield compounds with high activity and specificity for the anthrax toxins.

NONCYTOLYTIC RELEASE OF REOVIRUS FROM POLARIZED ENDOTHELIAL CELLS

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Bloodstream spread is a key step in the pathogenesis of many neurotropic viruses. Although many viruses are found in the blood, mechanisms that promote viremia are not well understood. Reoviruses are neurotropic viruses that disseminate hematogenously to the central nervous system. Junctional adhesion molecule A (JAM-A) is a tight junction protein that serves as a receptor for reovirus. JAM-A is required for the establishment of viremia in infected newborn mice and viral spread by the bloodstream to sites of secondary replication. We examined reovirus infection of polarized human brain microvascular endothelial cells (HBMECs) to determine how viruses might gain access to the circulatory system. Reovirus productively infects polarized HBMECs, but infection does not alter tight junction integrity. Apical infection of polarized HBMECs is more efficient than basolateral infection, which is attributable to binding of JAM-A and sialic acid. Viral release occurs exclusively from the apical surface in a noncytolytic fashion. These data suggest that infection of endothelial cells routes reovirus apically into the bloodstream for systemic dissemination in the host. Understanding mechanisms by which viruses invade the bloodstream may aid in the development of therapeutics that target this key step in neuropathogenesis.

UNCOVERING *STAPHYLOCOCCUS AUREUS* MECHANISMS OF RESISTANCE TO SMALL MOLECULE INHIBITORS THAT IMPAIR METABOLIC FLEXIBILITY

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Staphylococcus aureus is a Gram positive pathogen that is a major cause of morbidity and mortality worldwide. *S. aureus* develops resistance to antibiotic therapies at a rapid rate resulting in limited treatment options. One mechanism by which *S. aureus* develops antibiotic resistance is by adopting a small colony variant (SCV) phenotype. This altered growth phenotype is clinically relevant due to its ability to cause persistent infections. Previous studies in our lab have demonstrated that the respiratory inhibitors zinc protoporphyrin (ZnPPIX) and gallium protoporphyrin (GaPPIX) induce the SCV phenotype. Recently, we have also demonstrated that pigmentation production is required for the development of the SCV phenotype. Treatment of SCVs with the pigmentation inhibitor SKF-525a severely impairs staphylococcal growth. Consistent with this, dual treatment of wild type *S. aureus* with ZnPPIX or GaPPIX and SKF-525a inhibits growth. I have identified mutations within two genes, *fmtC* and *graS*, that provide resistance to ZnPPIX and GaPPIX. These mutants have aberrant autolytic activity suggesting that import of ZnPPIX and GaPPIX is impaired. Also, I have isolated SCVs that are more resistant to SKF-525a exposure supporting the idea that *S. aureus* can develop resistance to the dual treatment of respiratory and pigmentation inhibitors. These findings underscore the ability of this pathogen to adapt to antimicrobial treatments.

TRACING THE NEURAL CIRCUITRY OF AUDITORY FEAR CONDITIONING

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Purpose: For decades the mouse has served as a lab model and yet there is a dearth of information on the basic neural circuitry in the mouse brain. Typically, the mouse circuitry is assumed to have the homologous structure of a rat, where tracing studies are more common. However, there is increasing evidence of differences between the rat and the mouse. Here we present the foundational work attempting to address the dearth of tracing in the mouse by evaluating the proposed circuitry for auditory fear conditioning.

Methods: Classical anterograde tracing was performed by injecting biotinylated dextran amine (BDA, 10,000MW) into the infralimbic cortex, prelimbic cortex, medial geniculate, and lateral amygdala. Diffusion based magnetic resonance imaging with probabilistic tractography was also used to evaluate the connection of the aforementioned areas. The connectivity of these areas was then compared to the published tracing literature in the rat.

Results: The results show that there is the predicted connectivity between the infra/prelimbic cortex and the lateral amygdala (amongst other areas). Furthermore, there is also evidence of connectivity between the medial geniculate and lateral amygdala. There was also a strong concordance between classical and diffusion tracing. Only the most caudal areas of the diffusion data not replicate the classical tracing.

Discussion: Overall the results suggest that the mouse circuitry has the proposed connections of the auditory fear conditioning circuit, though a more comprehensive study is needed. The data also shows that diffusion based imaging had high fidelity in replicating the classical anterograde tracing results, though there were some false negatives and a lack of directionally. Finally, the results suggest that the connectivity of the circuit in the mouse is similar to that of the rat providing an anatomical reason for proposed homology.

INVESTIGATION OF GENE NETWORKS AND THEIR ROLE IN MOTOR PROGRAMS

Crystal Seldon

Processes such as breathing, locomotion, and ingestion require a well-connected network of cells that are in turn regulated by a network of genes. The nematode *C. elegans* allows us to integrate the study of the gene and cellular networks associated with locomotion. The cellular network that we are investigating involves a cross-inhibitory network composed of the DD and VD motor neurons (collectively termed the D motor neurons (mns)) and the muscles they innervate. The D mns contribute to the animal's sinuous pattern of locomotion by causing muscle relaxation. The gene network includes two transcription factors UNC-30 and ALR-1 and a large number of genes involved in the anatomical and physiological characteristics of the D mns. We have used two approaches to analyze the relationships between the gene and cellular networks: bioinformatics and genetics. In the bioinformatics approach, potential transcription factor binding sites in the upstream regulatory region of a neuropeptide gene in the D mns, *flp-11,* were analyzed using software, such as MUSSA and TESS. The two candidates that emerged were then analyzed using a genetic approach. We reasoned that if these two transcription factors regulated *flp-11*, then the pattern of a *pflp-11::gfp* reporter would be altered in a mutant background. *pflp-11::gfp* was crossed in an *alr-1* and an *unc-30* mutant backgrounds. The normal *pflp-11::gfp* pattern expression was observed in an *unc-30* mutant background, but not in an *alr-1* mutant background. In future studies, we will continue to bind these two approaches to study the relationship between gene and cellular networks.

COCAINE EXPERIENCE DYNAMICALLY ALTERS DNA METHYLATION AT PLASTICITY GENES WITHIN THE NUCLEUS ACCUMBENS

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Purpose: Epigenetics refers to a set of covalent modifications of chromatin that can impact gene expression without disturbing the primary DNA sequence. One of the most important epigenetic modifications involves methylation of cytosine bases in DNA. DNA methylation can provide enduring transcriptional control over gene expression, thereby making it an intriguing candidate for the regulation of long-- term cellular and behavioral memories. Given that drugs of abuse such as cocaine create especially potent memories that support the intractable nature of addiction, we hypothesize that cocaine induces alterations in DNA methylation mechanisms brain rain eward regions that critically regulate the transcriptional and behavioral effects of cocaine.
Methods: We first examined alterations in gene expression following acute cocaine (1 dose, 20mg/kg) and chronic cocaine administration (7 daily doses, 20mg/kg) using RT--PCR. Next, we evaluated gene specific changes in DNA methylation by MeDIP to see if methylation changes correspond to the alterations in gene expression observed.
Results: At one hour following acute cocaine, there is a downregulation of DNA methyltransferase 3a2 and upregulation of Gadd45b, a gene required for activity induced DNA demethylation. Similarly, repeated cocaine administration produced transient increases in important plasticity genes at one hour. At 24 hours after repeated cocaine treatment, there is a decrease in DNMT3b (an enzyme responsible for de novo methylation of DNA). Finally, locus specific alterations in DNA methylation at key plasticity genes were observed in response to repeated but not acute cocaine.
Discussion: These results provide novel evidence for modulation of DNA methylation/demethylation machinery in response to acute and repeated cocaine. Interestingly, there is a locus-specific decrease in DNA methylation within the promoters of several plasticity genes in response to repeated but not acute cocaine administration. These methylation changes persist for at least 24 hours, indicating that changes in methylation outlast the changes in gene expression observed here. These novel findings provide valuable information that advances our understanding of the molecular mechanisms underlying long--term neuroadaptions to cocaine.

PSAP INDUCES APOPTOSIS THROUGH A UNIQUE MITOCHONDRIAL PATHWAY

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Presenilin-associated protein (PSAP) was identified as a mitochondrial proapoptotic protein. However, the mechanism by which PSAP induces apoptosis remains unknown. To this end, we have established an inducible expression system. Using this system, we have examined the roles of caspases, Bcl-2 family proteins, cytochrome c, Smac (Smac/Diablo, second mitochondria-derived activator of caspases/direct IAP binding protein with low PI), and Apaf-1 (apoptotic protease-activating factor) in PSAP-induced apoptosis. Our results demonstrated that caspase activation is required for the execution of PSAP-induced apoptosis. Knockdown of caspase-9, but not caspase-8, abolished PSAP-induced PARP cleavage and the activation of other caspases, indicating that caspase-9 is the initiator caspase in the apoptotic cascades induced by PSAP. Knockdown of Apaf-1 abolished PSAP-induced caspase activation and PARP cleavage, indicating that the apoptosome formation triggered by cytochrome c is crucial for PSAP-induced apoptosis. Knockdown of Smac abolished PSAP-induced caspase activation and PARP cleavage, indicating that, in addition to Apaf-1 or apoptosome formation, Smac is also essential for PSAP-induced apoptosis; overexpression of Bcl-2 and Bcl-xL did not protect cells from PSAP-induced apoptosis, and knockdown of Bid, Bax, and Bak had no effect on PSAP-induced cytochrome c and Smac release, indicating that PSAP-induced apoptosis is not regulated by Bcl-2 family proteins. These results strongly suggest that PSAP evokes mitochondrial apoptotic cascades via a novel mechanism that is not regulated by Bcl-2 family proteins, but both the formation of cytochrome c-Apaf-1 apoptosome and the presence of Smac are absolutely required for PSAP-induced apoptosis.

UNCOVERING THE GENETIC REGULATION OF AN AXON SELF-DESTRUCT PATHWAY

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Purpose: Axon degeneration is a specialized self-destruct program that mediates axon breakdown and clearance in development, injury, and disease. Understanding the genetic regulation of axon degeneration will broaden our understanding of nervous system development and may offer novel paths to disease intervention.

Methods: We have developed a lentiviral RNAi-based screening platform to identify genes required for injury-induced axon degeneration in neurons. Primary sensory neurons are treated with shRNA-bearing lentiviral particles and injured by transection, and axon degeneration is quantified by automated microscopy and image analysis. Target genes identified from the primary screen are validated by qRT-PCR and genetic rescue.

Results/Conclusions: Our screen of ~16,000 mouse genes was recently completed and has yielded a variety of promising candidate genes that are currently being evaluated. The identification and validation of a *bona fide* axon degeneration signaling protein Sarm1 demonstrates the effectiveness of our screen and validation measures. Finally, the serendipitous identification of a large number of axon-protective shRNA sequences sharing common sequence "motifs" may provide unexpected insights into the nature of shRNA off-target effects in neurons.

THE INVOLVEMENT OF DNA METHYLATION IN CONDITIONED TASTE AVERSION

Mikael Guzman Karlsson

Epigenetic mechanisms have long been associated with cell differentiation and development. However, recent studies have also implicated epigenetic mechanisms in several brain regions involved in various types of learning and long-term behavioral changes in the adult. Typically, epigenetic mechanisms consist of DNA methylation and post-translational modifications of histones. Previous research from our lab and others suggest that DNA methyltransferase (DNMT) activity in hippocampal-cortical circuits is important for the consolidation and the maintenance of fear memory, however, little is known regarding how DNA methylation might be involved in taste learning. Our central hypothesis is that the encoding and storage of CTA memories is dependent on DNA methylation. In order to determine if DNA methylation is necessary for consolidation of CTA memories, we infused the DNMT inhibitor RG-108 into the insular cortex or the basolateral amygdala of rats during CTA training for saccharine. Furthermore, to determine if DNA methylation is necessary for the maintenance of CTA memories, we infused RG-108 into the insular cortex or basolateral amygdala 14 days after training. Neither of these RG-108 manipulations had an effect on the aversion index to saccharine, indicating the DNA methylation in the insular cortex or the basolateral amygdala is not necessary for the consolidation nor the maintenance of CTA memory. In future studies, we plan to investigate whether or not DNA methylation may have a role in other regions of the taste circuit, such as the central nucleus of the amygdala as well as the parabrachial nucleus, during the encoding and maintenance of CTA memories.

DECREASE OF NEUROINFLAMMATION MARKERS IN THE BENEFICIAL EFFECTS OF EXERCISE IN HEMI-PARKINSONIAN RATS.

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Parkinson's disease (PD) involves loss of dopaminergic neurons in the substantia nigra pars compacta (SNc), which is correlated to astrocytic and microglial changes. Physical exercise protects the brain, but its relationship to astrocytic and microglial behavior is still unclear. We investigated the exercise-induced changes on those inflammatory processes in a rat model of PD induced by striatal injections of 6-hydroxy-dopamine (6-OHDA). Adult male Wistar rats were divided into two groups: (1) sedentary (S) or (2) exercised (treadmill 3x/week for one month prior to surgery at the speed of 10 m/min for 40 min; EX). The rats were then divided into four sub-groups: (1) sedentary saline (S+SAL), (2) sedentary 6-OHDA (S+6-OHDA), (3) exercised saline (EX+SAL), (4) exercised 6-OHDA (EX+6-OHDA). Seven days after surgery, brains were collected for immunohistochemistry and immunoblotting for dopaminergic and glial markers into SNc and striatum (CPu). One-way ANOVA with the Tukey *post hoc* test ($p<0.05$) was used.

There were decreased levels of tyrosine hydroxilase in the SNc and CPu of the S+6-OHDA group. Astrocytic activation into SNc was higher in S+6-OHDA in relation to the other groups (ca. 100%), whereas microglia increased only in the CPu (ca. 36%). Levels of inducible nitric oxide synthase increased in S+6-OHDA in the SNc (ca. 133%) and CPu (ca. 87%). Exercised groups exhibited reduced damage to dopaminergic cells, as demonstrated by a higher tyrosine hydroxylase expression after lesions, and reduced neuroinflammatory markers.

These results indicate that physical exercise may reduce neuroinflammation after 6-OHDA and therefore reduce its impact on dopaminergic neurons.

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HEPARAN SULFATE PROTEOGLYCANS MEDIATE PATHOGENIC TAU SEED INTERNALIZATION

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Purpose: Tauopathies are a class of neurodegenerative disorders characterized by the pathological accumulation of microtubule-associated protein tau in the human brain. Emerging evidence demonstrates that tau aggregates are capable of transcellular spread whereby an aggregate formed in one cell is released freely into the extracellular space, enters a neighboring cell, and converts the natively folded tau protein into an aggregated, fibrillar form. Thus, misfolded tau aggregates capable of transcellular spread may directly serve as an agent of disease progression. However, the mechanism by which tau aggregates enter cells to induce misfolding of native tau protein remains unknown.
Methods: We used a combination of biochemical, flow cytometry, confocal and automated microscopy approaches to investigate the cellular uptake mechanism of tau aggregates. Pharmacological, enzymatic, and genetic tools were employed both *in vitro* and *in vivo* to identify the cell surface structure that mediates binding and internalization of pathogenic tau seeds.
Results: We identify heparan sulfate proteoglycans (HSPGs) as a critical receptor for aggregated tau. Inhibition of HSPGs suppressed tau fibril uptake in neural cell lines and primary hippocampal neurons. Additionally, inhibition of this pathway led to complete abolishment of the propagation of tau protein misfolding from cell to cell. Finally, we used heparin mimetics to inhibit the *in vivo* internalization of recombinant tau fibrils in the cortex of non-transgenic mice.
Conclusions: The present study delineates a critical cellular pathway required for tau fibril entry into neuronal cells and demonstrates that inhibition of this pathway can abrogate the propagation of tau protein misfolding.

EPIGENETIC CONTROL OF HOMEOSTATIC PLASTICITY

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The electrical architecture of the brain is highly complex and plastic. Neural circuits must be able to maintain a precise balance between excitation and inhibition in order to maintain proper informational integrity. Neurons must also have the ability to refine synapses in an activity-dependent manner. Both of these basic properties are important in higher-order functions, such as memory storage. In addition to plasticity at individual synapses, neurons also have the ability to regulate their total synaptic inputs. A specific example of this ability is synaptic scaling, in which neurons adjust their excitatory inputs up or down in response to network activity. This phenomenon has been shown to be dependent on Ca2+ flux in the postsynaptic cell, altering calcium/calmodulin-dependent protein kinase IV (CaMKIV) activity. This leads to an alteration of protein synthesis, finally resulting in the insertion or removal of AMPA receptors in a multiplicative manner across all synapses. We hypothesize that the changes in CaMKIV activity lead to an increase/decrease in postsynaptic AMPA receptors via epigenetic mechanisms. We will test this hypothesis using electrophysiological and molecular techniques in dissociated cultures of pyramidal neurons. We predict that we will be able to manipulate synaptic scaling with the use of DNA methyltransferase and/or histone deacetylase inhibitors. We also aim to investigate the types of epigenetic changes involved in synaptic scaling. Through the use of techniques such as high pressure liquid chromatography/mass spectrometry, western blotting, chromatin immunoprecipitation, methylation-dependent immunoprecipitation, and RT-PCR we will be able to measure the epigenetic changes at the global and individual gene levels and also assess how those changes effect gene expression.

LOCALIZATION AND TEMPORAL EXPRESSION PATTERNS OF PLASMINOGEN ACTIVATOR INHIBITOR-1 AND TISSUE PLASMINOGEN ACTIVATOR IN THE SUPRACHIASMATIC NUCLEUS

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The suprachiasmatic nucleus (SCN) of the hypothalamus is the location of the central pacemaker in mammals. Though circadian rhythms are endogenous, they can be exogenously entrained through various environmental elements, such as light. The SCN receives light input from the retina through signaling of the retinohypothalamic tract (RHT). Previous studies have demonstrated the importance of extracellular proteases in the central nervous system of mammals. Within the SCN, it has been shown that tissue-type plasminogen activator (tPA) cleaves plasminogen into plasmin, which then cleaves the pro-form of brain-derived neurotrophic factor (pro-BDNF) into its active, mature form (mBDNF). This mBDNF is then free to bind to the tyrosine kinase B (TrK B) receptor, with downstream effects including phase-shifting the SCN circadian clock when activated concurrent with NMDA receptor signaling. Plasminogen activator inhibitor-1 (PAI-1) has been shown to prevent this signaling through the inhibition of tPA. Little is known about the temporal-spatial expression of these proteins within the SCN. Using the immortalized SCN 2.2 cell line, we are seeking to determine the temporal expression of tPA and PAI-1 across 3 separate cellular compartments: the cellular fraction, the extracellular matrix, and the media produced by cell culture. Briefly, SCN 2.2 cells were cultured and synchronized. Samples were taken every 3 hours, separated into the 3 components, and stored in liquid nitrogen. Western blot analyses of the cellular fractions have been conducted, using antibodies for tPA and PAI-1. Current data indicates the rhythmic expression of both tPA and PAI-1 within the cellular fraction, with tPA expression greatest during circadian night and PAI-1 expression greatest during circadian day. Further study should determine their expression within the extracellular matrix and media.

KIDNEY DYSFUNCTION FOLLOWING ORTHOPEDIC TRAUMA IN OBESE VERSUS LEAN ZUCKER RATS

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Purpose: An increased incidence of multiple organ failure occurs following trauma in obese patients, but the extent and mechanism of exacerbated damage to specific organs is incompletely understood. The kidney, in particular, has not been well studied in obesity, despite acute kidney injury being a major cause of morbidity and mortality. We hypothesized that there would be a more severe impairment in renal function following traumatic injury in obesity.
Methods: Lean (LZ) and obese (OZ) Zucker rats (11-13 wk, n=6 per group) were used to test the hypothesis. Orthopedic trauma was mimicked with bilateral hind limb soft tissue injury, followed by an injection of crushed bone components to the area. Twenty-four hours after trauma, glomerular filtration rate (GFR), renal blood flow (RBF), blood pressure (BP), and urine albumin measurements were taken.
Results: RBF was found to be similar in all groups. LZ and OZ exhibited similar basal BP (122 ± 2.6 vs. 125 ± 4.5 mmHg, respectively) and GFR (1.51 ± .15 vs. 1.70 ± .13 ml/min/g). Trauma resulted in a significantly decreased BP (108 ± 2.5 mmHg) and GFR (0.89 ± .07 ml/min/g) in OZ with no changes in LZ. Urine albumin excretion was significantly increased compared to pre-trauma values in the twenty hours following trauma in OZ (.63 ± .30 vs. 1.60 ± .51 mg), but not in LZ (.27 ± .05 vs. .51 ± .12 mg).
Discussion: These results suggest that trauma has greater deleterious effects on kidney function in obesity, despite an unchanged blood flow to the kidney. Failure of the renal autoregulatory system may contribute to this dysfunction, but further studies are needed to elucidate the underlying mechanisms of this complex relationship. Inflammatory effects and microvascular changes following trauma are important areas of future research.

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MARCKS GENE EXPRESSION PREDICTS FOR IMPROVED SURVIVAL IN THE PRONEURAL SUBTYPE OF GBM AND IS ASSOCIATED WITH AN ATYPICAL MOLECULAR SIGNATURE

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Purpose: Efforts such as The Cancer Genome Atlas (TCGA) have been instituted to offer a more personalized approach to cancer treatment. Our previous results (Jarboe et al. Clin. Cancer Res 2012; PMID: 22619307) have indicated that increased Myristoylated Alanine Rich C-kinase Substrate (*MARCKS*) expression is associated with improved clinical outcomes in the Proneural molecular subtype of Glioblastoma Multiforme (GBM) (median survival of 47.2 months vs. 12.2 months). This effect was most pronounced in tumors with unmethylated *MGMT* status (median survival 65.3 months vs. 10.7 months). Here we analyzed differential gene expression between the latter two groups of tumors to identify potential molecular signatures associated with this significantly improved prognosis.
Methods: The *affy* packaqe (version 1.14.2) in Bioconductor was utilized. RMA (robust multichip average) was applied. Statistical testing was conducted using the *maanova* package with a two-way ANOVA model. The treatment term was tested by F test to identify differential expression. Greater than 2-fold difference was considered up or downregulation.
Results: Our analysis indicated further upregulation of markers of oligodendrocytic lineage and Proneural development in the tumors with high MARCKS expression including OLIG2, NKX2-2, TCF4, OCX, and ASCL1. Unexpectedly, the greatest upregulation of gene expression (4.2-fold) in these tumors was the astrocytic marker GFAP. Additionally, the highly specific neural marker stathmin-like 2 was upregulated, as well as the synaptotagamin SYT11. Markers of neural progenitor cells such as Nestin and SOX were also upregulated.
Conclusions: Our analysis demonstrates that this improved clinical outcome is associated with an increase in markers for the oligodendrocytic lineage. There is additional upregulation of astrocytic, neural, and neural progenitor markers suggesting an atypical molecular signature. MARCKS expression could therefore be a biomarker for prognosis in this subtype and further studies are warranted to identify how this molecular signature leads to such a significant survival benefit.

ELUCIDATION OF MEMBRANE PROTEIN STRUCTURE AND ANALYSIS OF SNP EFFECTS USING CORRELATION ANALYSES

Pedro Teixeira

Purpose: KCNQ1 is involved in repolarization during the cardiac cycle. The QT interval is the stage of the cardiac cycle, which includes the rapid depolarization and repolarization. Long QT intervals (and the Long QT Syndrome or LQTS) are associated with increased likelihood of arrhythmia, fainting, and sudden death. LQTS affects approximately 1 in 5,000 -10,000 people, and the first symptomatic episode is fatal in 30% of cases. Identification of LQTS single nucleotide polymorphisms before symptoms appear is crucial.

It is possible with mathematical analysis of multiple sequence alignments (MSA) to detect correlations between amino acid sites. Further analysis elucidates discrete and nearly independent correlation networks comprised of contiguous amino acids in a protein's 3-dimensional structure. Correlation networks provide valuable spatial information. Initial results using our folding program BCL::Fold augmented with correlations enriches for native-like topologies. Recent literature suggests such information is beneficial for membrane structure prediction. Membrane protein structures are especially difficult to determine but nearly half of pharmaceutical targets include a membrane domain.

Materials and Methods: MSA were created via HHBlits. In addition, I'll be using principle component analysis, statistical coupling analysis, and direct information to analyze primary sequences and predict protein sectors.

Results: Preliminary data shows accurate contact predictions made with direct information in serine protease (ROC curve with an AUC ~ 0.8). Promising results are also seen for *de novo* generated models after adding correlation based contact restraints for protein 1EYH.

Future Directions: Correlation networks or "protein sectors" imply strong functional significance for the contained amino acid sites. We believe it is possible to apply this knowledge of evolutionary entanglement between sites and the solved structure to evaluate more accurately a SNP's impact on protein function.

A SIMPLE RETROSPECTIVE NOISE CORRECTION FOR DIFFUSION KURTOSIS IMAGING

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PURPOSE: Diffusional kurtosis imaging (DKI) is a clinically feasible diffusion magnetic resonance imaging (dMRI) technique which can be used to measure the properties of water diffusion in biological tissues to assess tissue microstructure and pathophysiology. DKI is being investigated for clinical use in diseases such as stroke, Alzheimer's disease, ADHD, traumatic brain injury, Parkinson's disease, cancer, and others, but it is often limited to low spatial resolution due to noise, which can bias the measured signal. This study investigates the effects of noise on DKI parameters for noisy phantom data. A simple retrospective noise correction scheme is presented and applied to the images. The results are compared to reference DKI estimates.

METHODS: A previously validated dairy cream phantom was imaged with a protocol adapted to increase the effects of noise. Signal to noise ratio (SNR) was varied by changing the slice thickness, and a method of complex averaging was used to establish reference estimates for the mean kurtosis, a measure of microstructural complexity obtained with DKI. The unbiased dMRI signal was estimated from the measured signal and an estimate of noise level.

RESULTS: Decreasing slice thickness increased mean kurtosis estimates for the uncorrected analysis from 0.972(±0.003) to 1.261(±0.043) in the 10.0 mm and 2.0 mm datasets, respectively. Ground truth for the mean kurtosis was found to be 0.949, which differed from the 2.0 mm estimate by 33%. The mean kurtosis estimates following noise correction differed by no more than 2.7% from the reference estimate.

DISCUSSION / CONCLUSION: The noise correction technique demonstrated provides a convenient and straightforward method for removing the majority of noise bias in DKI. It may be particularly useful for datasets when high resolution images are acquired or when imaging anatomical regions with low dMRI signal due to short T2 values, such as the globus pallidus.

NOVEL MICROFLUIDIC TECHNOLOGY CAN IDENTIFY CHANGES IN LEUKOCYTE PHYSIOLOGY FOLLOWING SEVERE TRAUMA.

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INTRODUCTION: Diverse biomolecules, pathways, and environmental factors mediate the human response to critical illness or injury; the cell is the fundamental unit for integrating these factors into organ- and system-level responses. We implemented novel microfluidic and image processing technologies to study the dynamic physiology of human immune cells. These measurements may provide new insights into the pathophysiology of critical illness and injury, and illuminate the link between molecular phenomena and clinical symptoms. In this comparative pilot study, we tested the hypothesis that leukocyte calcium (Ca++) responses would differ between healthy volunteers and critically injured patients.
METHODS: We obtained whole blood samples from 3 healthy volunteers and 3 trauma ICU patients and isolated leukocytes using similar technique. Leukocytes were incubated with Flou-4, a Ca++ sensitive fluorescent dye, for 30 min. Cells were introduced into the multi-trap nanophysiometer (MTNP), a flow-through microfluidic device designed to hold cells stationary for extended experimentation. After a baseline period, cells were stimulated with ionomycin and Ca++ flux over time was measured fluorescently.
RESULTS: Ca++ fluxes were measured in each of 809 cells across all 6 subjects. Individual cellular Ca++ responses were higher in trauma cells (mean 99.8 v. 44.4, $p<.001$, $N=809$). Inter-patient mean Ca++ response was also higher in trauma samples but the difference was not statistically significant (mean 66.1 v. 23.7, $p=0.15$, $N=6$).
CONCLUSIONS: 1) The MTNP can isolate and measure physiological dynamics of human leukocytes; 2) Leukocyte Ca++ responses were altered in trauma ICU patients; 3) The MTNP permits a wide range of future studies to understand the dynamic cellular physiology of critical illness or injury.

COMBINATORIAL TREATMENT OF MALIGNANT PERIPHERAL NERVE SHEATH TUMORS WITH TYROSINE KINASE INHIBITORS HINDERS TUMOR PROLIFERATION AND SURVIVAL

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Purpose: Malignant peripheral nerve sheath tumors (MPNSTs) are the most common cause of death in patients with the genetic tumor susceptibility disorder neurofibromatosis type-1 (NF1). These highly aggressive sarcomas are mainly treated via resection, as radiotherapy is ineffective and no chemotherapeutics have been identified. Therefore, the development of targeted chemotherapeutics is critical to improving the survival of patients diagnosed with MPNSTs. Our lab has shown that dysregulated growth factor signaling by the Schwann cell mitogen neuregulin-1 (NRG1), via the erbB3 and erbB4 receptor tyrosine kinases, promotes the pathogenesis of MPNSTs. Treatment of tumor cells with the pan-erbB inhibitor canertinib leads to a decrease in tumor proliferation. However, we hypothesize that multiple receptor tyrosine kinases are co-activated in these tumors, thereby requiring combinatorial therapy to prevent the development of resistance.
Methods: Here we test the efficacy of five receptor tyrosine kinase inhibitors in different MPNST cell lines via calcein-AM survival assay. These drugs include canertinib; sorafenib, which inhibits platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR) and Raf kinases; crizotinib, which inhibits c-Met/hepatocyte growth factor receptor (HGFR) tyrosine kinase; sunitinib, which inhibits Kit, PDGFR and VEGFR; and nilotinib, which inhibits PDGFR, c-kit and Bcr-Abl.
Results and Discussion: The inhibitors canertinib and sorafenib were both able to blunt tumor proliferation and survival as individual treatments, while sunitinib and sorafenib were ineffective as primary chemotherapeutic agents. Our data show that multiple different growth factor receptors promote tumorigenesis, with erbB membrane tyrosine kinases being of primary importance. Additionally, tumor cells show additive decreases in proliferation when treated with both canertinib and sorafenib, suggesting that dual therapy may be a viable chemotherapeutic regimen for MPNSTs.

TARGETING HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR PATHWAYS TO RENDER TRIPLE NEGATIVE BREAST CANCER CELLS SUSCEPTIBLE TO PARP INHIBITION

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Background/Objectives

Few therapeutic options are effective for the highly aggressive triple negative breast cancers (TNBCs). Inhibitors of the DNA repair protein poly(ADP-ribose) polymerase (PARP), which target homologous recombination (HR) repair deficient cells, are being actively investigated in combination with systemic DNA damaging agents. We previously reported EGFR inhibition (EGFRi) attenuates HR. In this study, we hypothesized that EGFRi can induce a contextual synthetic lethality with PARP inhibition in TNBCs. Additionally, because EGFR activates DNA repair via nuclear translocation, we hypothesized the mechanism of EGFRi-mediated attenuation of HR involves alteration of protein subcellular localization and interaction with key DNA repair proteins, such as BRCA1.

Methods

Human TNBC cells MDA-MB-231, MDA-MB-453, and MDA-MB-468 were used in this study. EGFR and PARP inhibition was achieved using lapatinib and ABT-888, respectively. HR repair was assessed by immunohistochemistry for Rad51 foci and by a chromosomal-based repair assay. Cytotoxicity was assessed by ATPlite and colony formation assays, while cellular apoptosis was determined via annexin staining and assessment for cleaved caspase 3 and 9. Protein-protein interaction was determined by immunoprecipitation. Sub-cellular localization was assessed by cellular fractionation and western blot. Lastly, in vivo tumor growth delay was assessed in mice bearing orthotopic MDA-MB-231 xenografts.

Results

EGFRi induces a contextual synthetic lethality with PARPi both in vitro (70-99% cell kill, $p<0.01$) and in vivo (>3 fold tumor growth delay, $p<0.001$). This enhanced cytotoxicity involves activation of intrinsic apoptosis. Interestingly, EGFR and BRCA1 are in the same protein complex, which is reduced by EGFRi (35-70% reduction, $p<0.01$). EGFRi also increases cytosolic BRCA1 and EGFR, away from their nuclear DNA repair substrates (2-fold reduction, $p<0.01$).

Conclusions

These results reveal a contextual synthetic lethality between combined EGFR and PARP inhibition in TNBC that occurs via novel regulation of HR repair. Importantly, this contextual synthetic lethality may exist in other EGFR-dysregulated tumors.

GENDER, GENETICS, AND HEALTHY SEXUALITY: ASSOCIATIONS WITH SEXUAL RISK TAKING IN ADOLESCENT GIRLS.

Abstract Presenter: Erica Smearman, B.S.[1,2]
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Purpose. To develop optimally efficacious HIV prevention interventions for adolescent girls, research has focused on identifying biological, psychosocial and environmental factors that influence sexual risk-behaviors. The vast majority of research on adolescent sexual behavior is focused on the adverse consequences of sexual risk. Therefore, very little is known about factors related to fundamental dimensions of sexuality such as sexual arousal, satisfaction, and sensation seeking, despite evidence suggesting their influence on sexual risk-taking.
Methods. African-American females aged 14-20 were recruited from reproductive health clinics for an HIV intervention. Baseline survey and follow-up DNA data (N=304) was used to assess biological, psychological, life history (abuse) and sociocultural (peer norm) associations with the sexuality constructs of sexual arousal, satisfaction and sensation seeking.
Results. In multivariable linear regressions, the short serotonin allele was significantly associated with reduced levels of sexual arousability, satisfaction and sensation seeking while allelic variations of dopamine were not significantly associated. Higher depressive symptoms were associated with higher arousability. Higher social support was associated with greater sexual satisfaction. Impulsivity and risk-supportive peer norms were associated with increased sexual sensation seeking. These sexuality constructs were also significantly related to number of sex partners, frequency of vaginal sex, and number of unprotected vaginal sex acts in the past six months.
Conclusions. These findings emphasize the importance of increased understanding in adolescent sexuality. The findings suggest that certain individuals may have a genetic susceptibility for reduced sexual inclination and that serotonin may play a more prominent role then previously appreciated. In addition, social support and norms have influential roles in sexual satisfaction and sensation seeking. While the depression finding appears counter intuitive, some research has demonstrated a relationship between depression and sexual-risk behavior. Therefore, this relationship warrants further investigation. These findings affirm the need for research on factors influencing fundamental dimensions of sexual experience.

THE MONETARY VALUE OF BREASTMILK IN THE UNITED STATES

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Purpose: Breastmilk is an important but undervalued economic resource. National accounting practices that fail to consider human milk as a food resource and investment in human capital produce incomplete estimates of national food production.
Methods: To assess the monetary value of breastmilk in the United States, we analyzed data from the longitudinal Infant Feeding Practices Study II. Actual breastmilk production was compared to "optimal" production, defined as 90% of US families breastfeeding exclusively for the first 6 months and partially until 1 year of age. Four economic methods for valuing unmarketed products—market alternative cost, opportunity cost, replacement cost, and commodity cost— were used.
Results: In the United States, infants under 1 year of age consume over 40 million liters of human milk per year. The market alternative cost, which values breastmilk by using the price of human milk prevailing in "the market" through milk banks, is in excess of 5.4 billion dollars. Maternal time spent breastfeeding was used to estimate the opportunity cost, which values maternal time cost of extracting breastmilk, and the replacement cost, which estimates the cost of employing a wet nurse, at $2.08 billion and $1.51 billion dollars respectively. Finally, the annual commodity cost of breastmilk substitutes such as infant formula would be $1,130 per infant or 186 million dollars nationally. Optimal breastfeeding would produce an average of 220 liters of breastmilk per infant and save the economy 170 million dollars annually– 55% of which is borne by taxpayers through the Woman, Infants, and Children (WIC) formula provision program for low-income families.
Discussion: These results demonstrate the economic impact of breastmilk, an otherwise "politically invisible" food. Efforts to estimate the monetary value of human milk may assist in the promotion of breastfeeding, influence health policies that protect maternal time for breastfeeding, and recognize female economic activity and contribution to society.

PRELIMINARY MEDICATION ADHERENCE DATA OVER ONE YEAR IN A LONGITUDINAL COHORT OF POST-MENOPAUSAL WOMEN WITH EARLY STAGE BREAST CANCER INITIATING AROMATASE INHIBITORS

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BACKGROUND: In 2005, aromatase inhibitors (AIs) became standard care for postmenopausal early-stage breast cancer patients to prevent recurrence. AIs are taken orally daily for 3-5 years. There is concern about nonadherence to AIs in clinical populations. Our objectives are to measure AI adherence prospectively and better understand AI adherence predictors. METHODS: To date we collected data from 72 postmenopausal female oncology outpatients within 10 days of AI initiation, via self-report by paper surveys, completed every 2 weeks for 12 weeks, and at 52 weeks. We assess also clinical and sociodemographic characteristics, side effects, physical function, depression, and menopausal symptoms. Adherence is assessed via the Morisky Medication Adherence-Hormonal scale with scores ranging from 0-8. Scores <6 denote low adherence and =8 denote high adherence. We conducted univariate analyses and multivariable logistic regression modeling with robust standard errors. RESULTS: Seven women (10%) were classified as having low adherence, 17(24%) medium adherence, and 48(67%) high adherence over the 52-week period. In univariate analyses, we observed no association between adherence and any other factor measured. In the multivariable analysis, baseline income ($p<0.01$), physical function ($p=0.04$), and depression ($p=0.04$), and longitudinal out-of-pocket costs ($p<.01$) and menopausal symptoms ($p=0.04$) were associated with low adherence. DISCUSSION: If only two thirds of women taking AIs are adherent, reduced clinical effectiveness of AIs could lead to recurrence and mortality in cancer populations. In this cohort, we plan in future analyses to further investigate factors associated with adherence and to compare self-reported adherence with electronic monitoring and pill count data.

PERIPHERAL REGULATORY B CELL PHENOTYPE IN MULTIPLE SCLEROSIS PATIENTS

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Regulatory B cells (Bregs) are a unique $CD5^+/CD1d^+/IL10^+$ B cell subtype which has novel immunosuppressive capabilities. It remains to be determined if subjects suffering from autoimmune ailments such as multiple sclerosis (MS) display a defect in number or function of Bregs in association with their disease. Our hypothesis is that total B cell number, and Breg subset in particular, is distinct when comparing peripheral blood B cell populations from MS to healthy controls (HC). This study herein focuses on establishing a phenotypic baseline of the peripheral B cell profile. The total number of $CD19^+$ B cells in HC is 892±816 cells/µL (average ±SD, n=28), of which $CD5^+/CD1d^+$ Breg subset is 4.6±5.6 cells/µL. We found a greater number of B cells in MS subjects, 1441±1011 cells/µL (n=23, $p<0.05$, Student's t-test). However, the number of Bregs in MS patients is not significantly different from HC, 7.5±7.4 cells/µL ($p=0.13$). We further interrogated the phenotype of B cells in MS subjects and found that there is an increased number of $CD27^-IgD^+$ naïve/intermediate phenotype in MS, 1041±794 cells/µL, versus HC, 624±606 cells/µL ($p<0.05$). Our findings demonstrate that MS is not associated with a deficiency in the absolute number of $CD5^+/CD1d^+$ Bregs but rather a 60% increase in $CD27^-IgD^+$ B cells. We hypothesize that altered B cell distribution may be functionally associated to the underlying immune disease process of MS. This finding may inform the design of clinical trials examining B-cell depletion strategies for treatment of MS.

A POTENTIAL ROLE FOR INSULIN-LIKE GROWTH FACTORS IN CD4-T CELL DIFFERENTIATION AND FUNCTION

Daniel DiToro

Decades ago, insulin-like growth factors 1 and 2 (Igf1 and Igf2) were shown to be essential for the development and proliferation of myeloid and lymphoid cells. Produced in large amounts by bone marrow stromal cells and thymic epithelial cells, these cytokines function in part to drive proliferation of developing B and T cells. They have also been shown to influence proliferation of these cells following antigen stimulation. Early attempts to identify potential roles for these cytokines in CD4 T cell differentiation lead to mixed results, and the topic was eventually dropped. Recent studies, however, suggest a possible lineage-specific role for these cytokines. Microarray analyses of the various CD4 effector subsets by our lab and others consistently demonstrate differential expression of Igf-related genes. Th17 cells appear uniquely capable of responding to insulin-like growth factors, up-regulating both the signaling receptor, Igf1R, and a cytokine binding protein, Igfbp4, at least ten fold above naïve CD4s, while other subsets specifically down-regulate these genes. Validation of the microarray data by qPCR confirmed these observations and revealed the expression of insulin-like growth factor 2 in Th17 cells to be up-regulated 200 fold over naïve and 250 fold over Th1 cells, despite consistent failure of probes for this gene to generate signal in the various microarrays. Subsequent in vitro CD4 polarizations in the presence of either Igf1 or Igf2 lead to a significant increase in Il17A producing CD4s. Given the observations that Igf peptides reduce type 1 diabetes in NOD mice, slow the onset of EAE and protect from sepsis in multiple mouse models, and the essential role for CD4 T cells in all of these processes, we believe a more thorough understanding of the role these cytokines play in CD4 T cell differentiation and function may yield important opportunities for clinical applications.

IN VITRO TOOLS FOR IDENTIFYING SFB SIGNALS THAT STIMULATE TH17 CELL DEVELOPMENT

Marianne Ligon, Dan R. Littman

Commensal gut bacteria perform essential functions contributing to vertebrate homeostasis. In addition to metabolic activities, these commensal bacteria influence the development and maintenance of the host immune system. One such bacterial species, segmented filamentous bacteria (SFB), specifically stimulates the expansion of Th17 cells in the small intestine lamina propria. Th17 cells are important in maintaining immunity at mucosal barriers, yet can also contribute to systemic autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, and asthma. SFB, like many other commensal gut bacteria, are non-cultivable *ex vivo* and therefore present a challenge for identifying host-commensal signaling pathways that influence such host immunity. In this work, I adapt, develop, and optimize a cosmid cloning protocol for environmentally-derived bacterial DNA (eDNA) to generate cosmid libraries of commensal gut bacteria. These cosmid libraries will be useful resources to further understand the basic biology of non-cultivable commensal gut bacteria, the influence of the microbiome on health and disease, and the specific pathways involved in Th17 cell development and autoimmune disease pathogenesis.

INDUCIBLE B EFFECTOR CELLS (IBECS) FOR CANCER IMMUNOTHERAPY

Spencer Ng, Claudia Penafuerte and Jacques Galipeau

While cellular immunotherapy for malignances has traditionally focused on the development and manipulation of tumor targeted DCs, T and NK cells, B cells have remained vastly underutilized as a potential source for adoptive cell therapy. Here we demonstrate that B cells stimulated with FIST-2, a novel chimeric protein consisting of IL-2 fused to the ectodomain of the TGFβ receptor (type II), can adopt an effector phenotype with potent antitumor activity. Treatment with FIST-2 induces naïve splenic B cells to become B effector cells (iBECs), characterized by hyperphosphorylation of STAT3 and 5 downstream of the IL-2 receptor, upregulation of transcription factor, T-bet, and secretion of pro-inflammatory cytokines: IFNγ, TNFα and IL-6. iBECs retained their B cell identity by CD19 and PAX5 expression, but adopted an enhanced APC phenotype through upregulation of cell surface markers associated with antigen presentation and co-stimulation, including: MHC-II, CD80 and CD86. To determine whether iBECs conferred antitumor immunity, we utilized a mouse model of lymphoma expressing ovalbumin (EG.7-OVA). Syngeneic iBECs pulsed with OVA were able to activate OVA-specific OT-I and OT-II T cells *in vitro*, suggesting that they act as APCs. *In vivo* administration of OVA-pulsed iBECs protected immunocompetent C57BL/6 mice from EG.7-OVA tumor challenge, and promoted tumor regression in mice with pre-established tumors. These data support the concept of B cell-based adoptive immunotherapy.

GRAFTING THE TREE OF LIFE: RECURRENT HORIZONTAL GENE TRANSFER OF A *WOLBACHIA* LYSOZYME BETWEEN BACTERIA, ARCHAEA, AND EUKARYOTES

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Horizontal gene transfer is surprisingly common and is recognized as an important player in evolution, mixing genes between different species and even different domains of life. We describe here the likely horizontal transfer of a gene in bacteriophage WO, which infects the symbiotic bacteria *Wolbachia*, between various viruses, bacteria, archaea, and eukaryotes. This gene encodes a lysozyme that digests peptidoglycan and may therefore bestow antibiotic potential in all three domains of life. Phylogenetic analysis indicates the lysozyme is present in the archaean *Aciduliprofundum boonei* and the plant *Selaginella moellendorffii*, as well as numerous bacteria and fungi in addition to the previously reported insect *Acyrthosiphon pisum*. Genomic integration of the lysozyme gene was confirmed by PCR and sequencing in closely related species of the aforementioned taxa. Sequence alignments and protein modeling show that many of the residues lining the putative active site of this lysozyme are highly conserved amongst all members of the phylogeny. We therefore hypothesize that the lysozymes are functional and may be used by the non-bacterial species for antimicrobial defense. The lysozymes from three species are being cloned and purified to test their antimicrobial properties *in vitro*. Should these lysozymes remain active as predicted, this would suggest horizontal gene transfer of an antimicrobial gene between all three domains of life.

THE PARK18 SNP IS ASSOCIATED WITH ALTERED EXPRESSION AND REGULATION OF MAJOR HISTOCOMPATIBILITY COMPLEX II GENES IN PARKINSON'S DISEASE PATIENTS.

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Purpose: A single nucleotide polymorphism (SNP) in the first intron of the HLA-DRA gene, PARK18, was recently shown to be associated with a higher risk of Parkinson's disease (PD) in a genome-wide association study. Individuals homozygous for the rs3120882 G SNP (PARK18GG) have a 1.7 fold higher risk ($p=5x10^{-8}$) for PD than individuals homozygous for the A allele (PARK18AA). HLA-DRA is a located in the major histocompatibility complex II (MHCII) locus on chromosome 6 which encodes proteins that present antigens on the surface of antigen presenting cells (APCs) to activate the adaptive immune system. Implication of this genetic locus in PD is exciting because it provides a direct link to explain how adaptive immunity can exacerbate inflammation in the context of disease.

Results: Our examination of MHCII gene and protein expression in APCs, specifically B cells and monocytes, from the peripheral blood of PARK18GG PD patients compared to PARK18AA healthy age-matched controls has revealed interesting differences in the levels of HLA-DR and HLA-DQ expression. Monocytes from these patients also respond differently to interferon-γ stimulation with respect to MHCII expression. We have also examined gene sequences from the first intron of HLA-DRA in these patients for potential regulatory activity through a luciferase reporter assay in a human monocyte cell line. Furthermore, we have looked for other potential gene sequence polymorphisms that are also linked with PARK18 in promoter and regulatory sequences of the MHCII locus. Understanding the precise genetic and molecular regulatory mechanisms of how the PARK18 SNP is linked to these expression changes is currently underway.

Conclusion: Our initial findings indicate that this genetic marker is clearly linked to altered expression of these immune molecules in PD patients and provides evidence to clarify the role of adaptive immunity in disease progression [Funding: Emory Udall Center for PD Research (JMB/MGT); MJFox Foundation (JMB)].

RAB11A DIRECTS RHODOPSIN TRAFFICKING IN ROD PHOTORECEPTORS

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Precise vectorial transport of rhodopsin is essential for rod photoreceptor health and function. Mutations that truncate or extend the carboxy terminus of rhodopsin disrupt essential transport and lead to retinal degeneration in human patients and in mouse models. We hypothesize that these mutations disrupt an essential intermolecular interaction of rhodopsin with trafficking proteins. To test this hypothesis, we conducted pull-down assays using native rhodopsin from wild-type mice and mice homozygous for gene mutations encoding defective rhodopsin carboxy termini. Using this method, we show the novel finding that such mutations disrupt the binding interaction between rhodopsin and the small GTPase rab11a. To confirm this result in intact rod cells, we used the proximity ligation assay (PLA) to verify the rhodopsin-rab11a interaction in sections from wild-type mouse retina. In accordance with our *in vitro* data, the rhodopsin-rab11a interaction was severely diminished in sections from mice with homozygous mutations in the carboxy terminus of rhodopsin. To expand on these studies we conducted binding assays between GST-fusions of rab11a mutants and purified rhodopsin. These assays show the rhodopsin-rab11a binding is direct and does not depend on the nucleotide binding status of rab11a. To investigate whether the nucleotide binding status of rab11a is important *in vivo*, we expressed EGFP-tagged mutants of rab11a in *Xenopus laevis* tadpole photoreceptors. Expression of EGFP-rab11a or the constitutively active mutant Q70L rab11a did not cause rhodopsin mislocalization or photoreceptor degeneration. While the expression of the dominant-negative mutant S25N rab11a did not cause degeneration, the mutant rab11a ectopically accumulated in the outer segment. Expression of a different dominant-negative mutation N124I rab11a did cause rhodopsin mislocalization and photoreceptor degeneration. Taken together our results show the critical importance of rhodopsin-rab11a interactions to support the formation and maintenance of vertebrate photoreceptors.

SUBCELLULAR DISTRIBUTION OF MINERALOCORTICOID RECEPTOR IN HIPPOCAMPAL NEURON IN RAT

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Purpose: To study the subcellular distribution of mineralocorticoid receptor (MR) in hippocampal CA1 region. Methods: female Sprague-Darley rat brain was fixed with 3% paraformaldehyde and 0.5% glutaldehyde and sectioned by vibrotome. Brain tissues were incubated with specific mineralocorticoid receptor antibody (4G5, 2B7, 1-18) produced in lab and labeled by Nanogold (12nm). Each grid was analyzed with transmission electron microscope and Image-Pro plus 6.0 program. Result: Labeled mineralocorticoid receptors were found in cytoplasma, neural membrane, nuclei, mitochondria and neural synapses. Discussion/Conclusion: MR was known well expressed in rat hippocampal neuron. Under light microscope, immune-labeled MR can be found in both nuclei and cytoplasm. Like other steroid receptors, activated MR by corticosterone translocates into the nuclear compartment and functions as transcriptional factor to alter hippocampal neuron properties slowly but consistently. Recent physiological studies of corticosterone showed rapid effect through its receptors which were mainly through MR. However, anatomical location membrane-bound MR is unclear. This experiment clearly proved the fact that MR is also membrane-bound. MR distribution in neural synapses and mitochondria indicates its possible function in neural signal transduction and cell apoptosis in hippocampus.

SCREENING MOLECULES FOR AMYLOID IMAGING: A HIGH-THROUGHPUT ASSAY

***Richard A. McClure**, Chad Chumbley, Erik Todd, Richard Caprioli, Wellington Pham*

Specific Aim: The immediate aim of this research is the development of a high-throughput assay capable of screening candidate molecules for their ability to bind βA plaques. If successful, this novel assay could provide a means for identifying new classes of molecules capable of serving as diagnostic Positron Emission Tomography (PET) tracers of the hallmark lesion of Alzheimer's disease (AD). The anticipated long-term outcome of this research is the identification of a novel βA plaque binding molecule which can then be radio-labeled and utilized to screen patients for the early detection of AD using PET-based amyloid imaging.

Significance: Despite a myriad of efforts, a reliable screening diagnostic for AD, prior to the onset of symptoms, has remained elusive. PET-based imaging of β-amyloid (βA) plaques remains an ideal diagnostic methodology as it facilitates the imaging of low density targets while minimizing side effects due to the picomolar concentrations of tracer needed.[1] Currently, only Pittsburgh Compound-B has been shown capable of differentiating between normal brains and those affected by AD.[2] In addition, studies have shown that reliance on transgenic mouse models may be inappropriate for identifying PET tracers for amyloid imaging.[3] In summary, development of novel radio-labeled ligands for detection of βA plaques in-vivo has proven challenging and continues to suffer from a distinct paucity of screening methodologies necessary to refine candidate molecules prior to further investigation in humans. Our study will provide one such screening methodology and potentially lead to the discovery of a new class of compounds capable of functioning as PET tracers for amyloid imaging.

Innovation: Without high-throughput screening assays, the identification of molecules capable of binding βA plaques relies largely on formulating derivatives of known βA binding agents such as thioflavin.[4-5] However, this deductive approach limits our ability to select compounds based on other criteria such as the ability to cross the blood brain barrier (BBB). By developing an assay which evaluates a molecule's specificity for βA plaques, the necessity to adhere to molecules with similar chemical structure to known binding agents is obviated. Instead, candidate molecules can be screened based on their kinetics and ability to cross the BBB, thus substantially refining the parameters used to seek potential PET tracers for amyloid imaging.

Approach: Staining with thioflavin remains the most reliable methodology for visualizing βA plaques via fluorescence microscopy.[6] In the presence of βA, the fluorescent emission of thioflavin is dramatically increased when compared to thioflavin not complexed with plaques. By employing the 5XFAD mouse model, a brain lysate containing numerous βA plaques can be created in animals aged less than 8 months. By combining thioflavin with this amyloid lysate, the thioflavin emission is maximized and serves as a stable indicator of βA-binding. Molecules capable of binding βA plaques can then be identified through their ability to competitively bind βA plaques resulting in an attenuation of thioflavin emission due to the reduction of thioflavin-βA plaque complexes. The advantage of using this assay is that it can be optimized to achieve compatibility with plate readers facilitating high through-put screening. Using this assay, we have identified Promethazine and Resveratrol as two compounds which potentially bind βA plaques.

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Expression and function of proton-permeable cation channels in human glioma cells

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Gliomas are both the most common and the most aggressive primary brain cancer in humans, causing significant comorbidities and loss of life despite the best current treatments. They display striking extracellular pH (pH_e) heterogeneity due to a combination of factors including increased lactic acid production (due to hypoxia) and increased extracellular acidification (via various exchangers and pumps). We wondered if pH_e is purely a metabolic byproduct or if it can serve as a signal for glioma growth and invasion. To this end, we first found via Coulter Counter and BrdU-labeling experiments a marked inhibition in glioma cell proliferation at low pH_e (6.0) and a seemingly unbounded increase in proliferation at high pH_e (up to 8.8). We then hypothesized that ion channels could serve as the transmembrane chemosensor to translate the extracellular pH environment into intracellular changes, leading to pH_e-dependent glioma growth. We discovered large whole-cell pH_e-sensitive changes in both conductance and resting membrane potential (V_m) of human glioma cells. The underlying cation currents are tonically active, quinine- and 2-aminoethoxydiphenyl borate (2-APB)-sensitive, and reversibly abolished by acidic pH_e (6.0). From here, we speculated that it was these pH_e-dependent changes in ion flux and V_m that could be causing the changes in glioma growth. In this vein, both quinine and 2-APB affected glioma cell proliferation in a pH_e-dependent manner, implicating this cation conductance in cell physiology. In higher pH_e (and with larger cation conductance) the cells displayed greater drug sensitivity. Interestingly, in nominally Na^+- and K^+-free conditions, there exists a residual current that is pH_e- and 2-APB-sensitive and follows the reversal potential for protons, suggesting a direct proton permeability that is pH_e-dependent, with larger fluxes from lower external proton concentrations (higher pH_e). Hence we show evidence for a hitherto unrecognized cation conductance in glioma cells with properties that exploit the unique pH environment around these tumors.

ALTERED BEHAVIORAL AND SEROTONERGIC FUNCTION IN THE DHCR7 HETEROZYGOUS MOUSE MODEL FOR SMITH-LEMLI-OPITZ SYNDROME

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Purpose: Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder that is marked by a mutation in the cholesterol precursor 7-dehydrocholesterol reductase (7-DHC). Dhcr7+/- mice have elevated oxidative damage in the brain including higher levels of oxysterols. Dhcr7+/- mice also have fewer serotonergic (5-HT) cell bodies in the dorsal raphe nucleus, but increased 5-HT immunoreactivity. This represents an altered 5-HT system with potential compensatory upregulation. The purpose of this experiment was to assess changes in the 5HT system using pharmacological compounds for specific 5HT receptors to identify distinct changes in the Dhcr7+/- mice.
Methods: The current experiments were conducted in a mouse model for SLOS that carries one of the same mutations in the Dhcr7 as in humans. The mouse model the homozygous Dhcr7-/- genotype is embryonic lethal, therefore all experiments were undertaken in heterozygous mice (Dhcr7+/-). Mice were treated with 2,5-Dimethoxy-4-iodoamphetamine, (DOI) an agonist for the serotonergic receptor, 5HT-2A which elicits head twitch and face wash behaviors. Mice were treated (1mg/kg, i.p.) 10 minutes prior to behavior analysis. M-100907 a 5HT-2A receptor agonist and 8-OH-DPAT, a 5HT-1A agonist were also administered and analysis followed.
Results: Treatment with DOI leads to exaggerated head twitch behaviors in Dhcr7+/- and wild-type control mice but the effect is much greater in the heterozygous mice, and the difference was reduced by treatment with M-100907. The same altered response pattern was not found for 5HT-1a receptor agonist 8-OH-DPAT induced hypothermia.
Conclusions/ Discussion: These data have important implications for standard treatments for autistic patients, which include selective serotonin reuptake inhibitors, and which may not have the desired effects in SLOS patients with increased 5-HT sensitivity. This shows the importance of personalized medicine that is catered to each patient's case rather than treatment for a diagnosis.

ANTIDEPRESSIVE EFFECTS OF DNA METHYLTRANSFERASE INHIBITION

Joshua Cohen

Major depressive disorder (MDD) is a common and serious syndrome characterized by depressed mood, anhedonia, disturbed sleep, appetite, and energy, reduced concentration, excessive guilt, and suicidal. Despite its widespread prevalence little is known about the pathology of MDD. An emerging area of interest is the role that epigenetics may play in MDD as well as other mental illnesses.

Epigenetics refers to the state of DNA packing and chromatin structure, which controls transcription or silencing of genes by facilitating or blocking access to the gene by transcription machinery. Of particular interest, is a class of proteins called DNA methyltransferases (DNMTs) that methylate individual cytosine residues on DNA. Recent work by Sales *et al.* (2011) has shown that DNMT inhibition, by both systemic and intra-hippocampal administration, has anti-depressive effects in rats.

If DNMT inhibition is truly anti-depressive, then it is likely that epigenetic mechanisms play a role in the pathology of MDD. The goal of the present study was to replicate the findings of Sales *et al* in our selectively bred low responder (bLRs) model of depression. bLRs were bred based on low exploration behavior in novel environments, and have since been found to have enhanced anxiety and depressive-like behaviors/vulnerabilities. We show that intra-ventricular injections of the DMNT inhibitor RG-108 reduces time spent immobile by bLRs in a forced swim test, a measure of “depressive-like behavior,” and reduced overall cytosine methylation levels in the hippocampus.

"PATHOGENESIS OF NOVEL FMR1 MUTATIONS IN FRAGILE X SYNDROME"

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Fragile X Syndrome (FXS) is the most common cause of inherited intellectual disability and one of the leading known causes of autism. Most cases of FXS are caused by a trinucleotide repeat expansion within the 5'UTR of the gene *FMR1*. However, rare missense and indel mutations in the coding region of the gene have also been reported in a handful of patients. We have identified a novel variant, R138Q, in a patient with several FXS-like symptoms who tested negative for repeat expansion. To determine if R138Q is a pathological variant, we made *Fmr1* lentiviral constructs with this variant and tested its ability to rescue several phenotypes in *Fmr1* KO neurons. Interestingly, we found that R138Q is unable to rescue synaptic overgrowth at the *Drosophila* neuromuscular junction (NMJ), suggesting this variant may specifically impair FMRP function in the presynaptic compartment. In other assays testing the various cellular roles of FMRP, including AMPAR trafficking and neuronal stem cell differentiation, R138Q performed similar to WT, indicating this mutation does not cause global impairment in FMRP function. To confirm the presynaptic defect phenotype in a mammalian system, we plan to study the ability of the R138Q mutant to rescue induced growth cone collapse in *Fmr1* KO murine neurons. These results give important insights into the mechanisms of disease in this patient and provide clues about how the different domains and structure of FMRP may be involved in FMRP's normal functions, particularly at the presynapse.

MECHANISMS FOR PREPARING AND MAINTAINING TASK STATE COMPARED IN AUDITORY AND VISUAL CORTEX

Abdurahman Elkhetali

Attention and task state influence information processing in both visual and auditory cortex. One might expect that the mechanisms of this influence are similar in each region. On the other hand, because auditory and visual information go through different processing steps on the way to cortex, one might expect that the influence of attention on visual and auditory cortex would be different. We examined three measures of task state-related activity in visual and auditory cortex during performance of visual and auditory tasks that were as analogous as possible, in order to determine if the mechanisms for preparing and maintaining task state are similar in the two sensory regions.

Twenty participants performed a change detection task for auditory or visual stimuli. The visual stimuli were gray-scale horizontal gratings (Gabor patches), which cause sine wave patterns in the retina. The auditory stimuli, called "ripple sounds" cause sine wave patterns in the cochlea and are thought to be processed analogously (for example, effects of lateral inhibition on each type of stimulus are thought to be alike). The similarities in the way the stimuli activate the retina and the cochlea suggests that the steps of information processing needed to distinguish them are analogous across domains.

In some conditions ("Unimodal"), the stimuli were presented alone, and in other conditions ("Bimodal"), both types of stimuli were presented simultaneously, but the participants attended to only one. There were a total of four different trial types: Auditory Unimodal, Visual Unimodal, Auditory Bimodal and Visual Bimodal.

Regions of interest in visual and auditory cortex were created for each subject based on responses to visual or auditory stimuli. Three measures of attention-related, ongoing, non-stimulus-driven activity were measured in auditory and visual cortex. 1) Cue activity: Transient baseline shifts in activity elicited by cues instructing participants to attend to a relevant stimulus. 2) Task Maintenance activity: baseline shifts in activity maintained over successive trials. 3) Task Initiation activity: the transient activity associated with the onset of a task block.

We compared these three measures of attention-related activity for each of the four trial types. Data show that Cue activity and Task Maintenance activity in the visual cortex depend on task state and mirror each other. The auditory cortex showed a very different pattern of activity, however, indicating that visual and auditory cortex have different mechanisms for preparing and maintaining task state.

KINETIC CONTROL OF AMYLOID SELF-ASSEMBLY

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Purpose： We tested the hypothesis that amyloid self assembly can be directed to varies pathways by adding seeds with different structures, demonstrating the concept of kinetic regulation of Aβ self assembly in Alzheimer's Diseases.

Methods： The nucleation core of A beta protein Aβ（16-22）KLVFFAE exhibits fibril structure in neutral pH while its mutant KLVFFAL forms tubes. Sonicated fragments of KLVFFAL tubes are added to Aβ(16-22) monomers pre treated with HFIP. Their morphology was observed by Transmission Electron Microscopy (TEM) and Circular Dichroism (CD).

Results： From TEM image, The mutant seeded monomers grow into a transient product of tubes and later fibers sprout in the vicinity of the tubes. CD reading shows a dramatic increase of CD signature in Day 5 and a decrease in Day 7, confirming the conformation change.

Discussion/Conclusion：Our study demonstrate that the self assembly process can be regulated by kinetic processes and expands the existing understanding of amyloid aggregation process.

C-TYPE NATRIURETIC PEPTIDE: A POTENTIAL THERAPY FOR DIABETIC MACULAR EDEMA

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A central component of vision loss in diabetic retinopathy is the development of macular edema. Macular edema develops when the ability of retinal pigment epithelium (RPE) to actively remove fluid cannot compensate for the fluid entry from the retinal vasculature. Natriuretic peptides (NP) (atrial, brain, and C-type) play a central role in cardiovascular homeostasis and the receptors for these peptides are expressed in the retina. However their role in regulating retinal vasculature and the development of macular edema has not been investigated.

Methods: We used transepithelial resistance (TEER) to assess the barrier function of adult RPE cells (ARPE19) and primary human fetal RPE cells (hfRPE) monolayers cultured on transwell filters following human glycated albumin treatment in the absence or presence of different concentrations (1pM to 100 nM) of ANP, BNP or CNP . Immunohistochemistry and immunoblotting was used to further test the expression and localization of the NPR in RPE monolayers.

Results: Our data showed that glycated albumin can disrupt RPE barrier function. This response was concentration dependent with a maximal reduction in TEER of 40 ± 2% for ARPE-19 and 27 ± 7% for hfRPE at 100 µg/mL six hours post treatment. One hour pretreatment with ANP, BNP or CNP blocked this effect. The rank-order of agonist potency of natriuretic peptides CNP (IC_{50}=9.5 pM)> BNP (IC_{50}0.9 nM) ≥ ANP (IC_{50}=2.5nM) supports the idea that NP receptor 2 is the main mediator of this response. Immunohistochemistry and immunoblotting data are consistent with our functional studies.

Conclusion: Our data demonstrate that NPs, CNP being the most potent, can reverse the increase in permeability in RPE monolayers induced by glycated albumin. These studies present NP agonists as potential new candidates for treating retinal edema in diabetic patients.

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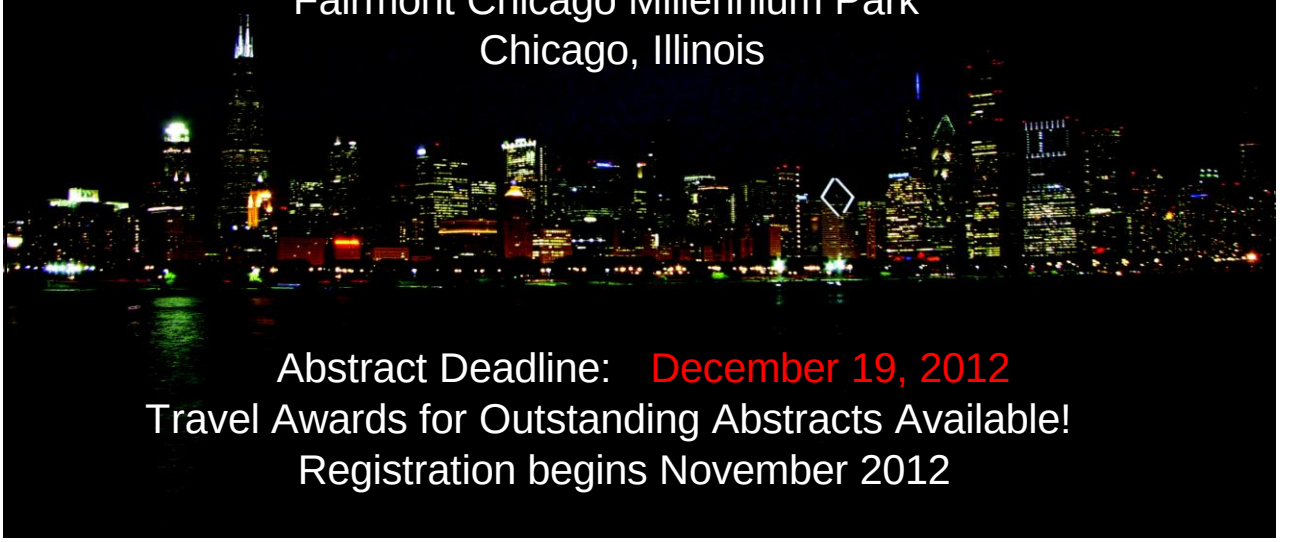

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