8TH ANNUAL SOUTHEASTERN MEDICAL SCIENTIST SYMPOSIUM (SEMSS)

SEMSS

SOUTHEASTERN MEDICAL SCIENTIST SYMPOSIUM

OFFICIAL PROGRAM

EMORY UNIVERSITY

SCHOOL OF MEDICINE

ATLANTA, GA

NOVEMBER 18-19, 2017

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Funding for this conference was made possible in part by NIH R13GM109532 from the National Institute of General Medical Sciences, a component of the National Institutes of Health. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention by trade names, commercial practices, or organizations imply endorsement by the U.S. Government.

SCHEDULE

SATURDAY, NOVEMBER 18, 2017

REGISTRATION — 12:00-12:30 PM

Location: Emory Conference Center – Emory Amphitheater

WELCOME AND OPENING REMARKS — 12:30-1:00 PM

Speakers: American Physician Scientists Association

SEMSS Committee

Welcome from Dr. Robert Gross, Emory MSTP Director

Location: Emory Conference Center – Emory Amphitheater

KEYNOTE SPEAKER 1 — 1:00-1:50 PM

Speakers: Craig Blackstone, MD, PhD

National Institutes of Neurological Disorders and Stroke (NINDS)

Location: Emory Conference Center – Emory Amphitheater

BREAKOUT SESSION 1 — 2:00-2:50 PM

- Undergraduate Track: Life of an MD/PhD Student
- Graduate Track: Career Options for Physician Scientists

Location: Emory Conference Center – Emory Amphitheater (Undergraduate Track)

Emory Conference Center – Oak Amphitheater (Graduate Track)

POSTER SESSION 1 — 3:00-3:50 PM

Location: Emory Conference Center – Lullwater Ballroom

BREAKOUT SESSION 2 — 4:00-4:50 PM

- Undergraduate Track: MD/PhD Admissions Information with MD/PhD Directors
- Graduate Track: Networking Meet-and-Greet

Location: Emory Conference Center – Emory Amphitheater (Undergraduate Track)

Emory Conference Center – Azalea Ballroom (Graduate Track)

SCHEDULE

SATURDAY, NOVEMBER 18, 2017 (CONTINUED)

KEYNOTE SPEAKER 2 — 5:00-5:50 PM

Speakers: Margaret Baron, MD, PhD

Icahn School of Medicine at Mount Sinai

Location: Emory Conference Center – Emory Amphitheater

POSTER SESSION 2 — 6:00-6:50 PM

Location: Emory Conference Center – Lullwater Ballroom

RECEPTION — 7:00-8:30 PM

Location: Emory Conference Center – Lullwater Ballroom

SUNDAY, NOVEMBER 19, 2017

BREAKFAST — 7:30-8:00 AM

Location: Emory School of Medicine, Rooms 190P, 178P, and 170A

The same breakfast will be served in each of the oral presentation rooms

ORAL PRESENTATIONS — 8:00-10:20 AM

Location: Emory School of Medicine, Rooms 190P, 178P, 170A

KEYNOTE SPEAKER 3 — 10:30-11:20 AM

Speakers: Lucienne Ide, MD, PhD

Healthcare innovator and CEO of Rimidi, Inc.

Location: Emory School of Medicine – Lecture Hall 120

CLOSING REMARKS, AWARDS, AND LUNCH — 11:30-12:00 PM

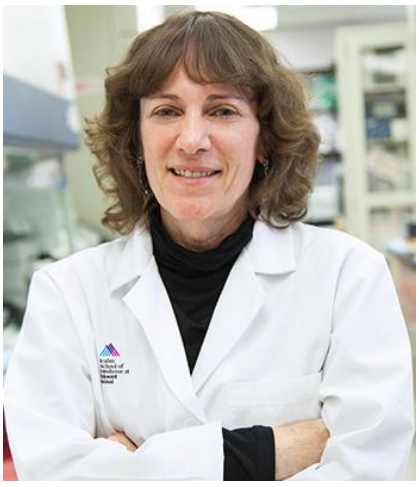
Location: Emory School of Medicine – Lecture Hall 120

KEYNOTE SPEAKERS

CRAIG BLACKSTONE, MD, PHD – NATIONAL INSTITUTES OF NEUROLOGICAL DISORDERS AND STROKE (NINDS)

Dr. Craig Blackstone serves as Senior Investigator and Section Chief of the Neurogenetics Branch of National Institute of Neurological Disorders and Stroke (NINDS) at the National Institutes of Health (NIH). Dr. Blackstone is also the Director of the NIH MD/PhD Partnership Training Program and Vice President of the American Neurological Association. Dr. Blackstone received his B.S. and M.S. degrees from the University of Chicago and his M.D. and Ph.D. degrees from Johns Hopkins University. He then completed his neurology residency at the Harvard-Longwood Neurology Program, followed by a concurrent clinical movement disorders fellowship at Massachusetts General Hospital and post-doctoral fellowship at Harvard Medical School. As a physician-scientist, Dr. Blackstone has established an impressive career studying the cellular mechanisms underlying neuromuscular and movement disorders. We are honored to have Dr. Blackstone present a keynote speech at SEMSS this year.

MARGARET BARON, MD, PHD - ICAHN SCHOOL OF MEDICINE AT MOUNT SINAI



Dr. Baron is the Fishberg Professor of Medicine in Medicine, Hematology, and Medical Oncology, Oncological Sciences, and Cell, Developmental and Regenerative Biology. She also serves as the Senior Associate Dean for Education and Director of the MD/PhD Program and Director of the Program in Hematology and Blood Disorders at Mount Sinai. After graduating from Harvard summa cum laude, Dr. Baron received her MD from Harvard Medical School and her PhD from M.I.T. through their Program in Health Sciences and Technology. She went on to Internal Medicine residency at Massachusetts General Hospital and a post-doctoral fellowship at Harvard University. Dr. Baron was an assistant and then associate professsor at Harvard before moving to Mount Sinai in 1997. Dr. Baron is an internationally renowned developmental biologist and her research has made important contributions to the understanding of hematopoietic development. Dr. Baron's work as a physician-scientist investigator exemplifies interdisciplinary and translational science, and her dedication to mentorship and teaching have helped her become a leader in her field. We are very grateful that Dr. Baron will share her career insights with us this year.

LUCIENNE M. IDE, MD, PHD - HEALTHCARE INNOVATOR AND CEO, RIMIDI

Dr. Ide is a healthcare innovator and CEO of Rimidi, Inc., and brings her diverse experiences in medicine, science, venture capital, and technology to transforming the delivery of healthcare for chronic diseases. Rimidi's cloud-based diabetes management platform provides a comprehensive solution for healthcare systems that connects healthcare providers with their patients. Rimidi enables providers to receive a clear snapshot of each patient's diabetes numbers and unique needs by merging patient and clinical data and provides them with decision support tools to make personalized treatment decisions. Dr. Ide founded Rimidi with the purpose of improving the health of the chronically ill and the healthcare system as a whole. "Our digital health technology helps health systems achieve the Quadruple Aim of improved outcomes, better patient experience, lower overall cost of care for patients, and improved clinician satisfaction," says Dr. Ide.

Prior to starting Rimidi in 2012, Dr. Ide worked as a physicist at the National Security Agency, Raytheon Systems Corporation and Monarch Capital Partners, a venture capital firm. She holds a joint M.D./Ph.D. degree from Emory University, and completed her medical training at the University of Pennsylvania Medical College's Magee Womens' Hospital in Pittsburgh. A speaker on women in technology and digital health innovation, Dr. Ide has been a guest speaker at many health IT conferences and has been published on Forbes.com. A member of the App Association, an industry trade organization advocating healthcare reform, she has spent time on Capitol Hill meeting with legislative officials about digital health. She also is a board member of the Georgia Community Leadership Board of the American Diabetes Association and a founder of I Can Be The Change, a not-for-profit focused on educating youth on obesity-related health issues. She serves as a Trustee of Middlebury College in Vermont. Rimidi has been named a Top 5 Company to Watch by the Georgia Pharmacists Association and a Top 10 Innovative Company in Georgia by the Technology Association of Georgia. Rimidi also is an ATDC Select Company. Additionally, the company received a "Platinum" ranking by BAnalytics as a socially conscious company.

Rimidi has been featured in REUTERS, THE ECONOMIST, THE HUFFINGTON POST, AND MOBIHEALTH NEWS, as well as local media, including THE ATLANTA JOURNAL-CONSTITUTION, and the ATLANTA BUSINESS CHRONICLE. Dr. Ide lives in Atlanta with her husband and their four sons. We very much look forward to learning more about Dr. Ide's unique and innovative career path at SEMSS this year.

PANELIST BIOS

UNDERGRADUATE TRACK: MD/PHD ADMISSIONS INFO WITH MD/PHD DIRECTORS

Bianca Islam – M3, Augusta University – Bianca is currently a third year medical student and her doctoral research focused on identifying PDE-5 inhibitors as novel therapeutic targets for inflammatory bowel disease and colon cancer. She hopes to pursue a career as a colorectal surgeon and to find new treatment and prevention strategies for inflammatory bowel diseases and colorectal cancer.

Hyunwoo (Tony) Kwon – G2, MUSC – Tony received his Honors Bachelor's Degree in Immunology at the University of Toronto in Canada. In 2014, he moved to Charleston to join the MSTP at Medical University of South Carolina (and to finally escape cold weather). Currently, he is a graduate student in Dr. Zihai Li's cancer immunology laboratory. In his spare time, he loves to travel, play racquetball and have great conversations with friends and families.

Annie Geller – G1, University of Louisville – Annie is a third year MD/PhD student at the University of Louisville. She went to the University of Pennsylvania where she studied bioengineering, and then went to Tufts for a masters in bioengineering. After getting her masters, she worked for a year at the NIH in Bethesda. Annie's research focuses on tumor immunology, where she is currently studying the immunomodulatory effects of B-glucan, and it's role in immune cell reactivity and trafficking to tumors. She is also studying PD-1/PDL-1 expression in tumors and immunotherapy. She plans to go into either hematology/oncology or transplant.

Elizabeth Ogunrinde – G2, MUSC – Elizabeth moved from Nigeria to the US in 2001 with her family. Her family spent two frigid years in Chicago before moving to the sunshine state (Florida) where they have been ever since. She spent four awesome years at Florida State University where she majored in biochemistry and was exposed to a variety of research experiences that led her to pursue an MD-PhD degree. Now at MUSC in Charleston, SC she is studying the role of sex hormones and the microbiome in lupus pathogenesis. In her spare time, she enjoys reading, cooking, learning new languages, and thinking about long-term solutions to inefficient systems at a local and global level.

Margaret Axelrod – G1, Vanderbilt – Margaret is a third year MSTP student (first year in graduate phase) in the Cancer Biology Department at Vanderbilt. She is studying tumor immunology in the lab of Justin Balko, PharmD., PhD. Her project focuses on tumor-cell specific Major Histocompatibility Complex Class II (MHC-II), a molecule canonically thought only to be expressed by professional antigen presenting cells. She hopes to figure out what tumor cells are doing with an immune molecule and how that might play into sensitivity to immunotherapy. She aspires to be a medical oncologist and run her own translational lab. Outside of the lab, Maggie is interested in health policy advocacy and rock climbing.

Lizzie Hale – G1, Vanderbilt – Lizzie is a third year MSTP student in her first year of graduate school training. She is in the Neuroscience department and studying the structural and functional connectivity of anxiety and addiction circuitry as a part of Dr. Jenni Blackford's lab. She is particularly interested in the insula and its role in the withdrawal and negative affect stage of addiction. Lizzie also spends a lot of time thinking about ways to enhance MD/PhD wellness both at Vanderbilt and nationwide. In her free time, she enjoys playing volleyball and exploring the Tennesseean outdoors.

Carey Jansen – G1, Emory (Moderator)- Carey Jansen is a G1 MD/PhD at Emory University. She is originally from near Athens, GA and graduated in 2015 from the University of Virginia. She is in the Cancer Biology PhD program, and she studies the biology of T cell responses in genitourinary cancers in the laboratory of Haydn Kissick, PhD. She was involved in starting Emory's Physician Scientist Interest Group and serves on the MSTP Student Association.

PANELIST BIOS

GRADUATE TRACK: CAREER OPTIONS FOR PHYSICIAN SCIENTISTS

Jacob VanHouten: Jacob VanHouten is fourth year medical student at Vanderbilt University School of Medicine. He was born and grew up in central Texas and graduated from Baylor University with a Bachelors in University Scholars. At Vanderbilt he completed a PhD in Biomedical Informatics and a Master's in Biostatistics as part of the Medical Scientist Training Program. His professional interests include preventive medicine, public health informatics, systems engineering, and decision analysis. His hobbies include watching social, cultural, and science documentaries, playing many kinds of board games, and looking forward to the next TV or movie adaptation of the comic books he has grown up reading (Marvel >> DC). He has applied for combined residencies in Internal Medicine/Preventive Medicine and Family Medicine/Preventive Medicine.

Evan McClure: Evan graduated from Emory's one-year MBA program in 2016 and Emory's MD program in 2017. He is a Partner at Life Science Partner Inc., a specialized executive search firm that recruits senior leaders for healthcare and life sciences companies across the country. He also owns a small consulting firm and serves as an operating advisor to a private equity firm investing in consumer-facing health and wellness companies. During his time at Emory, Evan worked in venture capital (HealthQuest Capital), biotechnology (Genentech), and co-founded a nonprofit organization to support the healthcare startup community in Atlanta (Forge Health). Evan completed his undergraduate degree in Neuroscience at Vanderbilt in his hometown of Nashville. Evan's personal interests include cooking, mountain biking, downhill skiing, graphic design, tennis, and guitar.

Sean McMaster: Sean McMaster is currently a Consultant in the Atlanta office of The Boston Consulting Group, focusing on work in the healthcare practice area. He graduated from the Emory University MD/PhD program in May 2017, completing his PhD in the Immunology and Molecular Pathogenesis Program. His dissertation focused on characterizing the factors necessary to establish and maintain lung resident memory T cells for eventual creation of a T cell mediated vaccine against the Influenza virus that patients would only need to get every 5-10 years. Prior to coming to Atlanta, he earned his BS in Natural Sciences from the University of Wisconsin-Madison, majoring in Biochemistry and Mathematics.

Laura Zambrano: Laura Zambrano is in her second year as an Epidemic Intelligence Service officer with the Centers for Disease Control and Prevention (CDC). Laura received her PhD in Environmental Health Sciences from Emory University, where she managed a large-scale cluster-randomized controlled trial of advanced point-of-use water filtration units and improved cookstoves in Western Province, Rwanda. This trial sought to mitigate the two largest contributors to childhood mortality in Sub-Saharan Africa: diarrhea and pneumonia. Before moving to Atlanta, Laura received her MPH in Epidemiology from the George Washington University Milken Institute School of Public Health and her BA in Biology from St. Mary's College of Maryland.

Brian Robinson: Brian Robinson is in his 5th year as a GI Research Fellow in the Clinical Pathology Residency program at Emory University. Brian graduated from the Emory MD/PhD program in 2013 with a PhD in Biochemistry, Cell, and Developmental Biology. For his PhD, Brian worked with Dr. Ken Moberg to study mechanisms of patterned growth in a fruit fly model.

Scott Krummey: Scott Krummey is in his 2nd year in the Clinical Pathology/Research Track residency program at Emory University. Scott graduated from the Emory MD/PhD program in 2016 with a PhD in Immunology and Molecular Pathogenesis. He received his BA in Molecular Biology from Colgate University and is originally from Pittsburgh, PA.

PANELIST BIOS

UNDERGRADUATE TRACK: MD/PHD ADMISSIONS INFO WITH MD/PHD DIRECTORS

Robin Lorenz, MD PhD (UAB) – Dr. Lorenz is currently the director of the UAB MSTP and the Physician Scientist Development Office. She received her MD and PhD in Immunology from Washington University in St. Louis. She completed her residency in Clinical Pathology and a postdoctoral fellowship in Gastrointestinal Biology.

Louis Justement, PhD (UAB) – Dr. Justement is the associate director of the UAB MSTP and a professor of Microbiology. Dr. Justement received his PhD in Microbiology with a special emphasis in Immunology from Ohio State University. HIs research interests include studying signal transduction processes underlying lymphocyte activation.

William Geisler, MD MPH (UAB) – Dr. Geisler is associate director of the UAB MSTP. He received in MD from the University of Tennessee and completed a residency in Internal Medicine at the University of Michigan. He completed a fellowship in Infectious Diseases and his research interests include investigating the characteristics of Chlamydia and the human host immune responses on clinical outcomes of chlamydial infection.

Nancy Demore, MD FACS (MUSC) – Dr. Demore is the program director of the MUSC MSTP. She received her MD from the University of Health Sciences, Chicago Medical School and completed her general surgery residency at Boston University Medical Center before completing a surgical research fellowship with Dr. Judah Folkman at Harvard University and a Surgical Oncology fellowship at Memorial Sloan Kettering Cancer Center. Her interests focus on all aspects of breast cancer, including breast cancer in young women, locally advanced and inflammatory breast cancer, breast sarcomas, and oncoplastic surgery.

Christopher Williams, MD PhD (Vanderbilt) – Dr. Christopher S. Williams, Associate Dean for Physician-Scientist Education and Training, Associate Professor of Medicine and Cancer Biology, and Director of the Harrison Society, serves as Director of the Vanderbilt MSTP. Dr. Williams is an alumnus of the Vanderbilt MSTP and obtained his residency in internal medicine at Vanderbilt School of Medicine followed by a fellowship in gastroenterology. Dr. Williams is a physician-scientist with clinical interests in epithelial biology, intestinal wound healing and carcinogenesis. Dr. Williams has served as an MSTP Advising College Leader since 2011 and was named Director in May 2016.

Megan Williams, PhD (Vanderbilt) – Dr. Megan A. Williams serves as Assistant Director of the MSTP. Dr. Williams earned her PhD in 2015 from the Neuroscience Graduate Program at Vanderbilt. Dr. Williams directs the recruitment and admissions process of the MSTP. Dr. Williams joined the MSTP Leadership Team in 2015.

Cathy Quinones, PhD (Emory) – Cathy Quiñones is Emory MD/PhD program's Associate Director of Administrative Affairs. Although she trained in evolutionary and organismal biology, her professional career has focused on science education programs that encourage participation and retention in science careers. Before joining the MD/PhD program, Dr. Quiñones administered research programs for undergraduate and post baccalaureate students and developed curriculum to develop professional skills relevant to science-intensive environments.

BREAKOUT SESSIONS

BREAKOUT SESSION 1

UNDERGRADUATE TRACK: LIFE OF AN MD/PHD STUDENT

Location: Emory Conference Center – Emory Amphitheater

Panelists: Bianca Islam – M3, Augusta University

Hyunwoo (Tony) Kwon – G2, MUSC

Annie Geller – G1, University of Louisville

Elizabeth Ogunrinde – G2, MUSC

Margaret Axelrod – G1, Vanderbilt

Lizzie Hale – G1, Vanderbilty

Carey Jansen (Moderator)

GRADUATE TRACK: CAREER OPTIONS FOR PHYSICIAN SCIENTISTS

Location: Emory Conference Center – Oak Amphitheater

Panelists: Evan McClure, MD, MBA – Partner at Life Science Partner, Inc.

Sean McMaster, MD, PhD – Consultant with Boston Consulting Group

Jacob Van Houten, PhD – M4 MD/PhD student at Vanderbilt completing a CDC Epidemiology elective and applying into Preventative Medicine.

Laura Zambrano, PhD, MPH – Epidemic Intelligence Service Officer, CDC

Brian Robinson, MD, PhD – Pathology & Laboratory Medicine Fellow, Emory

Scott Krummey – PGY2 Pathology Resident, Emory

Mojibade Hassan (Moderator)

BREAKOUT SESSIONS

BREAKOUT SESSION 2

UNDERGRADUATE TRACK: MD/PHD ADMISSIONS INFO WITH MD/PHD DIRECTORS

Location: Emory Conference Center – Emory Amphitheater

Panelists: Chris Williams, MD, PhD – Vanderbilt MSTP Director

Megan Williams, PhD – Vanderbilt MSTP Assistant Director for Admissions

Robin Lorenz, MD, PhD – UAB MSTP Director

Louis Justement, PhD – UAB MSTP Associate Director

William Geisler, MD, MPH – UAB MSTP Clinical Associate Director

Cathy Quinones, PhD – Emory MSTP Associate Director, Admin. Affairs

Nancy Demore, MD – MUSC MSTP Director

Saumya Gurbani (Moderator)

GRADUATE TRACK: NETWORKING MEET-AND-GREET

Location: Emory Conference Center – Azalea Room

Panelists: Margaret Baron, MD, PhD (MT. SINAI) Fishberg Professor of Medicine; Senior Associate Dean for Education and Director of the MD/PhD Program

Mehmet Asim Bilen, MD (EMORY) Assistant Professor, Hematology and Oncology

Craig Blackstone, MD, PhD (NIH) Senior Investigator and Section Chief, National Institute of Neurological Disorders and Stroke (NINDS)

Bassel El-Rayes, MD (EMORY) Professor and Vice Chair for Clinical Research, Hematology and Oncology

Sonya Heath, MD, MS (UAB) Associate Professor, Medicine; Program Director of Internal Medicine Research Pathway

Elizabeth Krupinski, PhD (EMORY) Professor and Vice Chair for Research, Radiology; Co-Director of Radiology Residency Research Track

Evan McClure, MD, MBA (LIFE SCIENCE PARTNER, INC)

John Petros, MD (EMORY) Professor, Urology and Pathology; Associate Chair for Urologic Research

Brian Pollack, MD, PhD (EMORY) Assistant Professor, Dermatology and Pathology

Vin Tangpricha, MD, PhD (EMORY) Associate Professor, Endocrinology; Director of Endocrinology, Metabolism, and Lipids Fellowship Program

Aimee Vester (Moderator)

ORAL PRESENTATIONS

SCHOOL OF MEDICINE **ROOM 170A**

8:00 AM Mfon Umoh, Emory – *A proteomic network approach across the ALS-FTD disease spectrum differentiates clinical phenotypes and genetic vulnerability in human brain*

8:20 AM Mackenzie Lemieux, Cornell – *Interacting With the Wrong Crowd: A CRISPR/Cas9-Edited Inducible Human Cell System Designed to Reveal the Chronology of Events Leading to Proteotoxic Stress in Tauopathies*

8:40 AM Arielle Valdez, Emory – *Regulated ubiquitination of FMRP as a molecular switch for protein synthesis at the synapse*

9:00 AM Peter Campbell, Louisville – *Formation of intrathalamic connections between visual thalamus and TRN*

9:20 AM Kenny Igarza, Emory – *Investigating the Activity Of Courtship Command-Neurons in Drosophila melanogaster*

9:40 AM Morgan Zipperly, UAB – *Dissection of Motivated Behavior and Reward: Analysis of Neural Activity in the Nucleus Accumbens*

10:00 AM Paige Souder, UAB – *Quantification of endocrine disruptor uptake in zebrafish embryos and larvae*

SCHOOL OF MEDICINE **ROOM 178P**

8:00 AM Bianca Islam, Augusta – *Type 2 cGMP-Dependent Protein Kinase Suppresses Tumorigenesis in the Mouse Colon*

8:20 AM Mary Barber, Belmont – *Modeling Breast Cancer Therapy-Induced Cardiotoxicity in Human Cardiomyocytes*

8:40 AM Muhan Hu, UAB – *The DAF-7/ TGF Cascade Affects Prostaglandin Metabolism, Sperm Guidance, and Sperm Gene Expression in the Adult Hermaphrodite Gonad*

9:00 AM Shan Parikh, Vanderbilt – *Thyroid and Glucocorticoid Hormones Promote Functional T-tubule Development in Human Induced Pluripotent Stem Cell Derived Cardiomyocytes*

9:20 AM Alina Uezko Antonova, Emory – *Improving Allogeneic Bone Marrow Stem Cell Transplantation Through the Beneficial Effect of Flt3L on Plasmacytoid Dendritic Cells*

9:40 AM Amanda Mener, Emory – *Complement regulates CD4 independent and dependent antibody responses to RBC antigen*

10:00 AM Matthew Kudelka, Emory – *Cosmc is an X-linked inflammatory bowel disease risk gene that spatially regulates gut microbiota and contributes to sex-specific risk.*

ORAL PRESENTATIONS

SCHOOL OF MEDICINE **ROOM 190P**

8:00 AM Jeffrey Singer, UAB – *Host and Commensals Cooperate in the Neonatal Intestine to Prevent Dysbiosis and Late Onset Sepsis*

8:20 AM John Diehl, Emory – *A Potential Role of Vitamin D in Reducing Inflammation and Lowering Cellular Adhesion Proteins to Protect from Cerebral Malaria*

8:40 AM Mariko Peterson, Emory – *Plasmodium vivax malaria: tissue burden and disease severity in Saimiri bolviensis model*

9:00 AM Daniel Chopyk, Emory – *Ethanol-induced Dysregulation of Junctional Adhesion Molecule-A Contributes to Barrier Disruption in Intestinal Epithelial Cell Monolayers*

9:20 AM Henry Skelton, Morehouse – *Morphologic Changes in Mouse Retinal Pigment Epithelium Subsequent to Light Induced Retinal Damage*

9:40 AM Corey Duke, UAB – *Light-activated epigenetic control of gene expression*

10:00 AM Steven Yarmoska, Georgia Tech – *Beyond Bubbles: Laser-Activated Perfluorocarbon Nanodroplets for Diagnostic Ultrasound Imaging and Therapy*

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	NAME	ABSTRACT TITLE	SCHOOL
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2	Amara Ejikemeuwa	A New Protocol for the Transcription and Capping of HIV-1 RNA	University of West Florida
3	Christina Schoose	Determining the Specificity of the MIS Complex and its Role in the Methylation of mRNA.	The University of Alabama
4	Trisha Dalapati	Effects of Plasmodium falciparum Derived Hemozoin on Expression of Inflammatory and Coagulation Factors in BeWo Cells	University of Georgia
5	Yasminye Pettway	Early life stress in male mice induces vascular TLR4 expression in adulthood	University of Alabama at Birmingham
6	Ryan Day	Creating a Smaller Synthetic Muscleblind-like 1 Protein	University of Florida
7	Amer Abu-kwaik	Modification of a Peptide Sequence with Selective Binding to Helix 69 to Increase Cell Permeability	Wayne State University
8	Edie Osuma	High Throughput Measurement of Mitochondrial and Glycolytic Activity in Planarians Reveals Activation of Glycolysis During Regeneration	Wesleyan College
9	Zahna Bigham	Shear Stress Induces Calcium Influx via TRPV4 in Rat Lung Microvascular Endothelial Cells	Wesleyan College
10	Ethan Wang	Efficacy of 18F FDG PET/CT in predicting local control of spinal tumors following SSRS.	University of Texas at Austin
11	Gregory Schwing	A novel bioinformatics approach for mapping the genomic landscape of pediatric Glioblastoma Multiforme	University of New Orleans
12	Omer Ashmaig	Bayesian Optimization of Asynchronous Distributed Microelectrode Stimulation and Spatial Memory	Emory University
13	Syed Zaidi	A Model-based Analysis of Changes in Neural State due to Optogenetic Stimulation in the Septohippocampal Network	Emory University
14	Shivani Ananthasekar	Estimation of Mechanical Work by Pulmonary Endothelial Cells	University of Alabama at Birmingham
15	Courtney Swain	The Analysis of the Apoptotic Behavior of Neutrophils in Antipsychotic-induced Neutropenia	University of West Florida
16	Anna Sharabura	Collagen Increases Proliferation and Drug Resistance of Papillary Thyroid Cancer Cells Harboring BRAFV600E Mutations	Hendrix College
17	Anne Jessica Roe	An Analogue of Securinine with Therapeutic Potential Against Acute Myeloid Leukemia	Case Western Reserve University
18	Ashley Williams	Understanding the Mechanistic Role of Integrin Alpha 6 in Tumor Development	Georgia Southern University
19	Jonathan Jenkins	Collagen Increases Apoptosis Resistance of Papillary Thyroid Cancer Cells with BRAFV600E Mutations	Hendrix College
20	Rachel Miles	Collaboration of IDH2 and Spliceosomal Gene Mutations in Acute Myeloid Leukemia	Vanderbilt University
21	Samuel Chang	Targetable Alterations in Global Lipid Metabolism upon Arginine Deprivation in ASS1 Deficient Sarcomas	Washington University in St. Louis
22	Ian George	Utilizing CRISPR Cas9 Technology to Knock Out T-cell Gene Expression For Use With CAR T-Cell Cancer Immunotherapy	Emory University

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24	Aaron Sandoval	A Histological Progression of Ear Skin and Muscle Regeneration in Acomys and Mus	University of Florida
25	Christopher Cunningham	Iodine mediated one-pot successive cyclization-alkylation reaction strategy for the synthesis of biologically useful sulfur heterocycles	University of West Florida
26	Natalie Fallows	Copper and Sodium Salts mediated Electrophilic Halocyclization for the Synthesis of Benzo[b]thiophenes	University of West Florida
27	Vanessa Amabo	Synthesis and Characterization of Fluorescent Substrates for Undecaprenyl Pyrophosphate Synthase.	University of North Carolina at Charlotte
28	Alice Li	Patterns in Post-Discharge Venous Thromboembolism	Vanderbilt University
29	Samantha Fortier	Locally-Advanced Breast Cancer in Casablanca, Morocco: An Epidemiological and Clinical Classification Overview	Boston University
30	Darrell Morris	Dysfunctional Uterine Bleeding in Women with Sickle Cell Disease Should Raise Alarms: Further Evidence of Decreased Incidence of Uterine Fibroids in the Setting of Sickle Cell Disease	Emory University
31	Anita Qualls	SLC39A14-Related Manganism: Treatment Outcomes Over a 4-Year Period	University of Georgia
32	Craig Hinson	The Specific Activity of Acid Phosphotase in Arabidopsis	Emory University
33	Maryann Aniekwe	A Behavioral Genetics Approach to Understanding Risky Driving	Georgia Gwinnett College
34	Joline Hartheimer	Cellular Mechanisms Underlying Gene-Nutrient Interactions Affecting Longevity	The University of Alabama (Tuscaloosa)
35	Fares Hosseinzadeh	Cytotoxic CD8 T-Cells in Von-Hippel Lindau Renal Tumors	Emory University
36	Amanda Schaefer	Microfluidics for environmental control and quantitative analysis of mouse stem cell aggregate differentiation to motor neurons	Georgia Institute of Technology
37	Katherine Quintin	Modulating Inflammasome Activity by using FcyRIIa	University of West Florida
38	Elizabeth Quaye	Synthesizing Curcumin Loaded Silver Nanoparticles as a Novel Anti HIV-1 Therapeutic	University of Buffalo
39	Emilie Fisher	Antimicrobial Peptide Resistance Mechanisms in Staphylococcus aureus Infection	National Institute of Allergy and Infectious Diseases
40	Nilang Shah	The Effect of Antibiotics on Non-growing Bacterial Populations	Emory University
41	Lily Brooks	Immune response of the bed bug, Cimex lectularius, to simulated traumatic insemination	Mississippi State University

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43	Brooke Beasley	Insulin-Like Growth Factor 1 Receptor Expression in Postmortem Brain Tissue of Autism Spectrum Disorder	East Tennessee State University
44	Long Di	Gene-based synaptic inhibition: a proof of principle study for sustained botulinum toxin expression in the treatment of muscle spasticity	Emory University
45	Samuel Cockey	IL-10 Expression Leads to Early Mortality Without Affecting Dopaminergic Neurons in a Mouse Model of Alpha Synucleinopathy	University of Florida
46	Solana Liu	Effects of Dopamine 2 Receptor Agonist Treatment on Microglia Polarization following Cerebral Ischemia in Mice	Emory University
47	Tyler Behymer	NIHSS and Variation of Infarct Volume by Hemisphere	University of Cincinnati
48	Amanda Watters	Pre-gestational oxycodone exposure impacts maternal motivation but not maternal caregiving or drug seeking in the postpartum mouse	Sewanee: The University of the South
49	Gregory Overbeek	A Combined 7T Stroop fMRI and MRS Study in First Episode Schizophrenia	University of Alabama at Birmingham
50	Derian Pugh	Validating the Effect of RBFOX1 Expression on the Regulation of MAPT Splicing in vitro	Xavier University of Louisiana
51	Juhi Shah	The Effects of Gestational Psychological Stress on Neonatal Mouse Intestinal Development	University of Alabama at Birmingham
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ABSTRACTS – ORAL PRESENTATIONS

A proteomic network approach across the ALS-FTD disease spectrum differentiates clinical phenotypes and genetic vulnerability in human brain

Mfon E. Umoh1,4, Eric B. Dammer2,4, Duc M. Duong2,4, James J. Lah1,4, Allan I. Levey1,4, Marla Gearing1,3,4, Jonathan D. Glass1,3,4 and Nicholas T. Seyfried1,2,4

1 Department of Neurology, Emory University School of Medicine; 2 Department of Biochemistry, Emory University School of Medicine; 3 Department of Pathology and Laboratory Medicine, Emory University School of Medicine; 4 Center for Neurodegenerative Diseases, Emory University School of Medicine, Atlanta, GA, 30322

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are neurodegenerative diseases with overlap in clinical presentation, neuropathology, and genetic underpinnings. The molecular basis for the overlap of these disorders is not well established. We performed a comparative unbiased mass spectrometry-based proteomic analysis of frontal cortical tissues from post-mortem cases clinically-defined as ALS, FTD, ALS and FTD (ALS/FTD), and healthy controls. We also included a subset of patients with the C9orf72 expansion mutation, the most common genetic cause of both ALS and FTD. Our systems analysis of the brain proteome integrated both differential expression and co-expression approaches to assess the relationship of these differences to clinical and pathological phenotypes. Weighted correlation network analysis (WGCNA) revealed 15 modules of co-expressed proteins, 8 of which were significantly different across the ALS-FTD disease spectrum. These included modules associated with RNA binding proteins, synaptic transmission, and inflammation with cell-type specificity (neuronal, microglial and astrocytic), that showed correlation with TDP-43 pathology and cognitive dysfunction. Modules were also examined for their overlap with TDP-43 protein-protein interactions (PPIs), identifying one module enriched with RNA-binding proteins and other causal ALS genes that increased in FTD/ALS and FTD cases. A module enriched with astrocyte and microglia proteins was increased in ALS cases carrying the C9orf72 mutation compared to sporadic ALS cases, suggesting that the genetic expansion is associated with inflammation in the brain even without clinical evidence of dementia. Together, these findings highlight the utility of integrative systems level proteomic approaches to resolve clinical phenotypes and genetic mechanisms underlying the ALS-FTD disease spectrum in human brain.

Interacting With the Wrong Crowd: A CRISPR/Cas9-Edited Inducible Human Cell System Designed to Reveal the Chronology of Events Leading to Proteotoxic Stress in Tauopathies

Mackenzie Lemieux, Louisa Wang, Declan Williams, Gerold Schmitt-Ulms

Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada.

Purpose: A key open question in the tauopathy field is how tau proteins aggregate and cause proteotoxic stress in neurons. We recently published a first in-depth tau interactome, which revealed that tau carrying the P301L frontotemporal dementia mutation exhibits reduced interactions with heat shock proteins and the proteasome. Our previous work captured steady-state interactions of a specific mutated and plasmid-encoded tau isoform. However, as disease evolves dynamically, critically needed are next generation models that can be induced to reveal the chronology of events underlying tau's aggregation and proteotoxicity.

Methods: We are poised to fill this unmet need with novel CRISPR/Cas9-generated cell models that can be induced to express human tau alleles. Specifically, inducible tau IMR-32 human neuroblastoma cells were created in a two-step gene engineering workflow: first, a pair of foundation lox sites was inserted into the AAVS1 human genome safe harbor locus via a CRISPR/Cas9 nickase strategy. Second, wild-type or P301L tau C-terminally fused to the enhanced green fluorescent protein were inserted into the primed AAVS1 locus via Cre recombinase-mediated heterologous gene exchange using the foundation lox sites. The system can be used for time-course transcriptome and proteome analyses. Accurate transgene insertion was confirmed by genomic PCR and sequencing. Marked induction and tight leakage control of the inducible model were validated by Western blotting after treating the cells with doxycycline for 0-18 hr.

Results: A preliminary mass spectrometry analysis confirmed that tau interacts with a large segment of the cellular ribonucleoproteome and chaperones. Data collected to date establish the successful implementation of an inducible tau-EGFP expression system that will allow us to dissect the time-course of aberrant tau interactions underlying proteotoxic stress.

Discussion/Conclusions: Targeted insertion minimizes the deleterious effects of random integrations, and the flexible two-step process can potentially accommodate any genes of interest. In the future, we can use this cell model to find suitable time intervals for in depth interrogation and also polish the system to express both the 3 and 4 repeat tau domains to even better recapitulate the endogenous pathological brain state.

Regulated ubiquitination of FMRP as a molecular switch for protein synthesis at the synapse

Arielle N. Valdez*+, Austin Lai*, Gary J. Bassell *

* Department of Cell Biology, Emory University School of Medicine, Atlanta, GA; +Medical Scientist Training Program, Emory University School of Medicine, Atlanta, GA

For learning and memory to occur, neurons need to synthesize synaptic proteins in direct response to stimulation. One of the ways that cells regulate protein synthesis is ubiquitination. Disrupted ubiquitination can lead to debilitating forms of human disease, such as Angelman Syndrome and Autism Spectrum Disorder. Dysregulated ubiquitination may be a shared mechanism amongst neurodevelopmental diseases. One neurodevelopmental disease that currently has no FDA-approved treatments is Fragile X Syndrome (FXS). FXS is caused by the loss of only one protein, the Fragile X Mental Retardation Protein (FMRP). Despite lacking only FMRP, symptoms of FXS are complex and multifaceted. This suggests that FMRP must play a vital role in multiple cellular processes. Indeed, FMRP has been observed to repress mRNA translation. Few studies have demonstrated how the repressive activity of FMRP is regulated. Stimulation of metabotropic glutamate receptor 5 (mGluR-5) has been observed to increase the translation of FMRP-targeted mRNAs. Here, we show that the stimulation of mGluR-5 leads to an increase in the ubiquitination of FMRP. Thus, ubiquitination may be a mechanism of regulating the repressive activity of FMRP. We then wanted to identify an enzyme that ubiquitinates FMRP. Using immunoprecipitation, we demonstrate that the Cdh1 subunit of the E3 ligase anaphase-promoting complex (APC) binds to FMRP. Inhibition of Cdh1-APC leads to an increase in the amount of modified FMRP and ubiquitinated FMRP following mGluR-5 stimulation. The effects of inhibition suggest Cdh1-APC may not ubiquitinate FMRP, but it does play a role in FMRP regulation. Our data demonstrate that FMRP is ubiquitinated following stimulation of the same receptor that leads to the translation of FMRP-targeted mRNAs. Furthermore, we have identified Cdh1-APC as an E3 ligase that plays a regulatory role in this pathway. Our data support the novel hypothesis that ubiquitination serves as a dynamic mechanism to regulate FMRP.

Formation of intrathalamic connections between visual thalamus and TRN

Peter W. Campbell, William Guido, PhD

University of Louisville, Department of Anatomical Sciences & Neurobiology

Purpose: The reciprocal connections between the dorsal lateral geniculate nucleus (dLGN) and the thalamic reticular nucleus (TRN) play an important role in regulating thalamocortical activity. Axon collaterals of thalamocortical neurons provide feedforward excitatory input onto GABAergic TRN neurons, which in turn convey feedback inhibition to dLGN relay neurons. Here we examined when and how these circuits arise during early postnatal life in the presence and absence of retinal inputs to the brain.

Methods: We employed mouse transgenics to visualize when inputs appear and optogenetics to assess when functional patterns of connectivity emerge in both controls and animals devoid of retinal inputs. To determine when projections arrived in their target nucleus, we examined coronal sections across multiple early-postnatal ages using confocal microscopy. Functional connections were assessed by combining optogenetics with in vitro whole cell patch clamp physiology.

Results: TRN terminals arrive in dLGN at early postnatal ages and span the entire nucleus by the end of the first postnatal week. Weak inhibitory postsynaptic activity from TRN to dLGN appears during the first postnatal week, and grows steadily in amplitude to reach adult-like levels by the third postnatal week. Feedforward excitatory connections from dLGN to TRN appear near the end of the first postnatal week. Soon after, weak excitatory responses appear and mature rapidly, reaching adult-like levels by the third postnatal week. The developmental plan described herein was largely unchanged by removing retinal inputs; however, TRN terminals did arrive earlier in dLGN compared to control mice.

Conclusion: These data suggest that reciprocal connections between dLGN and TRN develop in a coordinated manner, with feedback inputs arising somewhat earlier that feedforward ones. The emergence of this intrathalamic loop occurs well after retino-geniculate and geniculo-cortical connections are established and at about the same time as descending corticothalamic connections are made with TRN and dLGN.

Investigating the Activity Of Courtship Command-Neurons in Drosophila melanogaster.

Kenny J. Igarza, College of Arts and Sciences, Emory University, Atlanta, GA; Daisuke Hattori, PhD. Department of Neuroscience, Columbia University, New York, NY; Kurtis J. Swartz, BS. Department of Neuroscience, Columbia University, New York, NY; Richard Axel a,b,c, MD

(a) Department of Neuroscience, (b) Department of Pathology & Cell Biology, (c) Department of Biochemistry & Molecular Biophysics, Columbia University, New York, NY.

All organisms respond to environmental stimuli with distinct behaviors. One class of behaviors may be considered innate due to the hard-wired neural circuitry that governs the execution and performance of each step of stereotyped responses to specific stimuli from early life stages. Innate behavior is often driven by the activity of command-neurons. A population of male-specific command-neurons termed P1 in Drosophila melanogaster is responsible to execute and produce all steps of courtship behavior, a highly-stereotyped ritual performed solely by males toward females. However, while the behavioral role of P1-command neurons is sufficient and necessary to evoke courtship behavior, little has been studied about the specific contexts required to trigger the activity of this network. Here, we investigate the contexts and stimuli required to activate P1 interneurons. We examined the activity of P1 neurons by means of the SplitGAL4-UAS method and a newly developed neural activity marker, CRTC, and asked whether P1 neurons are activated by the presence of a female fly, and if so, whether physical contact is necessary for the activation. Additionally, we studied whether elevated courting experience affects P1 neuronal activity. We find that P1 neurons may be variably activated by either male or female flies, hinting to potential new roles for this network beyond courting behavior. Also, we see that physical contact with a female is not necessarily needed to trigger the activity of P1 neurons, providing an insight into the new contexts that activate command neurons. Finally, we observe that courting experience leads to variable levels of P1 activity, indicating that they may facilitate a more efficient execution of the hard-wired behavior.

Dissection of Motivated Behavior and Reward: Analysis of Neural Activity in the Nucleus Accumbens

Morgan E. Zipperly[12], John J. O'Malley[2], Andrew C. Brane[2], Kaela J. Kelly[2], Jeremy J. Day, PhD[2]

[1]Medical Scientist Training Program, University of Alabama at Birmingham, Birmingham, AL;
[2]Department of Neurobiology, University of Alabama at Birmingham, Birmingham, AL.

Purpose: Addiction is an increasingly prevalent problem in the United States, associated with progressively higher rates of morbidity and mortality. Exposure to drugs of abuse leads to reorganization of neural circuits and alteration of synapses, which outlive the direct effects of the drug and may contribute to addiction. The nucleus accumbens (NAc) has a significant role in motivation, pleasure, and reward, and has been identified as a key area in the development and maintenance of addiction. The present study aims to determine how neuronal activity in the NAc is altered in response to cocaine. Our central hypothesis is that administration of cocaine will increase neuron firing in the NAc, and that optogenetic photoactivation of neurons in the NAc will elicit reward-seeking behavior and drug-evoked neuroplasticity.

Methods: In order to assess how neuronal activity in the NAc is altered as a result of drug exposure, cell firing was recorded in vivo from electrode microarrays bilaterally implanted in the NAc of naive male Sprague Dawley rats that have been exposed to either cocaine (10mg/kg) or saline. In an additional group of animals, channelrhodopsin (ChR2) was virally expressed in the NAc and optogenetic guides were surgically implanted to determine if photostimulation of neurons in this area is sufficient for reward-related behavior.

Results: As predicted, acute cocaine exposure increased activity of a subpopulation of neurons in the NAc, independent of environment or context. Additionally, preliminary data show that photostimulation of ChR2 in the NAc core, but not in the NAc medial shell, drives motivated behavior as measured by real-time place preference.

Conclusions: By elucidating how cocaine exposure alters the activity of specific cell populations and specific neuronal circuitry involving the NAc, we may identify important mechanisms underlying the etiology of addiction and relapse, as well as propose novel targets for preventive and therapeutic interventions.

Quantification of endocrine disruptor uptake in zebrafish embryos and larvae

J. Paige Souder, Daniel A. Gorelick

Department of Pharmacology and Toxicology, University of Alabama at Birmingham, Birmingham, AL.

Zebrafish are a powerful model system to assess the molecular and cellular effects of exposure to toxic chemicals during embryonic development. To study the effects of endocrine disruptor compounds (EDCs), embryos and larvae are commonly exposed to supraphysiologic concentrations of these compounds in the water, but their bioavailability is largely unknown. To test the hypothesis that supraphysiologic concentrations of EDCs are required to achieve physiologic levels in vivo, we developed an assay using radiolabeled estradiol ([3H]E2) to measure compound uptake at multiple concentrations and exposure durations in zebrafish from 0-5 days post fertilization. We then used this assay to measure the uptake of two other EDCs, bisphenol A (BPA) and ethinyl estradiol (EE2). We found that the uptake of each compound increased with increasing concentration, duration, and developmental stage, but that percent uptake from the total exposure solution remained constant with increasing concentration. When comparing compound uptake, we found that E2 and EE2 uptake was similar under the same exposure conditions, while BPA had comparatively lower uptake. These results support the hypothesis that exposing zebrafish embryos and larvae to supraphysiologic concentrations of EDCs is required to achieve physiologically-relevant concentrations in vivo. An application of this assay is to test factors that influence EDC uptake. One hypothesis is that environmental persistent organic pollutants (POPs) inhibit ABC transporters that would normally efflux EDCs and their metabolites, inducing toxicity in aquatic organisms. To test this hypothesis, we used our assay to measure [3H]E2 levels in zebrafish in the presence or absence of the POP PDBE-100, and cyclosporin A, an inhibitor of ABC transporters. We found that neither chemical affected [3H]E2 levels in zebrafish, suggesting that zebrafish can maintain estradiol efflux with ABC transporter inhibition. Using the isotopic uptake assay developed here, future studies will test whether other chemicals influence EDC levels in vivo.

Type 2 cGMP-Dependent Protein Kinase Suppresses Tumorigenesis in the Mouse Colon

Bianca Islam, PhD, Sarah Sharman, Justin Ashby, Yali Hou, and Darren Browning, PhD.

Department of Biochemistry & Molecular Biology, Augusta University, Augusta, GA

Colorectal cancer is one of the most prevalent and lethal cancers, making chemoprevention relevant due to large populations with established risk factors, and a poor prognosis for advanced disease. Signaling downstream of cGMP plays a central role in intestinal homeostasis and is emerging as a potential target for colon cancer prevention. However, little is known about the tumor suppressive mechanism of cGMP, but understanding the signaling will facilitate clinical application. As the main effector of cGMP in the colon, the present study tested the significance of type 2 cGMP-dependent protein kinase (PKG2) in colon homeostasis and carcinogenesis. PKG2 knockout (KO) mice exhibited 52% higher proliferation in colonic crypts and twice the apoptosis in the luminal epithelium relative to wild-type mice. Polyp formation was quantified in the colons of mice subjected to the AOM/DSS model of colon cancer. PKG2 KO mice had 62.5% more polyps per mouse than wild-type, but there was not significant difference in tumor size. Taken together these findings suggest that PKG2 has a tumor suppressive role in the colon epithelium, and that reducing the proliferative compartment may be part of the mechanism.

Modeling Breast Cancer Therapy-Induced Cardiotoxicity in Human Cardiomyocytes

Mary C. Barber*+^, Donald Okoye*+^, Javid Moslehi*+^

*Division of Cardiovascular Medicine, Vanderbilt University Medical Center, Nashville, TN; +Cardio-Oncology Program, Vanderbilt University Medical Center, Nashville, TN; ^Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN

The explosion of novel, targeted cancer therapies has heralded a new era in cancer treatment and introduced survivorship as a new theme in oncology. However, both traditional chemotherapies and newer targeted therapies cause cardiotoxicities. Cardiovascular issues are particularly a problem in breast cancer treatment. Traditional therapies such as anthracyclines or radiation and newer therapies such as HER2 inhibitors (e.g. trastuzumab) cause cardiovascular issues including cardiomyopathy. Our lab is developing various models that recapitulate cardiotoxicities to screen new cancer therapies for clinical use and to understand the mechanism of cardiomyopathy caused by novel cancer treatments. My project focuses on using human induced-pluripotent stem cells differentiated into cardiomyocytes (hiPSC-CM) as a high-throughput way to assess for cancer therapy-induced cardiotoxicity. This process is adapted from a protocol for hiPSC-CM differentiation and maturation developed at Vanderbilt resulting in mature cardiomyocytes in 34 days. Following maturation, hiPSC-CMs are treated with clinically relevant dosages of single or combination HER2 inhibitor therapy, including trastuzumab alone, trastuzumab and pertuzumab, T-DM1, or trastuzumab and lapatinib. After a treatment period, data is obtained on apoptosis, viability, and cytotoxicity and individual cardiomyocyte contractility and cell length. Our preliminary data show that while doxorubicin (an anthracycline) results in direct cardiomyocyte death, HER2 targeted therapies cause a significant decrease in cardiomyocyte contractility but have no effect on apoptosis or cell viability. A subset of patients who receive HER2 inhibitors for breast cancer develop heart failure, so contractile dysfunction in cardiomyocytes after treatment may closely model what occurs clinically. This model will enable cardiologists, oncologists, and drug developers to assess cardiotoxicities prior to clinical use and will provide insight into the mechanism of heart failure caused by traditional and new breast cancer therapies.

The DAF-7/ TGF Cascade Affects Prostaglandin Metabolism, Sperm Guidance, and Sperm Gene Expression in the Adult Hermaphrodite Gonad

Muhan Hu, Michael Miller, PhD

Medical Student Training Program; Department of Cell, Developmental and Integrative Biology, University of Alabama at Birmingham

Successful fusion of the sperm and egg is fundamental to the development of sexually reproducing animals and is critical for the maintenance of genetic diversity. It is well established that oocytes of certain marine species secrete chemoattractants to promote sperm guidance. Accumulating evidence suggest that activated sperm of internally fertilizing animals also respond to chemotactic cues while searching for the oocyte in the female reproductive tract. In C. elegans, we have identified a group of structurally similar F-series prostaglandins (PGFs) that help guide the sperm towards the spermatheca. These PGFs are synthesized via a novel mechanism and are found in mammalian ovaries, suggesting that PGF regulatory mechanisms may be conserved. Previous studies showed that the DAF-7/ TGF pathway is essential for sperm guidance and PGF levels (McKnight et al., 2014). The purpose of this project is to uncover the mechanism by which DAF-7/ TGF regulates PGF levels. Using liquid chromatography tandem mass spectrometry, I measured PGF levels in wild-type, daf-1(m40), and daf-1 (m40); daf-3 (mgDf90) mutant adults. The data indicate that the DAF-3 co-SMAD transcription factor negatively regulates PGF biosynthesis or positively regulates PGF breakdown. As the type I TGF- receptor daf-1 is partially required in the germ line, I hypothesized that DAF-3 transcriptional activity is critical in the germ line to affect PGF levels. To test this hypothesis, I conducted RNA-sequencing on wild type, daf-1(m40), and daf-1(m40); daf-3 (mgDf90) mutant adults. I identified over 1000 genes that are significantly altered in the experiments. I focused on a set of 179 genes that are expressed in the germ line (Reinke et al. 2004). RNAi screening of these 179 genes identified 32 genes that might act downstream of DAF-3. Of particular interest, 25 of 32 positive RNAi clones encode for genes that are highly enriched in developing spermatocytes (i.e. sperm genes). My data thus far support the model that DAF-3 promotes increased sperm gene expression in the adult hermaphrodite germ line, thereby down-regulating PGF levels. Current efforts are underway to understand how these sperm genes affect PGF metabolism and fertilization.

Thyroid and Glucocorticoid Hormones Promote Functional T-tubule Development in Human Induced Pluripotent Stem Cell Derived Cardiomyocytes

Shan S. Parikh1, 2, Daniel J. Blackwell1,3, Nieves Gomez-Hurtado1,3, Michael Frisk4, Lili Wang1,3, Kyungsoo Kim1,3, Christen P. Dahl5, Theis Tønnessen4,6, Dmytro O. Kryshtal1,3, William E. Louch4, Bjorn C. Knollmann1,2,3

Affiliations: Vanderbilt Center for Arrhythmia Research and Therapeutics1, Departments of Pharmacology2, Medicine3, Vanderbilt University Medical Center, Nashville, Tennessee, USA.4Institute for Experimental Medical Research, Oslo University Hospital and University of Oslo, Oslo, Norway, 5Department of Cardiology, Oslo University Hospital Rikshospitalet, Oslo, Norway, 6Department of Cardiothoracic Surgery, Oslo University Hospital Ullevål, Oslo, Norway

Introduction: Human induced pluripotent stem cells (hiPSCs) are increasingly used for modeling heart disease and are under development for regeneration of the injured heart. Incomplete structural and functional maturation such as a lack of t-tubules, relatively immature excitation-contraction (EC) coupling, and inefficient Ca-induced Ca release (CICR) remains a major limitation.

Hypothesis: Thyroid and glucocorticoid hormones are critical for fetal heart maturation. We hypothesized that their addition to standard protocols promotes t-tubule development and EC coupling maturation in hiPSC-cardiomyocytes (CM).

Methods: HiPSC-CMs were generated using a standard chemical differentiation method that was supplemented with triiodo-L-thyronine (T3) and/or dexamethasone (Dex) during days 16-30. Cells were then matured for 5 days on Matrigel mattress and studied using confocal microscopy and whole cell patch clamp.

Results: HiPSC-CMs treated with T3+Dex, but not with either T3 or Dex alone, developed an extensive t-tubule network (5.9±2.1%, $p<0.0001$ vs vehicle). T-tubule density was greater than that found in adult human atrial-CM but below that of adult human ventricular-CM. Notably, Matrigel mattress was necessary for t-tubule formation. Consistent with ventricular-like EC coupling, transverse line scans demonstrated uniform Ca release in T3+Dex cells compared to U-shaped Ca release in control cells. Simultaneous measurement of L-type Ca current and intracellular Ca release confirmed enhanced functional coupling between L-type Ca channels and RyR2 in T3+Dex cells (EC coupling gain 0.15 ± 0.11 vs 0.057 ± 0.05 $\Delta F/F0$ /ICa, $p < 0.01$).

Conclusions: Our results suggest a permissive role of combined thyroid and glucocorticoid hormones during the cardiac differentiation process which, when coupled with further maturation on Matrigel, is sufficient for robust functional t-tubule development. The new maturation technique reported here overcomes a major barrier in the stem cell field, which will help improve the utility of hiPSC-CM for disease modeling and provides mechanistic insight to the molecular pathways underlying t-tubule development.

Improving Allogeneic Bone Marrow Stem Cell Transplantation Through the Beneficial Effect of Flt3L on Plasmacytoid Dendritic Cells

Alina Ulezko Antonova*, Mojibade Hassan*, Edmund K. Waller*+

*Emory University, +Winship Cancer Institute

Hematological malignancies can be cured with hematopoietic stem cell transplantation (HSCT). The source of hematopoietic stem cells (HSC) from adult donors is either bone marrow (BM) or granulocyte colony-stimulating factor mobilized peripheral blood (G-PB). A randomized, multi-center clinical trial (BMTCTN0201) showed better overall survival (OS) and less graft-versus-host disease (GvHD) among recipients of grafts containing higher numbers of plasmacytoid dendritic cells (pDC). This is only true of BM grafts, but not G-PB grafts. Thus, BM pDC may be more effective than G-PB pDC in regulating post-transplant GvHD. Unfortunately, not all BM grafts have optimal pDC content. We hypothesized that FMS-like receptor tyrosine kinase 3 ligand (Flt3L) could be used as a method to increase pDC number in all BM grafts, because Flt3L upregulates pDC proliferation and maintains pDC homeostasis. We tested whether subcutaneous administration of Flt3L to mice would increase the content of pDC in BM grafts and whether transplantation of Flt3L-stimulated BM (F-BM) would result in better transplant outcomes in a murine model of HSCT. Flow cytometric analysis of F-BM versus unstimulated BM showed a 3-fold increase in BM pDC frequency. Transplantation of 0 or 4 million allogeneic donor T cells with either T-cell depleted control BM or F-BM resulted in better OS among recipients of F-BM than control BM alone (F-BM: 100%, 100% survival vs. BM: 80%, 30% survival, respectively), as well as less GvHD ($p<0.001$). Because G-PB grafts are utilized clinically more than BM grafts, we tested whether the beneficial effect of Flt3L was seen in combination with G-PB. Mice were transplanted with BM, F-BM, G-PB or Flt3L-treated G-PB with or without 4 million T cells. Interestingly, again, recipients of F-BM had the highest OS. These results suggest that donor BM graft treatment with Flt3L may improve HSCT outcomes by increasing pDC content and their GvHD-controlling activity.

Complement regulates CD4 independent and dependent antibody responses to RBC antigen

Amanda Mener*, Seema Patel*, Connie Arthur*, Sean Stowell*

*Emory University School of Medicine Department of Pathology and Laboratory Medicine

Purpose: While intrinsic antigen composition can influence whether a humoral response occurs through T cell-independent or dependent pathways, innate immune factors have also been suggested to impact the pathway by which humoral immunity can occur. This may be particularly important during the development of an anti-red blood cell (RBC) alloantigen antibody response, where complement component 3 (C3) deposition on transfused RBCs may serve as the primary adjuvant to drive antibody formation. As a result, we examined the impact of C3 on dictating CD4 T cell requirement during an alloantibody response to the model RBC alloantigen, KEL.

Methods: Using RBCs transgenically express the KEL antigen, we transferred KEL RBCs into C57BL/6 wild-type or mice deficient in C3 (C3 KO) or complement receptors 1 and 2 (mCR2 KO) in the presence or absence of CD4 T cells. Serum was collected at days 3, 5, 7, 14, 21 and 28 post-transfusion. Antibody development in WT, C3 KO or mCR2 KO recipients was examined by flow crossmatch, where serum was incubated with KEL RBCs. Following incubation, RBCs were washed and incubated with fluorescently-tagged secondary antibody and analyzed using flow cytometry. To determine the impact of CR1/2 expression on hematopoietic or non-hematopoietic cells, bone marrow transplantation (BMT) was performed utilizing CD45.1 WT bone marrow and CD45.2 mCR2 KO bone marrow transplanted into either WT or mCR2 KO recipients in the presence or absence of CD4 T cells.

Results: Similar to WT mice, C3 KO recipients unexpectedly generated a robust anti-KEL IgM response following KEL RBC transfusion. However, in contrast to WT mice, C3 KO mice were completely reliant on CD4 T cells to generate anti-KEL IgG. As previous studies demonstrate that C3 binds to CR1/2 on the B cell as part of the B cell activation complex, we next sought to determine whether C3 binding to CR1/2 in WT recipients may mediate a T cell-independent anti-KEL IgG response. To test this, mice deficient in CR1/2 (mCR2KO) were transfused with KEL RBCs in the presence or absence of CD4 T cells. Similar to C3 KO mice, mCR2KO mice generated an anti-KEL IgM response, but failed to produce anti-KEL IgG in the absence of CD4 T cells, further suggesting that the interaction between C3 and CR1/2 may act as a switch between T-independent and dependent responses to KEL. Transplantation of WT bone marrow into lethally irradiated mCR2KO mice demonstrated that CR1/2 expression specifically on B cells is important for the development anti-KEL IgG independent of CD4 T cell help.

Conclusion: These results suggest that the complement pathway may be a novel molecular switch that allows the same antigen to elicit either a T-independent or dependent antibody response.

Cosmc is an X-linked inflammatory bowel disease risk gene that spatially regulates gut microbiota and contributes to sex-specific risk.

Matthew R. Kudelka 1,2,3, Benjamin H. Hinrichs 2*,Trevor Darby 2*, Carlos S. Moreno 2, Hikaru Nishio 2, Christopher E. Cutler 3, Jianmei Wang 2, Huixia Wu 2, Junwei Zeng 3, Yingchun Wang 1, Tongzhong Ju 1, Sean R. Stowell 2, Asma Nusrat 4, Rheinallt M. Jones 2, Andrew S. Neish 2# and Richard D. Cummings 3#.

*,# Equal contributions. 1 Department of Biochemistry, 2 Department of Pathology, Emory University, Atlanta, GA. 3 Department of Surgery, BIDMC, Boston, MA. 4 Department of Pathology, University of Michigan, Ann Arbor, Michigan.

Purpose: Inflammatory bowel disease (IBD) results from aberrant immune stimulation against a dysbiotic mucosal but relatively preserved luminal microbiota and preferentially affects males in early onset disease. However, factors contributing to sex-specific risk and the pattern of dysbiosis are largely unexplored. Core 1 β3GalT-specific molecular chaperone (Cosmc), which encodes an X-linked chaperone important for glycocalyx formation, was recently identified as an IBD risk factor by genome-wide association study. We sought to understand the role of Cosmc in IBD and in regulation of the gut microbiome.

Methods: Cosmcf/+ females were crossed with B6.SJL-Tg(Vil-cre)997Gum/J transgenic males to generate Cosmc-KO, mosaic, and WT mice. Immunoblot, enzyme assays, tissue staining, ELISAs, and DSS colitis were performed as described. 16S sequencing was performed by MiSeq (Illumina) and MacQIIME. Gene expression was analyzed in mouse IECs with Illumina mouse ref 8v2.0 expression chips.

Results: We deleted Cosmc in mouse intestinal epithelial cells (IECs) and found marked reduction of microbiota diversity in progression from the proximal to the distal gut mucosa, but not in the overlying lumen, as seen in IBD. This loss of diversity coincided with local emergence of a proinflammatory pathobiont and distal gut restricted pathology. Mechanistically, we found that Cosmc regulates host genes, bacterial ligands, and nutrient availability to control microbiota biogeography. Loss of one Cosmc allele in males (IEC-Cosmc-/y) resulted in a compromised mucus layer, spontaneous microbe-dependent inflammation, and enhanced experimental colitis; however, females with loss of one allele and mosaic deletion of Cosmc in 50% of crypts (IEC-Cosmc+/-) were protected from spontaneous inflammation and partially protected from experimental colitis, likely due to lateral migration of normal mucin glycocalyx from WT cells over KO crypts.

Discussion/conclusion: These studies functionally validate Cosmc as an IBD risk factor and implicate it in regulating the spatial pattern of dysbiosis and sex bias in IBD.

Host and Commensals Cooperate in the Neonatal Intestine to Prevent Dysbiosis and Late Onset Sepsis

Jeffrey R. Singer, Emily G. Blosser, Ranjit Kumar, Casey D. Morrow, David A. Randolph, and Casey T. Weaver

Primary succession of microbial species that populate the mammalian intestinal tract is critically important to host health. The microbiome of premature infants lacks significant microbial diversity and is dominated by facultative anaerobic bacteria. Newborn mice share a similarly aerobic intestine and offer an underutilized model to study colonization dynamics of the premature intestinal tract. In this developing ecosystem, opportunistic pathogens commonly overgrow and translocate into the bloodstream, causing Late Onset Sepsis (LOS). Clinical investigations have suggested a role for the microbiome in the pathogenesis of LOS, but the exact mechanisms remain unclear. Using a novel murine model of LOS we sought to determine whether or not host or the microbiome itself offered the major buffering capacity against intestinal blooms to prevent blood-borne infection. Through gnotobiotic rearing and maternal antibiotic exposure, we found that a rich microbiome community is necessary to prevent opportunistic overgrowth and sepsis. Susceptible pups could be rescued with fecal microbiome transplantation (FMT), suggesting the microbiome was also sufficient for protection. However, differences between FMT input and engrafted communities demonstrate host factors strongly dictate early colonization dynamics. This is further demonstrated by the differing probiotic activity and colonization dynamics of various phylogenetically diverse Lactobacillus strains. Taken together, these data suggest that the pioneering members of the microbiome offer an important buffering capacity to prevent overgrowth and blood-borne infections by potentially invasive organisms. However, these organisms require host cooperation to support their colonization. Perturbing colonization dynamics of the microbiome through the use of broad-spectrum antibiotics may dramatically reduce this capacity and lend an already vulnerable host further susceptible to opportunistic infections.

A Potential Role of Vitamin D in Reducing Inflammation and Lowering Cellular Adhesion Proteins to Protect from Cerebral Malaria

John W. Diehl1,6, Lynda E. Polgreen2,6, Robert O. Opoka3, Troy C. Lund4, Chandy C. John5, Sarah E. Cusick4.

1 Emory University School of Medicine 2 Department of Pediatrics, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA, USA 3 Department of Pediatrics, College of Health Sciences, Makerere University, Kampala, Uganda 4 Department of Pediatrics, University of Minnesota, Minneapolis, MN, USA 5 Departments of Pediatrics and Microbiology and Immunology, Indiana University, Indianapolis, IN, USA 6 Equal contribution

Purpose: Cerebral malaria (CM) and vitamin D deficiency are prevalent in children in equatorial Africa. Vitamin D deficiency has been associated with inflammation, penetration of the blood brain barrier, and CM in animal models. We sought to determine if 1) lower total 25(OH)D is associated with a pro-inflammatory environment, and 2) lower 25(OH)D is associated with increased endothelial activation in Ugandan children with and without CM.

Methods: We measured plasma concentrations of 25(OH)D by LC-MS/MS, pro-inflammatory markers (TNF-alpha, IL-6, CRP, IFN-gamma), anti-inflammatory markers (IL-10, TGF-beta), and cellular adhesion markers (VEGF, vWF, VCAM, ICAM) in children 1.5-5 years of age admitted with CM (n=68) to Mulago Hospital in Kampala, Uganda from June 2010 to December 2013, and in age-matched community children (CC, n=60). We compared median values of biomarker data between groups using Wilcoxon rank-sum tests and evaluated the association of 25(OH)D with biomarker data using linear regression.

Results: Median 25(OH)D was significantly lower among children with CM than CC [50thp (25thp, 75thp): 21.0 (17.0, 26.5) vs. 26.0 (21.0, 32.0) ng/mL]; ($p<0.001$), and the prevalence of 25(OH)D deficiency (defined as 25[OH]D ≤ 20 ng/mL) was twice as high in the CM group as CC (46% vs. 23%; $p=.010$). Children with CM had significantly higher levels of pro-inflammatory markers TNF-alpha, IL-6, and CRP (all $p<0.05$) and higher concentrations of adhesion molecules (ICAM, VCAM, vWF, VEGF) compared to CC. We found an inverse association of 25(OH)D with IL-10 and CRP, and a positive association of 25(OH)D with TGF-beta, ICAM, and VCAM (all $p<.05$).

Discussion/Conclusion: We have shown for the first time in children in a malaria-endemic region a connection between low 25(OH)D and increased inflammation and endothelial activation. Considering these are essential steps in the pathogenesis of CM, vitamin D supplementation may have a role in the prevention of childhood CM.

Plasmodium vivax malaria: tissue burden and disease severity in Saimiri bolviensis model

Mariko S Peterson*; Regina J Cordy*; Chester J Joyner*; Jorge Salinas*,+; Stacey A Lapp*; Esmeralda V Meyer*; Malaria Consortium*,#; AnaPatricia Garcia*,**; Galinski MR*,**
*Corresponding authors

Emory Vaccine Center and Yerkes National Primate Research Center*; Division of Infectious Diseases, Emory University School of Medicine+; Pathology Division, Yerkes National Primate Research Center**; Malaria Host-Pathogen Interaction Center#

Plasmodium vivax is responsible for over 40% of malaria cases outside of Africa and causes a wide spectrum of clinical manifestations ranging from mild to lethal disease. In humans, organ damage may occur as a result of a variety of factors, including inflammatory processes. Plasmodium vivax has not been implicated in deep vascular tissue sequestration like P. falciparum, but modeling, experimental, and case studies point to potential 'concealment' of P. vivax parasites in organs such that they are removed from the peripheral blood circulation. We utilized a nonhuman primate model to investigate the effect of P. vivax parasites on different organs, and identified inflammatory processes that were associated with P. vivax-mediated pathology. Black-caped squirrel monkeys (Saimiri boliviensis) are susceptible to P. vivax infection, and have been used historically to obtain parasite source material and for vaccine studies. Using this monkey model to study short-term acute clinical blood-stage infections with P. vivax, we describe significant pathology, quantify apparent parasite tissue preferences, and make associations with immunopathogenesis. Organ damage was associated with inflammatory processes, with significant immune infiltrate composed predominantly of mononuclear cells, namely in the kidneys, lungs, and liver. Saimiri boliviensis infected with P. vivax suffer similar pathologies comparable to humans and they are a valuable model for immune response and pathogenesis studies. This is the most comprehensive study to date of acute P. vivax infections and tissues using a nonhuman primate model.

Ethanol-induced Dysregulation of Junctional Adhesion Molecule-A Contributes to Barrier Disruption in Intestinal Epithelial Cell Monolayers

Daniel. M. Chopyk*; Pradeep Kumar*, PhD; Khalidur Rahman*, PhD; Yunshan Liu*; Tekla Smith*; and Frank A. Anania*, MD

*Emory University School of Medicine, Department of Medicine, Division of Digestive Diseases, 615 Michael St. NE., Suit 265, Atlanta, GA, 30322, USA

Purpose: Ethanol exposure causes significant disruption of the intestinal tight junction (TJ), the structure responsible for the formation and regulation of epithelial barrier function. TJs consist of three groups of transmembrane proteins: occludin, claudins, and junctional adhesion molecules (JAMs). Our lab recently demonstrated that mice lacking JAM-A, the prominent JAM expressed in the intestine, have elevated serum lipopolysaccharide (LPS) levels compared to wild-type littermates after 8-weeks of high-fat diet. This observation supports a hypothesis that JAM-A regulates paracellular permeability to macromolecular solutes. JAM-A has been proposed to control the contractile tone of the apical cytoskeleton via modulation of myosin light chain kinase (MLCK) activity by a Rap2c-RhoA-MLCK signaling axis. Despite the demonstration of an important role of JAM-A for TJ function, the role of JAM-A in ethanol-induced gut barrier dysfunction has been left understudied. Here, we hypothesized that dysfunction of JAM-A contributes to ethanol-induced epithelial barrier disruption.

Methods: Caco-2 cells, a commonly used colon cancer cell line, were grown on semipermeable transwells inserts over 16-20 days to form differentiated epithelial monolayers. Alterations in protein expression and epithelial barrier function following ethanol treatment were measured by monitoring transepithelial electrical resistance (TEER) and apical-to-basal flux of fluorescein isothiocyanate (FITC)-dextran. MLCK activity was measured by immunoprecipitation followed by luminescence kinase assay. Rap2 activity was measured by pulldown followed by western blot. Additionally, JAM-A overexpression and knockdown were achieved by transfection and lentiviral transduction, respectively.

Results: Ethanol induced a dose- and time-dependent reduction in JAM-A protein expression. We also observed a reduction in active Rap2, followed by an increase in MLCK activity. Furthermore, stable knockdown of JAM-A enhanced paracellular flux of 40-kDa FITC-dextran following ethanol treatment, whereas it was reduced by JAM-A overexpression.

Conclusions: Together, our results suggest that dysregulation of JAM-A contributes to the mechanisms by which ethanol disrupts the intestinal epithelial barrier.

Morphologic Changes in Mouse Retinal Pigment Epithelium Subsequent to Light Induced Retinal Damage

Henry M Skelton*, Kevin J Donaldson^, Hans Grossniklaus^, MD, John Nickerson^, PhD

*Morehouse School of Medicine, Atlanta, GA; ^Emory Eye Center, Emory University, Atlanta, GA

Purpose: Light-induced retinal damage (LIRD) has shown promise as an animal model for dry age-related macular degeneration (AMD). However, its effects on the retinal pigment epithelium (RPE) layer have not been well characterized, despite the central role of the RPE in AMD. This study attempts to identify the changes in RPE morphology following light damage in order to provide for a better understanding of the pathology of photooxidative retinal damage and possibly suggest morphologic markers for diagnosis and treatment of AMD. Preliminary results suggested that after light damage the RPE exhibited increased presence of abnormally shaped RPE cells that were termed "potholes" and appear histologically as enlarged areas between tight junctions with many nuclei.

Methods: Mice (129SV) were light damaged with 30,000 lux white light for 3 hours. At set timepoints post light damage (3, 7, and 14 day groups) mice were sacrificed with their RPE-choroid-sclera flatmounted and stained for tight and adherens junctions. The entire flatmounts were imaged on a confocal microscope and analyzed using machine learning techniques to identify potholes in the cell sheet.

Results: At the 7 day timepoint, the light damaged eyes exhibited 17 potholes per 10,000 RPE cells, compared to 2 per 10,000 for the dim control ($p=0.02$). The 3 and 14 day timepoints were not significantly different from the dim control.

Discussion/Conclusion: The prevalence of pothole morphology was shown to increase transiently in the mouse RPE sheet following LIRD treatment. Further characterization of the underlying cellular processes could help elucidate the mechanisms of stress-induced retinal remodeling. The pothole morphology itself may serve as a feature of interest for in-vivo imaging of human RPE, as that technology approaches clinical viability.

Light-activated epigenetic control of gene expression

Corey G. Duke, Katherine E. Savell, Faraz A. Sultan, M.D.-Ph.D., Jeremy Day, Ph.D.

Department of Neurobiology, Evelyn F. McKnight Brain Institute, University of Alabama at Birmingham, Birmingham, Alabama

The control of gene transcription is critical to normal cellular function, and disruption of the regulatory mechanisms enabling this control has been implicated in most disease states. Thus, a thorough understanding of these mechanisms will provide new opportunities for therapeutic interventions for many disorders. Investigations into these regulatory mechanisms have proven difficult due to the complex interplay of several levels of molecular control, the difficulty of making targeted alterations at specific gene loci, and an inability to achieve the temporal precision required to mimic endogenous waves of gene expression. Here, we present several tools that enable the temporal and gene location precision necessary to investigate the role of specific epigenetic and transcriptional regulatory mechanisms at endogenous loci. Harnessing the Light-Activated CRISPR Effector (LACE) system, we have engineered several tools that allow manipulations of DNA methylation, histone acetylation, and the recruitment of transcription factors to specific gene loci in a time-dependent manner. To demonstrate the utility of these tools, we have employed the LACE system to investigate the key regulatory mechanisms utilized by several genes in multiple cell types, including genes critical for plasticity and memory formation in primary neuronal cultures. These novel fusion proteins will enable the investigations of gene expression regulation and epigenetic modifications with a precision that is critical to understanding how different mechanisms are utilized in normal physiologic function and in disease states.

Beyond Bubbles: Laser-Activated Perfluorocarbon Nanodroplets for Diagnostic Ultrasound Imaging and Therapy

Steven K. Yarmoska*, Daniela Y. Santiesteban*, Kristina A. Hallam*, Heechul Yoon+, Stanislav Y. Emelianov, Ph.D.*,+

*Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University School of Medicine, Atlanta, GA; +School of Electrical and Computer Engineering, Georgia Institute of Technology, Atlanta, GA

Purpose: Traditional ultrasound contrast agents (i.e., gas microbubbles) provide a wealth of anatomical and functional information within the vasculature. However, because microbubbles are only stable at sizes between 1 to 10 micrometers, they cannot extravasate or easily traffic within the extravascular space. Thus, molecular markers and therapeutic targets for neoplastic and neurological diseases are inaccessible to traditional microbubbles: outside of leaky tumor neovasculature and across the blood-brain barrier (BBB), respectively. To overcome this limitation, we have developed laser-activated perfluorocarbon nanodroplets (PFCnDs), agents that are stable at sub-micron sizes and can be triggered to undergo a phase change for on-demand ultrasound contrast and controlled delivery and release of cargo. These works demonstrate the diagnostic and therapeutic potential of laser-activated PFCnDs.

Methods: PFCnDs were synthesized via established sonication-based techniques and conjugated by adapting a directional nanoparticle bioconjugation protocol. Size studies utilized a dynamic light scattering instrument (Malvern) and a custom ultrasound-photoacoustic (USPA) imaging system (Verasonics and Opotek). Targeting studies used SK-BR-3 cells and confocal microscopy. Cancer imaging studies used nude mice with orthotopic MDA-MB-231 or 4T1 xenografts and a commercial USPA imaging system (Visualsonics). Neuroimaging studies used a custom fiber irradiation setup (Opotek) and the Visualsonics system.

Results: PFCnDs can be consistently synthesized at sizes permissive of extravasation. They can also be successfully functionalized with monoclonal antibodies, enabling molecularly-specific diagnostic ultrasound. PFCnDs show the potential to provide functional information about primary tumor vasculature and the ability to detect sentinel lymph nodes. Further, they can disrupt the BBB to deliver diagnostic agents and therapeutic cargo into the brain. Lastly, they can provide multiplexed information to interrogate multiple molecular targets simultaneously.

Discussion: Laser-activated PFCnDs demonstrate the potential for use in an array of extravascular imaging applications. Ongoing studies will investigate these agents in preclinical models of metastatic breast cancer, glioblastoma, and Alzheimer's disease.

ABSTRACTS – POSTER SESSION 1

Identification of the Replication Fork Proteome

Aditya A. Sathe, Sarah R. Wessel, Ph.D , Kareem N. Mohni, Ph.D, David K. Cortez, Ph.D

Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN

The information in our DNA is essential for life and must be copied billions of times each cell cycle. Although remarkably precise, replication is challenged by DNA lesions, difficult to replicate sequences, and exogenous sources of damage. A large number of DNA binding and accessory proteins facilitate this high-fidelity duplication. The Cortez Lab developed a revolutionary technique called iPOND (isolation of proteins on nascent DNA) to distinguish proteins bound to active replication forks. Most proteins from iPOND were correctly identified to be major components of replication machinery, yet many still remain uncharacterized. It will be important to establish a complete inventory of the proteins associated with the replication fork and validate those with previously undefined roles. In doing so, we may gain insight into new regulators of our genome and potential candidates for therapy. iPOND purification is used to capture proteins associated to replication forks. The enrichment of these proteins is quantified by mass spectrometry. Proteins are grouped into confidence categories based on these enrichment ratios. As an initial validation assay, siRNA pools are used to silence each protein coding gene and perform DNA combing analysis. Nucleotides incorporated into nascent DNA are labeled with an analog and stained with antibodies. The lengths of the elongating DNA for each gene knockdown is measured and compared to a non-silencing control to assess defects in elongation rates. DNA combing experiments show defects in elongation for a majority of proteins in the highest confidence category. Additionally, siRNA silencing experiments in the presence of replication stress agents greatly reduce cell viability. We present a comprehensive list of the replication fork proteome and support the iPOND data with validation experiments. Our list will serve as a database and resource for the lab and other scientists to aid in the characterization of novel regulators of genome integrity.

A New Protocol for the Transcription and Capping of HIV-1 RNA

Amara S. Ejikemeuwa*, Joshua D. Brown+, Michael F. Summers+PhD

Department of Biology, University of West Florida, Pensacola, Florida*; Department of Chemistry, University of Maryland, Baltimore County, Baltimore, Maryland+

The human immunodeficiency virus (HIV-1) is a retrovirus containing RNA as genomic material. The 5' leader region of the viral RNA genome is post-transcriptionally capped by a N7-methylated reverse-linked guanosine. This guanosine serves as protection against degradation from exonucleases, and has been found to significantly affect the overall structure and function of the HIV RNA genome. In order to study the native HIV RNA genome, large quantities of the capped RNA must be produced. The purpose of this project is to generate a procedure that 1) decreases the loss of RNA from an in vitro transcription and capping procedure, and 2) decreases the amount of time it takes to do so. Most RNAs are prepared in the lab by an in vitro transcription reaction, which results in a pure uncapped RNA product after a multi-day gel and washing purification. An additional in vitro capping reaction takes place to add the modified guanosine to the purified transcriptional product, followed by another round of gel and washing purification. The second round of purification can result in up to 50% of RNA loss. The transcription and capping procedure has a significant effect on the yield of RNA, and takes up to eight days. The new protocol utilizes filtration to combine the transcription and capping procedure into a single reaction with no gel purification being necessary in between. After in vitro transcription, the product is filtered with an amicon tube to remove reagents and residues, then the capping and purification procedure begins. This new protocol shortens the purification procedure by three to four days, decreases loss of RNA, and increases the pace of progress for capped RNA biophysical studies.

Determining the Specificity of the MIS Complex and its Role in the Methylation of mRNA.

Christina N. Schoose, Jack A. Dunkle PhD

Department of Chemistry, University of Alabama, Tuscaloosa, AL.

The MIS complex is composed of three proteins Mum2, Ime4, and Slz1. The reported in vivo function of the MIS complex is to modify mRNA within the cell converting specific adenosine residues in messenger RNA to 6-methyl adenosine, commonly termed m6A. This methylation is believed to play a role in regulating meiosis. The goal of this project is to perform in vitro assays with Ime4 or the MIS complex to understand its activity and specificity. We seek to determine how domain-wise truncations of Ime4 will affect its ability to modify mRNA. The Dunkle Research Group is investigating multiple recombinant expression systems for full length Ime4 and a library of truncation mutants. We report progress in molecular cloning, recombinant expression, protein expression, and purification of Ime4 and the truncation mutants.

Effects of Plasmodium falciparum Derived Hemozoin on Expression of Inflammatory and Coagulation Factors in BeWo Cells

Trisha Dalapati*, Catherine D. Smith*, Julie M. Moore, PhD*

*Department of Infectious Diseases and Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA

Plasmodium falciparum infection during pregnancy, which commonly progress into placental malaria (PM), is estimated to cause over 200,000 infant deaths annually. PM is characterized by sequestration of parasite-infected red blood cells (iRBCs) in the maternal space of the placenta. Pathogenesis is additionally marked by aberrant inflammation, adversely affecting nutrient and gas exchange in the syncytiotrophoblast, the outermost multinucleated cell layer of the placenta. Alongside inflammation, increased expression of tissue factor (TF) leads to activation of the extrinsic coagulation cascade and subsequent blood clotting. PM has been marked by excessive clotting, leading to low fetal birth weight and growth restriction. Protease activated receptors including PAR-1 and PAR-2 may be involved in positive feedback of inflammation and coagulation. Although inflammation and coagulation are often viewed separately, activation is concurrent. Because the pathology of PM remains ambiguous, quantifying gene expression of inflammatory and coagulation factors may provide essential mechanistic information. BeWo, a choriocarcinoma cell line, was used as an in vitro model to mimic in vivo host-parasite interactions. After reaching proper confluency, BeWo cells in duplicate samples were syncytialized and stimulated using lipopolysaccharide (LPS) or hemozoin. LPS, the positive control, is found on the outer membrane of gram-negative bacteria and elicits immune response. Hemozoin is the primary parasite by-product from the digestion of hemoglobin. After stimulation, cells were scraped at various time points for RNA isolation, cDNA generation, and real-time PCR. Three genes were chosen for study: TF, PAR-1 and PAR-2. Analysis of gene expression of these targets provide preliminary insight into the inflammatory and coagulatory responses during PM.

Early life stress in male mice induces vascular TLR4 expression in adulthood

Yasminye D. Pettway, Dao H. Ho, PhD, and Jennifer S. Pollock, PhD

Cardio-Renal Physiology & Medicine, Division of Nephrology, Dept. of Medicine, University of Alabama at Birmingham, Birmingham, AL

Purpose: Early life stress (ELS) has been correlated with an increased risk of developing cardiovascular disease (CVD) in adulthood; however, the mechanism by which ELS induces CVD risk is unknown. Research has implicated toll-like receptor (TLR) activation as a key link between inflammation and cardiovascular disease development, specifically the activation of TLR4. We used a mouse model of maternal separation with early weaning (MSEW) to study the effect of ELS on TLR4 expression and activation. In this model, we previously showed that ELS induces superoxide-mediated vascular endothelial dysfunction and increases circulating proinflammatory factors in the thoracic aorta. We hypothesized that ELS may contribute to CVD risk in adulthood in a TLR4-dependent manner.

Methods: MSEW mice were separated 4hr/day during postnatal day (PD)2-5, 8 hr/day during PD6-16, and weaned at PD17. Control mice were left undisturbed and weaned at PD21. Experiments were conducted on 3-6 month old male mice. The mRNA expression of TLR4 isoform in the thoracic aorta were determined via qRT-PCR. Free heme present in plasma was measured via ELISA.

Results: Compared to controls, MSEW mice display increased TLR4 mRNA expression. MSEW mice also have increased free heme in plasma, an activator of TLR4. Treatment with either TAK-242, a TLR4 receptor antagonist, or hemopexin, a heme scavenger, reduces superoxide produced by mouse aortic endothelial cells (MAECs) treated with MSEW plasma to levels comparable to control.

Discussion/Conclusion: These results suggest that ELS-induced superoxide production in mice may be dependent upon a heme/TLR4 signaling pathway. Future studies include investigating the effect of TLR4 on vascular endothelial dysfunction and mechanisms for increased free heme and TLR4 expression observed in MSEW mice.

Creating a Smaller Synthetic Muscleblind-like 1 Protein

Ryan C. Day, Melissa A. Hale, Andy Berglund

Muscleblind-like(MBNL) proteins are alternative splicing factors that regulate alternative exon use during development. MBNL proteins are sequestered by toxic repeat RNA transcripts produced in myotonic dystrophy (DM) patients. This sequestration of proteins leads to mis-splicing events responsible for disease phenotypes. MBNL1 consists of four different zinc finger (ZF) RNA binding motifs that fold into two distinct domains (ZF1-2 and ZF3-4). These two domains are connected by an unstructured linker region (LR) made up of approximately 76 amino acids. Although the ZF binding domains of MBNL1 are critical for this protein's functions, little is known about the importance of the flexibility between these two RNA binding domains offered by the LR. To determine how the LR impacts MBNL1 activity, we created MBNL1 proteins with shortened LRs of 57, 38, and 19 amino acids (L57, L38, and L19, respectively). Preliminary data from minigene splicing assays indicates that the three MBNL1 proteins with modified LRs have similar splicing activities, with a small activity decrease as the size of the LR decreases. These modified LRs were also introduced into synthetic MBNL1 constructs that contain either two ZF (1-2) domains or two ZF (3-4) domains. Additional splicing assays with all MBNL1 proteins containing modified LRs will be performed to further determine the functionality of these proteins. A reduced LR that orders MBNL1 into a more rigid compact structure may be advantageous over the full size MBNL1 protein in potential protein therapeutics for DM patients.

Modification of a Peptide Sequence with Selective Binding to Helix 69 to Increase Cell Permeability

Amer E. Abu-kwaik, Hyosuk Seo, Nisansala Muthunayake and Christine S. Chow*

Department of Chemistry, Wayne State University, Detroit, MI.

Purpose: Much research has been done to combat the emerging problem of antibiotic resistance. In the Chow lab, helix 69 (H69), a region on rRNA (ribosomal ribonucleic acid) in the bacterial ribosome that is crucial to cellular function has been studied as a potential therapeutic target. Peptides show promise as potential drug candidates. In the Chow lab, NQAANHQ was found to have moderate binding affinity and selectivity towards H69. However, peptides such as NQAANHQ have difficulty penetrating the cells of bacterial pathogens. The purpose of this study was to overcome this problem by attaching an alkyl-group to the N-terminus of the NQAANHQ (S1) peptide in order to increase membrane permeability, thus increasing the efficiency of cellular transport of the peptide.

Methods: The synthesis of the peptides was done utilizing solid phase fluorenylmethyloxycarbonyl (FMOC)-chemistry and modifications were added via click-chemistry. Peptide purification was performed via reverse-phase high performance liquid chromatography (HPLC) and purity was verified via matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS). Minimum inhibitory concentration (MIC) assays were performed to determine peptide efficacy utilizing an Acyl-[acyl-carrier-protein]-UDP-N-acetylglucosamine O-acyltransferase mutant Escherichia coli (LPXA E.Coli) strain.

Results: The MIC results indicate that the modified Alkyl-peptide (S1) required slightly lower peptide concentrations to inhibit bacterial growth when compared to the unmodified peptide.

Discussion: Overall, the modified peptide (S1) did not exhibit any significant improvement in efficacy when compared to NQAANHQ. Future studies will involve different modifications on NQAANHQ, such as altering the length of the chain, the nature of the attached functional groups, or the location of the modification (e.g. C-terminus). Future studies will also include H69 binding assays to examine the effect of modifications on binding strength and selectivity toward H69.

High Throughput Measurement of Mitochondrial and Glycolytic Activity in Planarians Reveals Activation of Glycolysis During Regeneration

*Edie A. Osuma,3,4 , Daniel W. Riggs2, Andrew A. Gibb,2,4 , and Bradford G. Hill1,2,4

1Institute of Molecular Cardiology, 2Diabetes and Obesity Center, Department of Medicine, University of Louisville, Louisville, KY, 3Wesleyan College, Macon, GA, and 4Department of Physiology, University of Louisville, Louisville, KY

Planarians are outstanding models for studying mechanisms of regeneration; however, there are few methods to measure changes in their metabolism. Examining metabolism in planarians is important because the regenerative process is dependent on numerous integrated metabolic pathways, which provide the energy required for tissue repair as well as the ability to synthesize the cellular building blocks needed to form new tissue. Therefore, we standardized an extracellular flux analysis method to measure mitochondrial and glycolytic activity in live planarians during normal growth as well as during regeneration. Small, uninjured planarians showed higher rates of oxygen consumption compared with large planarians, with no difference in glycolytic activity; conversely, glycolysis was increased during planarian regeneration. Exposure of planarians to koningic acid, an inhibitor of glyceraldehyde 3-phosphate dehydrogenase, completely abolished extracellular acidification with little effect on oxygen consumption, which suggests that the majority of glucose catabolized in planarians is fated for aerobic glycolysis. These studies describe a useful method for measuring respiration and glycolysis in planaria species and provide data implicating changes in glucose metabolism in the regenerative response.

Shear Stress Induces Calcium Influx via TRPV4 in Rat Lung Microvascular Endothelial Cells

Zahna Bigham - Division of Pulmonary and Care Medicine, Johns Hopkins University PCCM Summer Internship Program, Baltimore, Maryland
Laura Servinsky - Division of Pulmonary and Care Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland
Karthik Suresh, M.D. - Division of Pulmonary and Care Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland
Larissa Shimoda, Ph.D. - Division of Pulmonary and Care Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland

In pulmonary arterial hypertension (PAH) vascular remodeling of the small pulmonary arteries, leads to elevated right heart pressures and ultimately, heart failure. This luminal remodeling is driven in part by abnormal migration and proliferation of endothelial cells (ECs) that line the small pulmonary arteries. Though the mechanisms underlying abnormal EC function in PAH is not fully known, increased intracellular calcium (Ca 2+) levels are thought to play role. PAH ECs are exposed to increased shear stress, and in other vascular beds, increased shear stress has been shown to increase intracellular Ca 2+ . However, the effect of shear stress on intracellular Ca 2+ levels in PAH ECs is not known. To better understand the mechanisms underlying EC dysfunction in PAH, we isolated rat lung microvascular endothelial cells (RLMVEC) from control rats (N-RLMVEC) and rats undergoing Sugen/Hypoxia, an experimental model of PAH (SuHx- RLMVEC). To determine the link between shear stress and Ca 2+ in PAH, N- and SuHx- RLMVECs were exposed to increasing rates of shear stress and changes in intracellular Ca 2+ was observed. Since shear stress-induced Ca 2+ influx has been previously shown to involve the transient receptor potential vanilloid-4 (TRPV4) calcium channel, we examined the effect of TRPV4 activation and inhibition on Ca 2+ levels in N- and SuHx-RLMVEC at various levels of shear stress. We found that shear stress-induced Ca 2+ responses were higher in SuHx-RLMVEC, and that these responses were attenuated with TRPV4 inhibition. Additionally, Ca 2+ response following treatment with TRPV4 agonist GSK were significantly higher in SuHx-RLMVEC. Our data suggests that membrane bound TRPV4 plays a role in increased intracellular Ca 2+ levels in response to shear stress in SuHx-RLMVEC.

Efficacy of 18F FDG PET/CT in predicting local control of spinal tumors following SSRS.

Ethan B. Wang(1), Amol Ghia, MD(2), Andrew J. Bishop, MD(2), Jing Li, MD, PhD(2), Laurence D. Rhines, MD(3), Claudio E. Tatsui, MD(3), Debra N. Yeboa, MD(2), Behrang Amini, MD, PhD(4)

(1) University of Texas at Austin, Austin, Texas; (2) Department of Radiation Oncology, Division of Radiation Oncology, MDAnderson Cancer Center, Houston, Texas; (3) Department of Neurosurgery, Division of Surgery, MDAnderson Cancer Center, Houston, Texas; (4) Department of Diagnostic Radiology, Division of Diagnostic Imaging

Purpose: This study sought to determine the correlation between pre-treatment Standardized Uptake Value (SUV) in fludeoxyglucose-18 (18F FDG) PET/CT scans and local recurrence of spinal metastases following spine stereotactic radiosurgery (SSRS). We hypothesized that spinal lesions registering moderate SUV would exhibit the greatest local control.

Methods: We reviewed 446 patients treated with SSRS from 1/6/2014 to 6/30/2017 at M.D. Anderson Cancer Center. Patients were screened on the basis of receiving a 18F FDG PET/CT within 30 days prior to SSRS and having more than six months of follow-up post-SSRS. 41 patients remained. MIM Vista was used to gather data on SUVmean, SUVmax, total lesion glycolysis (TLG), and volume for all treated spinal lesions. A logarithmic function was applied to normalize SUVmax distribution. Local control was determined through PET/CT and/or MRI scans, and Kaplan-Meier curves were constructed for survival analysis.

Results: 14 patients (34%) had local failure, and there was no statistically significant difference in the SUVmax of lesions that exhibited local failure versus those that did not ($p>0.05$). Patients in the 3rd quartile of SUVmax (6.67-10.34, $p = .000$) performed significantly worse than the 1st (2.74-5.37, $p=.001$) and 4th quartiles (10.34-43.04, $p =0.009$). Using the third quartile to detect local failure yielded poor sensitivity, poor positive and negative likelihood ratios, and modest specificity (sensitivity = 0.357143, specificity = 0.81481, LRpos = 1.928571, LRneg = 0.78896).

Conclusion: SUVmax in the third quartile (6.67-10.34) is not sensitive but may be specific for predicting local failure. PET/CT may be useful in predicting SSRS treatment response and in guiding SSRS planning, but further study is needed to determine PET utility as an imaging biomarker for predicting local control rates following SSRS.

A novel bioinformatics approach for mapping the genomic landscape of pediatric Glioblastoma Multiforme

Gregory J. Schwing*, Chindo Hicks+, PhD

Department of Computer Science, University of New Orleans, New Orleans, LA; +Department of Bioinformatics and Genomics, Louisiana State University Health Sciences Center - New Orleans, New Orleans, LA

Purpose: A high-resolution integrated analysis contrasting adult and pediatric Glioblastoma Multiforme (pGBM) found substantial molecular differences between the two diseases. These differences justify an integrated analysis of pGBM as a distinct biological entity, which to our knowledge has not yet been done. The objective of this study is to map the genomic landscape of pGBM.

Methods: Publicly available gene expression data was downloaded from GEO. The raw CEL files of the eligible samples were retrieved. The samples were preprocessed by Robust Multi-Chip Average (RMA). The unadjusted p-value and False Discovery Rate were obtained. Single-nucleotide polymorphisms (SNPs) were taken from a Whole-Genome Sequencing study conducted at St. Jude Children's Research Hospital, classifying the probes as mutants and non-mutants. Probes meeting these thresholds were uploaded to Ingenuity: mutants < 1.0 x 10-7 and non-mutants < 1.0 x 10-10.

Results: Fifty-two experimental arrays and sixteen control arrays were included in the study. 2308 probes (mutants = 815; non-mutants = 1503) were uploaded to Ingenuity. The top networks were (1) Developmental Disorder, Hereditary Disorder, Neurological Disease; (2) RNA Post-Transcriptional Modification, Nucleic Acid Metabolism, Small Molecule Biochemistry; (3) Post-Translational Modification, RNA Damage and Repair, Protein Folding ; (4) Cell Signaling, DNA Replication, Recombination, and Repair, Nucleic Acid Metabolism; and (5) Cancer, Cellular Assembly and Organization, Cellular Function and Maintenance.

Discussion/Conclusion: These networks are based on experimentally observed interactions of the highest confidence. Networks (2) and (3) suggest a majority of the dysregulation in pGBM is not genomic but transcriptomic. Of particular interest is the last member of (2), Small Molecular Biochemistry, one of the hottest areas of cancer therapeutics. In the future, Molecular Dynamics will be applied to the biological system to search for small molecule ligands in the GROMACS software package.

Bayesian Optimization of Asynchronous Distributed Microelectrode Stimulation and Spatial Memory

Omer Ashmaig, Mark Connolly, BS, Robert E Gross, MD/PhD, Mahmoudi Babak, PhD

Purpose: There is a great need for a therapy to treat epileptic patients that successfully reduces seizure incidence with minimal side effects. One novel treatment developed in the tetanus toxin rat model of mesial temporal lobe epilepsy, asynchronous distributed microelectrode stimulation (ADMETS) in the hippocampus has been shown to significantly reduce seizure frequency. Preliminary results have demonstrated that ADMETS has a negative effect on spatial memory that scales with the amplitude of stimulation. Critical to advancing this therapy will be identifying the trade-offs between therapeutic efficacy and side effects. Given the high dimensional space of possible stimulation parameters, it is difficult to construct a mapping from variations in stimulation to behavioral effect. In this project, we present a novel, principled approach using closed-loop Bayesian optimization to tune stimulation to maximize a desired objective – performance on a spatial memory assay.

Methods: Three rats were implanted in the hippocampus with a 16-channel microelectrode array. Surgical details and stimulation parameters were consistent with prior publications. Each animal was evaluated using a spatial object recognition task separated by at least one hour. After each trial, the stimulation parameters and performance of the animal was added to a subject-specific model. Bayesian optimization then determined the next stimulation parameter based on a balance between exploration of unknown regions of the parameter space with exploitation of the suspected optimum.

Results: The optimization was performed for 13 iterations. In each animal, convergence of the Bayesian optimization algorithm indicated a maximum memory performance between 0.0 and 0.69V. Additionally, exploration of the parameter space found that above this threshold, memory was severely impaired.

Conclusion: We have demonstrated a behavior-domain closed-loop optimization approach that can successfully identify stimulation parameters with desirable behavioral effects. This approach can readily be scaled to higher dimensional parameter spaces where manually tuning stimulation parameters is intractable.

A Model-based Analysis of Changes in Neural State due to Optogenetic Stimulation in the Septohippocampal Network

Syed Ali Zaidi*, Mark J. Connolly B.S.+, Robert E. Gross, M.D., Ph.D.-, Babak Mahmoudi, Ph.D'.;

*Emory University; +Department of Biomedical Engineering, Emory/ GA Institue of Technology; -Emory School of Medicine Department of Neurosurgery; ' Emory School of Medicine Department of Biomedical Informatics

Purpose
Neural modulation is an alternative treatment for pharmaco-resistant functional neurological disorders, such as epilepsy, where the goal is to control the state of the dynamic, and ever changing, brain. The objective of our work is to use a biophysical computational model and state estimation to understand the underlying mechanism of modulating the septohippocampal pathway.

Methods
An unscented Kalman filter (UKF) is a method of nonlinear state estimation that uses two sets of Gaussian inputs and approximates probabilistic distributions for the future state. Using this method in conjunction with a classical Jansen and Ritt neural mass model of the hippocampus, we were able to analyze the effect of optogenetic stimulation of the septohippocampal pathway on the hippocampus in a non-pathological rodent model.

The GABAergic neurons of the rat medial septum (MS) was transfected through stereotaxic injection of an adeno-associated virus containing the excitatory ChR2 opsin with an hSyn promoter. The stimulation protocol consisted of twenty 60-second experiments: 20 seconds each of pre-baseline, stimulation, post-baseline). Using the UKF we were able to track the synaptic excitability of the inhibitory population as well the time constant variable for the population.

Results
Our results showed a significant increase in the excitability and a shorter synaptic time delay of the inhibitory population upon stimulation which persisted post-stimulation. The observed effect suggests that the recorded local field potential may describe the activity of the afferent MS neuron, rather than the local hippocampal neurons

Discussion
This insight gives us confirmation of the exact effect the modulation is having on the circuitry and sheds light on the biological underpinnings of the recorded neural signals.

Estimation of Mechanical Work by Pulmonary Endothelial Cells

Shivani Ananthasekar*, Neel G. Patel+, Dhananjay T. Tambe+^, PhD

*Department of Biomedical Sciences, University of South Alabama, Mobile, AL; +Department of Mechanical Engineering, University of South Alabama, Mobile, AL; ^Department of Pharmacology, Center for Lung Biology, University of South Alabama, Mobile, AL

Purpose: Fifty million people worldwide suffer from chronic lung diseases with the only available treatment being lung transplantation. An alternative is engineering bioartificial lungs which are generated by decellularizing donor lungs followed by autologous recellularization. However, achieving a functional vasculature is a reigning problem. Vessel recellularization requires each cell to go through processes of attachment, migration, division, and barrier formation. The goal of this study is to develop an assay for quantitative assessment of above processes in the heterogeneous mechanical microenvironment of cells.

Methods: We explore the extent to which cellular states can be quantified around a measurable mechanical property, mechanical work, by a cell. Key components of mechanical work comprise of forces that the cell exerts on the substrate and its neighbors and deformation of the substrate. These properties can be obtained using the technique called Monolayer Stress Microscopy (MSM). We report mechanical work by three different cell types: pulmonary artery endothelial cells (AEC), microvascular endothelial cells (MEC), and pulmonary vein endothelial cells (VEC).

Results: In an advancing sheet of pulmonary endothelial cells, the AECs had most uniform cobblestone morphology and remained most quiescent. On the other hand, VEC had non-uniform mesenchymal morphology and were least quiescent. The three cell types showed a systematic order where the most quiescent cell type, AEC, was also doing the least amount of work on their substrate, while the least quiescent cell type, VEC, was doing the most work. The most uniform distribution of mechanical work across the cell monolayer gave most productive migration to MECs.

Discussion/Conclusion: Using advancing sheets of pulmonary endothelial cells, we have built the analytical framework and opened a window onto the relationship of mechanical work with the processes including their ability to become quiescent and to cover the surface area. Quiescence was found to be inversely proportional to the amount of mechanical work. The arrangement where hardest working cells are dispersed across the monolayer provide fast coverage of surface area.

The Analysis of the Apoptotic Behavior of Neutrophils in Antipsychotic-induced Neutropenia

Swain, C.A., Cavnar, P.J., PhD

Department of Biology, University of West Florida, Pensacola, FL

Purpose: Atypical Antipsychotics used in behavioral drug therapies are known to cause severe side effects that have no known risk factors or direct causes. The antipsychotic clozapine, used to treat schizophrenia, is restricted due to it causing drug-induced neutropenia, or the condition of an abnormally low count of neutrophils in the peripheral bloodstream. Neutrophils are essential white blood cells of our innate immune system. Neutropenia leads to a weakened immune system and it can progress into agranulocytosis, a more severe form of neutropenia. The pathogenesis of neutropenia is the early and excessive programmed cell-death, or apoptosis, of neutrophils. Mitochondria play a role in neutrophil apoptosis and the mitochondrial membrane potential is a viable indicator of apoptotic behavior. Many factors can induce or inhibit apoptosis by interacting with the mitochondrial membrane. This project investigates the role of atypical antipsychotics in neutrophil apoptosis through analyzing the polarization of the mitochondrial membrane and cell viability.

Methods: The atypical antipsychotics clozapine, aripiprazole, olanzapine, and quetiapine was studied. Using the human neutrophil cell line PLB-985, JC-1 apoptosis assays and Annexin V/PI flow cytometry was used to study neutrophil mitochondrial health and apoptosis in response to these compounds.

Results: JC-1 one-hour incubation assays had shown that aripiprazole has a variable effect on neutrophils at all concentrations (1-20 μM). At incubation intervals of 2 to 24 hours, aripiprazole and quetiapine exhibit a dose-dependent, pro-apoptotic effect. Clozapine and olanzapine show very little effect on the mitochondrial health of neutrophils under all conditions.

Conclusions: These findings will contribute to the knowledge of the cellular mechanisms underlying the depletion of neutrophils in antipsychotic-induced neutropenia, leading to better initiatives in the use of atypical antipsychotics in behavioral health. Further studies are currently being conducted using flow cytometry to study possible apoptotic cellular markers expressed upon the treatment of these compounds.

Collagen Increases Proliferation and Drug Resistance of Papillary Thyroid Cancer Cells Harboring BRAFV600E Mutations

Anna Sharabura, Jonathan Jenkins, Laura J. MacDonald, Ph.D

Department of Biology, Hendrix College, Conway, AR

Purpose: Thyroid cancer is the most common endocrine cancer, and incidence is increasing worldwide. Thyroid cancer can be classified as either well-differentiated or poorly differentiated. Of well-differentiated thyroid cancers, papillary thyroid cancer is most common, and is associated with activating BRAF mutations. While our understanding of the genetic basis for thyroid cancer is fairly extensive, less is known about how the tumor microenvironment alters tumorigenic characteristics of thyroid tumor cells. Recently, Jolly et al. reported that papillary thyroid tumors derived from cells harboring activating BRAFV600E mutations and PTEN deletions are enriched with fibrillar collagen which is associated with decreased survival. In this study, we investigated whether growth on collagen enhanced tumorigenic characteristics of papillary thyroid cancer cell lines with BRAFV600E mutations.

Methods: Three distinct cell lines derived from mouse papillary thyroid cancer tumors were grown in the presence and absence of collagen. Morphology was assessed using brightfield microscopy. Proliferation was assessed by trypan blue staining and ATP concentration, while growth inhibition assays were used to assess response to chemotherapy drug resistance.

Results: Interestingly, our results suggest that growth on collagen contributes to a more mesenchymal morphology, increased proliferation, and decreased sensitivity to chemotherapy drugs.

Discussion/Conclusion: These and other results implicate an important role for collagen in the progression of thyroid cancer.

An Analogue of Securinine with Therapeutic Potential Against Acute Myeloid Leukemia

Anne Jessica T. Roe*, Sheela Karunanithi, PhD*, Ruifu Liu*, Yongchun Hou, PhD*, David N. Wald, MD, PhD*

*Department of Pathology, Case Western Reserve University, Cleveland, Ohio

The defining feature of Acute Myeloid Leukemia (AML) is a block of differentiation of hematopoietic progenitor cells. Traditional chemotherapeutics that have now been used for over 40 years target rapidly dividing cells instead of the differentiation block and exhibit both high toxicity and poor efficacy. Our therapeutic approach is a differentiation therapy that overcomes the maturation arrest safely and effectively. We previously identified securinine, a plant-derived alkaloid, as a promising AML differentiation agent. Here, we show that newly modified analogues of securinine exhibit favorable drug-like properties due to their low nanomolar potency on AML cells. To screen the new securinine analogues for high potency (EC50 <500nM) a cell growth assay on a variety of AML cell lines (ex- HL-60, OCI-AML3, MOLM-13) was performed. To begin to move the compounds forward, we also performed a series of drug development assays including in vitro liver microsomal stability assays, mouse pharmacokinetic studies, and toxicity testing. Compounds 250 and 317 that exhibited a t1/2>25min in vivo were used for further testing in a circulating AML (OCI-AML3-luciferase cells) model in immunodeficient mice (NSG). Mice injected with compounds 250 or 317 exhibited significant reductions in leukemic burden. Our preliminary studies into the molecular mechanism of these analogues suggest that their molecular target is thioredoxin reductase (TrxR). TrxR inhibition has been shown to lead to reactive oxygen species (ROS) production and disruption of the mitochondrial metabolism for which cancer cells are more dependent. We are currently exploring the links between both ROS production and metabolic changes in the leukemic cells as triggers for the 250 and 317-mediated growth inhibition and differentiation. Our future work also involves conjugating securinine-250 to viral nanoparticles to both improve the stability of the compound and to better target them to the tumor cells.

Understanding the Mechanistic Role of Integrin Alpha 6 in Tumor Development

Ashley B. Williams*, Reid D. Loveless*, Shauntell N. Luke*, Vinoth K. Sittaramane*, Ph.D.

*Department of Biology, Georgia Southern University, Statesboro, Georgia

Present day cancer incidence and mortality rates indicate the need for effective cancer diagnostic tools and targeted cancer therapeutic strategies. Recent studies have focused on the biological pathways of cells and tumor microenvironments to identify putative biomarkers and potential drug targets as diagnostic and therapeutic tools. Human integrins, adhesion receptors, have become the focal points in these studies, specifically Integrin Alpha 6 (ITGA6) which has been implicated in major tumor progression roles: metastasis and angiogenesis. These characteristics make ITGA6 an excellent candidate for a potential drug or diagnostic target, however, the mechanism by which ITGA6 facilitates tumor progression remains unclear. Cell culture studies have indicated ITGA6 could be cleaved extracellularly to increase metastasis but, zebrafish with organismal structures and vascular network, present a complete in vivo model to track metastasis. In this study, we aim to identify the role of ITGA6 in tumor development by using a humanized zebrafish model, where CM-DiI labeled human breast cancer cells (MDA-MB-231) are transplanted into Tg (Fli1a: gfp) embryos that are overexpressed with human ITGA6 constructs. To test the domain-specific role of ITGA6, human full-length or truncated extracellular or mutated non-cleavable ITGA6 RNA is injected into the zebrafish tumor xenograft. Our studies indicate that truncated ITGA6 overexpression significantly upregulates tumor metastasis compared to full-length ITGA6 overexpression. Similarly, mutated ITGA6 significantly decreases tumor metastasis. These results suggest that cleaved ITGA6 increases tumor metastasis, potentially aiding in extracellular matrix remodeling. The cellular role of ITGA6 will be evaluated by transplanting ITGA6 siRNA and DNA transfected MDA-MB-231 cells into zebrafish tumor xenografts. We anticipate these experiments will help establish the cell and non-cell autonomous roles of ITGA6 during tumor development. Further, we expect to use high-resolution imaging techniques to track the migration of single cancer cells in an in vivo system to understand the dynamics of metastasis.

Collagen Increases Apoptosis Resistance of Papillary Thyroid Cancer Cells with BRAFV600E Mutations

Jonathan Jenkins, Anna Sharabura, and Laura J. MacDonald, Ph.D.

Department of Biology, Hendrix College, Conway, AR

Purpose: Thyroid cancer is the most common endocrine malignancy, and projected increases in occurrence suggest it will exceed that of colon cancer by 2030. Of thyroid cancer subtypes, papillary thyroid cancer is most common and is associated with BRAFV600E mutations, which lead to constitutive activation of the MAPK signaling pathway. Previous studies in mouse models demonstrate that papillary thyroid tumors driven by BRAFV600E mutations and PTEN deletions are enriched in fibrillar collagen. Additionally, increased collagen expression in patient samples correlated with poor survival, suggesting its presence is important for papillary thyroid cancer progression. In this study, we investigated whether growth on collagen increased resistance to apoptosis and altered related signaling pathways.

Methods: Three cell lines derived from murine papillary thyroid cancer tumors were grown on collagen and assessed for increased resistance to staurosporine-induced apoptosis through Western blotting and immunofluorescence microscopy.

Results: Interestingly, our results suggest that collagen increases resistance to apoptosis in an AKT-dependent manner. Additionally, we found that growth on collagen lead to decreased sensitivity to chemotherapy drugs, which may be due to increased resistance to apoptosis.

Conclusions: Collectively, our results suggest collagen plays a key role in regulating tumor cell characteristics.

Collaboration of IDH2 and Spliceosomal Gene Mutations in Acute Myeloid Leukemia

Rachel Miles(1,2), Akihide Yoshimi, MD/PhD (3), Omar Abdel-Wahab, MD (3)

1Gateways to the Laboratory Program, Weill Cornell / Rockefeller / Sloan Kettering Tri-Institutional MD-PhD Program 2Vanderbilt University, 3Memorial Sloan Kettering Cancer Center

AML is characterized by rapid proliferation of immature white blood cells, affecting normal hematopoiesis. Mutations of the IDH2 gene are common in leukemias. In wild-type individuals, IDH metabolizes isocitrate into alpha-ketoglutarate, whereas mutant IDH further processes this compound into the oncometabolite 2-hydroxyglutarate (2HG) and leads to DNA hypermethylation via 2HG-mediated inhibition of TET family proteins. Mutations in the spliceosome also play a role, specifically in SRSF2; ~50% of IDH2-mutant patients have a co-existing mutation in this gene. In testing for a collaborativeis effect, both retroviral bone marrow transplantation assays and double knock-in mice expressing these genes from endogenous loci recapitulated the same phenotype: lethal mixed MDS/MPN neoplasms. Additionally, RNA sequencing of patients with both mutations revealed significant intron retention for the INTS3 gene, which encodes a component of the integrator complex that participates in snRNA processing. Alterations in processing of U2 and U4 snRNAs are similar when there is a double SRSF2/IDH2 mutation and when INTS3 is directly downregulated. We also are pursuing the therapeutic potential for combined mutant AML using AG-221, an inhibitor of the mutant IDH protein, H3B-8800, a spliceosome inhibitor, and combined treatment; a substantial decrease in pre-leukemic cells was observed in mice treated with both drugs.

Targetable Alterations in Global Lipid Metabolism upon Arginine Deprivation in ASS1 Deficient Sarcomas

Samuel F. Chang*, Bethany C. Prudner*, PhD, Lenny Rogers*, Brian A. Van Tine*, MD PhD

*Division of Medical Oncology, Washington University in St. Louis, St. Louis, MO.

PURPOSE: Argininosuccinate Synthetase 1 (ASS1) is silenced in ~90% of sarcomas. ASS1 silencing leads to deficiency in the urea cycle resulting in a dependency on extracellular arginine for continued cell growth and survival, termed arginine auxotrophy. Arginine starvation induced by pegylated arginine deiminase (ADI-PEG20) alters global metabolism. We investigated several fatty acid pathways to elucidate which of these pathways sarcomas become reliant on during ADI-PEG20 treatment. By examining the mechanisms leading up to reliance on fatty acid (FA) metabolism, we sought to identify various points within these pathways that are capable of being therapeutically exploited.

METHODS: Three Cell lines--SKLMS1, SKUT1, and LMS1--were treated with ADI-PEG20 at 1uM. Cells were stained with BODIPY, and the accumulation of lipid droplets was measured via fluorescence microscopy. Cell lines were then treated with a combination of ADI-PEG20 and perhexiline at 10uM (beta-oxidation inhibitor) to determine synthetic lethality. Finally, pathway analysis was performed via western blot to look at proteins within the endoplasmic reticulum (ER) stress response such as GRP98, CHOP, and PERK.

RESULTS: There were clear alterations in lipid metabolism induced by arginine deprivation. Elevated lipid droplet counts demonstrate increased dependency on lipid metabolism upon ADI-PEG20 treatment. Cell culture experiments with ADI-PEG20 and perhexiline demonstrated synthetic lethality. And moreover, ADI-PEG20 treatment led to increased expression of GRP78 on the cell surface.

CONCLUSIONS: An increased dependence on lipid metabolism is capable of being targeted by small molecule inhibitors, namely beta-oxidation inhibitors, with the result being an induction of synthetic lethality. And the accumulation of GRP78 on the cell surface upon ADI-PEG20 treatment points to the possibility of antibody-drug conjugate driven therapies. By understanding the molecular mechanism of change in the lipidome induced by arginine deprivation, we hope to build more specific, multi-agent synthetic lethal therapies for sarcoma based on metabolism that avoids chemotherapy.

Utilizing CRISPR Cas9 Technology to Knock Out T-cell Gene Expression For Use With CAR T-Cell Cancer Immunotherapy

Ian A. George, Lauren C. Fleischer, H. Trent Spencer, PhD

Gene and Cell Therapy, Aflac Cancer and Blood Disorders Center of Children's Healthcare of Atlanta, Emory University, Atlanta, GA.

Chemotherapeutics have long been the basis of cancer treatments, but despite years of research these treatments continue to be limited by their ineffectiveness, highlighting the need for novel therapeutics. Immunotherapies such as CAR (chimeric antigen receptor) T-cell therapies offer an attractive alternative by genetically engineering T-cells isolated from the host immune system to express surface proteins that target specific antigens expressed on cancerous cells. This study seeks to test the efficacy of utilizing a CRISPR (Clustered Regulatory Interspaced Short Palindromic Repeats) Cas9 system to induce a specific mutation that will knock out gene expression within these CAR T-cells to further specialize the therapy. Monoclonal colonies of EGFP (Enhanced Green Fluorescent Protein) positive Jurkat cells were created through a limiting dilution of a heterogenous population, and two EGFP positive clonal populations were isolated and subsequently nucleofected with either the CRISPR Cas9 vector targeting the EGFP gene, or a blank solution. At 19 days post-nucleofection the two clones that had undergone nucleofection with the CRISPR Cas9 vector presented EGFP negative populations of 19.8% and 6.2%, while the mock nucleofection populations presented EGFP negative populations of .4% and .6% percent, respectively. This significant reduction in EGFP expression indicated that the gene editing strategy was successful, theoretically allowing one to isolate the edited population in order to grow a stable colony of edited cells for therapeutic use. Due to this promising data we were able to design a new CRISPR Cas9 vector targeting our principal gene of interest. To expand the technology further, a cloning strategy was created in order to compare this Cas9 protein to a novel human codon optimized Cas9 protein, which will be tested in future experimentation to determine how to best optimize the technology for future use in human derived T-cells.

Intercellular adhesion molecule-2 decreases cell growth, anchorage-independent colony formation, and migratory abilities of MYCN-amplified IMR-32 neuroblastoma cells

Skyler V. Hendrix 1,2, Aubrey L. Miller 1, Joseph M. Feduska 1, Karina J. Yoon 1

1 Department of Pharmacology and Toxicology, University of Alabama at Birmingham, Birmingham, AL; 2 UAB Honors College, University of Alabama at Birmingham, Birmingham, AL

Purpose: Neuroblastoma (NB) arises from neural crest cells of the sympathetic nervous system, and accounts for ~15% of cancer-related deaths in children. High-risk NB cases are particularly deadly due to their tumor cell heterogeneity and tendency towards metastasis. The mechanisms by which NB cells metastasize remain relatively unknown. Our lab, however, has shown that Intercellular Adhesion Molecule-2 (ICAM-2) confers a non-metastatic phenotype in NB cells. The purpose of this research is to examine if ectopic expression of ICAM-2 results in a non-metastatic phenotype in NB cells lines using cell-doubling time, anchorage-independent colony growth, and modified Boyden Chamber migration assays.

Methods: To test the effect of ICAM-2 expression on NB cells, SK-N-AS (without MYCN amplification) and IMR-32 cell lines (MYCN-amplified) were used. Cell-doubling times were calculated by counting cell numbers over 120 hours. Colony growth assays were performed in 1.6% agarose gel and analyzed after a two-week incubation period. Migratory ability was examined using Modified Boyden Chamber plates and quantifying cells after a 48 hour incubation.

Results: IMR-32 ICAM-2 expressing cell lines were shown to have decreased cell-doubling times. All ICAM-2 expressing cell lines showed decreased colony formation abilities by approximately half, and an approximate three-fold decrease in migration. Western blot data also indicated that ectopic ICAM-2 expression inhibited the phosphorylated FAK and AKT cellular pathways involved in cell survival.

Discussion/conclusion: This study showed that ectopic ICAM-2 expression in NB cells decreased cell growth, inhibited colony formation, and decreased migratory abilities. In addition, western blot data indicated ICAM-2 involvement in inhibiting cellular pathways involved in cell survival and proliferation. Further functional assays will be conducted in order to investigate the role of ICAM-2 in MYCN-amplified NB cells, and evaluate ICAM-2 expression on the efficacy of chemotherapeutics.

A Histological Progression of Ear Skin and Muscle Regeneration in Acomys and Mus

Aaron W. Sandoval; Jason O. Brant, PhD; Malcolm Maden, PhD

Department of Biology, University of Florida, Gainesville, FL.

Regeneration has been studied almost exclusively in lower invertebrates as most mammals are only able to regenerate fetal tissue. The African spiny mouse (Acomys) represents the first time advanced regeneration has been observed in an adult mammal. Acomys has evolved a defense mechanism which involves fragile skin that tears easily when caught by a predator, allowing the mouse to escape. Subsequently, the mouse regenerates extensive parts of its body. The regenerative capabilities of Acomys are being further studied by comparing it to a normal mouse (Mus). In order to compare the progression of ear regeneration, ears of both species were wounded using a four-millimeter punch to remove the epidermal and dermal tissue layers. The ears were subsequently harvested and trichrome stained. Microscopic analysis revealed that although the cartilaginous layer eventually degenerated in both species, extensive degeneration was present much earlier in Mus. Furthermore, Acomys was able to regenerate its cartilage and hair follicles, whereas Mus was not. In order to compare the progression of muscle regeneration, the Tibialis Anterior (TA) muscles of both species were injected with cardiotoxin (snake venom) to damage the myocytes. At different time points, the mice were sacrificed and had their TA surgically removed and mounted. The slides were treated with antibodies for Collagen I and XII through immunocytochemistry and examined via immunofluorescent microscopy. It was found that regeneration is present in both, but it is much quicker in Acomys. TA muscle in Acomys returns back to a normal organizational pattern by Day 8, while Mus is still not normal by Day 16. The results of further study of Acomys could prove integral in gaining a comprehensive understanding of the regenerative process. Findings could ultimately improve the entire healthcare field by allowing for the regeneration of human tissue.

Iodine mediated one-pot successive cyclization-alkylation reaction strategy for the synthesis of biologically useful sulfur heterocycles

Christopher M. Cunningham, Katherine E. Whalen, Tanay Kesharwani, PhD,

Department of Chemistry, University of West Florida, Pensacola, Florida

Three reagents were combined to make a one-pot, two-step reaction synthesizing benzo[b]thiophenes; which is the core backbone of the compound. Benzo[b]thiophene are known to have physiological and biological purposes: FimH antagonists, anti-tumor, anti-fungal, kinases inhibitor, anti-inflammatory, anti-mitotic, anti-depressants, and estrogen receptor modulator. Benzo[b]thiophene can be applied to the field of organic material, because of the soluble and durable attributes of sulfur. The electrophilic iodocyclization methodology was used to synthesize benzo[b]thiophene by using iodine as the electrophile and as the catalyst for an alkylation reaction. The 1,3-diketone is the nucleophile, forming a noteworthy way of producing benzo[b]thiophene in one-pot, two-step reaction sequence. The reaction goes through completion under ambient conditions resulting in high yields of the disubstituted benzo[b]thiophene core structure which eliminates the use of excess reagents and did not result in any significant byproducts.

Copper and Sodium Salts mediated Electrophilic Halocyclization for the Synthesis of Benzo[b]thiophenes

Natalie L Fallows, Christopher A Walter and Tanay Kesharwani PhD

Department of Chemistry, University of West Florida, Pensacola, Florida

A new method was developed and optimized to synthesize benzo[b]thiophenes using electrophilic halocyclization. This method uses a copper(II) salt and simple sodium salts which act as an electrophile to perform the halocyclization and an external oxidant. A number of experiments were performed to test various substrates and proved that the method would retain high yields with an assortment of functional groups. Benzo[b]thiophene are naturally occurring molecules that have abundant applications in medicine and material science. Most recently, molecules with a benzo[b]thiophene core structure are used as antidepressants, antibacterial inhibitors, and estrogen receptor mediators. Applications within material science include phosphorescent OLED's and dye-sensitized solar cells. This new method is a simplistic process to form highly functionalized 3-halobenzo[b]thiophene derivatives.

Synthesis and Characterization of Fluorescent Substrates for Undecaprenyl Pyrophosphate Synthase.

Vanessa Amabo*, Amanda Reid*, and Jerry Troutman* (PhD)

*Department of Chemistry, University of North Carolina at Charlotte

Undecaprenyl Diphosphate Synthase (UppS) is an enzyme critical for the production of peptidoglycan, teichoic acid and capsular polysaccharides, which are essential for bacterial survival, interactions and pathogenicity. It catalyzes the condensation of eight isopentenyl diphosphate (IPP) with one Farnesyl diphosphate (FPP) to give a bactoprenyl diphosphate (BPP), a C55 molecule. Because UppS is crucial at the beginning stage of bacterial polysaccharide biosynthesis, it has become an attractive antibiotic target. Our interest is to determine the substrate selectivity of UppS from different bacterial species. In this report, we synthesized a library of FPP analogues with different sizes and polarity by substituting individual isoprene moieties with different fluorescent compounds such as benzyl anilines, biphenyls, acetophenones and anthracenes. In determining the photophysical properties of these analogues, we notice solvatochromic properties. This allows the use of a plate reader based fluorescence assay to test the substrate selectivity of UppS from different bacterial species. Since UppS is an antibacterial target, understanding its selectivity between different bacterial species can be exploited to target specific groups of bacterial species over another.

Patterns in Post-Discharge Venous Thromboembolism

Alice Li,* Anneliese Schleyer, MD, MHA, SFHM,+~ Ellen Robinson, PT~;

*Vanderbilt University, Nashville, TN; +Department of Medicine, University of Washington, Seattle, WA; ~Department of Quality Improvement, Harborview Medical Center, Seattle, WA

Venous thromboembolism (VTE), which encompasses both deep vein thrombosis (DVT) and pulmonary embolism (PE), refers to a blood clot that starts in a vein. Although VTE is a major cause of morbidity and mortality in the United States, many VTE cases within healthcare settings are considered to be preventable, especially for patients who were previously hospitalized within 90 days. To characterize this group of patients and achieve a better understanding of current hospital practices regarding VTE prophylaxis, a descriptive case series was conducted on 92 patients (70 patients previously hospitalized as inpatients, 22 as Emergency Department (ED) patients) who presented with post-discharge VTE at Harborview Medical Center in Seattle, WA between October 2015 and May 2017. The most common VTE risk factors found in this patient sample included age>40 (80.4%), hospitalized within 30 days prior to VTE admission (67.4%), acute medical illness (44.6%), smoking (35.9%), prior VTE (31.5%), obesity (29.3%), and surgery within 1 month (26.1%). Among principal diagnoses (recorded using ICD-10 codes) from the previous hospitalization, the two most common categories were 1) injury, poisoning, and certain other consequences of external causes (21.7%), followed by 2) diseases of the musculoskeletal system and connective tissue (12.0%). Strikingly, although nearly 90% of the 70 inpatients received VTE prophylaxis during hospitalization, less than 25% received VTE prophylaxis after discharge. Among the 22 patients whose prior hospitalization was an ED visit, only 13.6% received prophylaxis during hospitalization or at discharge. The results present new possibilities for further research seeking to improve VTE risk assessment and prevention models.

Locally-Advanced Breast Cancer in Casablanca, Morocco: An Epidemiological and Clinical Classification Overview

Samantha Fortier MPH. Karima Bendahhou MD, Amr S. Soliman MD/PHD

Ibn Rochd Oncology Center, Casablanca, Morocco, University of Nebraska, Department of Public Health, Boston University, Department of Pubic Health

Purpose: Breast cancer is the most common cancer among women in most middle-income countries. Morocco is one of those countries where the incidence of women breast cancer is about 47 per 100,000. Because many of cancer patients in developing countries seek care at last stage of the disease presentation and not all types of breast cancer are aggressive, we conducted a study to investigate the demographic, epidemiologic, and clinical characteristics of locally-advanced breast cancer (LABC) in the population-based cancer registry of Casablanca, Morocco. Methods: This retrospective study was conducted during the period of May-July 2017 and included about all breast cancer cases (4800 cases) from the registry for the period of 2008-2012 as well as all cases from the logbooks of the largest referral hospital in Casablanca for (Ibn Rochd Oncology Center) for the period of 2013-2015. Locally Advanced Breast Cancer (known as LABC) involving abstracting information from the Population-based registry located in Casablanca, Morocco, from 2008 to 2015. Results: The preliminary results show that LABC represents about 4.5% of all breast cancer patients in the registry and 6.3% of all breast cancer patients in the Ibn Rochd center. Because LABC is not an aggressive type of breast cancer, around half of the patients were followed for at least 2 years' post-diagnosis. Discussion/Conclusion: A proportion of patients were misdiagnosed as inflammatory breast cancer because of the inflammation-like symptoms encountered with the neglected disease. Future analysis will focus on characterization of the risk factors and tailoring screening and prevention programs for early detection and management of LABC in Morocco and middle-income countries.

Dysfunctional Uterine Bleeding in Women with Sickle Cell Disease Should Raise Alarms: Further Evidence of Decreased Incidence of Uterine Fibroids in the Setting of Sickle Cell Disease

Darrell Morris BS*, Janice Newsome MD+, Zachary L. Bercu MD+, Jonathan G. Martin MD+

*Morehouse School of Medicine, Atlanta, GA +Department of Radiology and Imaging Sciences, Emory University, Atlanta, GA

Purpose: Uterine fibroids (UF) are the most common benign tumor in women, with significantly increased incidence in premenopausal African American (AA) women. When a premenopausal AA woman presents with dysfunctional uterine bleeding (DUB), UF are primarily suspected. Based on initial data from an academic hospital system, we propose an inverse correlation between sickle cell disease (SCD) and UF. We seek to corroborate this data in a large county hospital patient population. If demonstrated, this recommends a more vigorous search for alternative sources of the bleeding, such as adenomyosis or malignancy, in women with SCD.

Methods: Four years of interventional radiology procedures at Grady Hospital were reviewed in this IRB approved retrospective study. A total of 79 AA women underwent uterine artery embolization (UAE) for symptomatic UF, and 10 AA women with SCD underwent disease-related procedures. Patient charts were analyzed for patient demographics, hematologic diseases, presence of fibroids, fibroid-related symptoms, and contraceptive and hormone use. Patient imaging was reviewed for primary and secondary signs of disease.

Results: Of the 79 AA women who underwent UAE, none (0%) had a diagnosis or secondary evidence of SCD. Of the 10 AA women with SCD, none (0%) had a diagnosis or secondary evidence of UF.

Discussion/Conclusions: In the United States, at least 80% of AA women will have UF by the age of 50. AA women also have a 3-fold increased age-adjusted incidence rate and 3-fold increased relative risk of UF. Thus, in the setting of DUB or bulk symptoms in AA women, the presence of UF is highly suspected.

SLC39A14-Related Manganism: Treatment Outcomes Over a 4-Year Period

Anita E. Qualls 3,4; Lance H. Rodan 1,2, MD; Siqi Cao 2,4, BS; Andrea L. Gropman 5
Pankaj B. Agrawal 1,3,4, MD

1Division of Genetics and Genomics, 2Department of Neurology, 3Division of Newborn Medicine, Boston Children's Hospital, Boston, MA 4The Manton Center for Orphan Disease Research, Boston Children's Hospital and Harvard Medical School, Boston, MA 5Division of Neurogenetics and Developmental Pediatrics, Children's National Medical Center, Washington, D.C.

Purpose: To date, 9 individuals in 5 families have been reported with a homozygous splice site variant of SLC39A14, resulting in chronically elevated blood manganese (Mn) levels, treatment refractory dystonia and brain mineralization. SLC39A14 encodes for a ZIP divalent biliary metal transporter that, when mutated, leads to blood Mn dyshomeostasis and deposition in the globus pallidus and substantia nigra of the basal ganglia. We report the clinical case history and 4-year novel treatment outcome for an additional patient identified with this genetic disorder.

Methods: Whole-exome sequencing (WES) revealed an intronic mutation in SLC39A14 c.751-9 C>G (IVS5-9C>G) that was initially missed on Sanger sequencing of the gene. Whole genome sequencing was also performed while the WES data was analyzed. The identified variant was confirmed by Sanger sequencing the family and confirmed to be only present in the affected proband.

Results: The patient was treated with oral chelators, botox injections, novel nutritional treatments including manganese limited and manganese free diets, and 5-day IV NaCaEDTA chelation therapy over a 30-month cycle.

Discussion: Despite persistent treatment over the past 4 years, the patient's serum manganese levels remain essentially unchanged. While each course of chelation caused temporary improvement in dystonia and motor function, overall her dystonia and dysarthria continue to worsen. She is now a candidate for deep brain stimulation (DBS). The benefits and limitations of each treatment are important to understand in comparing the gradual disease progression of our patient to the other SLC39A14 hypermanganesemia patients who demonstrated some long-term relief of symptoms with the chelation therapy.

The Specific Activity of Acid Phosphotase in Arabidopsis

Craig X. Hinson and Larry L. Lowe.

Biology, Chemistry, and Environmental Health Science Department, Benedict College, 1600 Harden Street, Columbia SC 29204.

Acid phosphatases are a family of enzymes present in many animal and plant species. In animals it is found in high concentrations in the epithelial cells of the prostate, but is also found in the cellular components of bone, spleen, kidney, liver, intestine, and blood. In plants it causes the hydrolysis of phosphate esters within the plant to assist in the energy metabolism and metabolic regulation of plant cells and plant growth. The purpose for conducting this research is to determine if Salk insertional mutations affects the specific activity of acid phosphatase in Arabidopsis plants compared to the non-insertional controls, and compare these results to overall plant growth and developmental phenotypes. The method used to determine acid phosphatase specific activity was by measuring the conversion of p-nitro phenol phosphate (PNPP) to p-nitro phenol (PNP) at 405 nm using the Beckman Model DU-640 UV/VIS spectrophotometer. The results of this initial investigation showed an average specific activity of acid phosphate of the Sigma control was 0.492 units/mg of total protein and the kale control plant was 0.013 units/mg of total protein. These results are significant to future work by establishing baseline acid phosphatase specific activity to be compared to acid phosphatase specific activity in Salk control and mutant Arabidopsis plants.

A Behavioral Genetics Approach to Understanding Risky Driving

Maryann O. Aniekwe, Matthew T. Schmolesky PhD, Jennell M. Talley PhD, Jill K. Penn PhD

School of Science and Technology, Georgia Gwinnett College, Lawrenceville, GA

Novelty seeking is a personality trait in Cloninger's (1993) Temperament Character Inventory that is associated with people's tendency to engage in risky behavior. The purpose of this study was to examine the relationships between college students' novelty seeking personality trait, gender, race/ethnicity, and genotype, and, ultimately, their propensity to drive recklessly based on these factors. The gene of interest, SLC6A4, codes for the serotonin transporter. Polymorphisms (S-short allele; L-long allele) in this gene may be related to transcription rates of the transporter in the human brain that could influence personality, cognition, and behavior. Participants (n=199) completed the Temperament Character Inventory to assess novelty seeking as well as two inventories to measure their frequency of engagement in, and risk assessment of, three driving behaviors: riding a motorcycle, driving after drinking alcohol, and reckless driving. DNA was collected using cheek swabs and the buccal samples were genotyped using the polymerase chain reaction and agarose gel electrophoresis. The results of this study showed no correlation between novelty seeking scores and risky driving. There was, however, a negative correlation ($r = -0.22$, $p < 0.05$) between participants' engagement in reckless driving and their risk assessment of that behavior. In our sample, gender did not predict the frequency of driving recklessly but race/ethnicity did. Based on initial genotyping (n=34), there was no significant difference between the average reported engagement or risk assessments for the S/S, S/L, or L/L genotypes for driving recklessly or for driving after drinking alcohol; however, preliminary data suggest S/S participants report higher rates of riding motorcycles. Genotyping is still in progress.

Cellular Mechanisms Underlying Gene-Nutrient Interactions Affecting Longevity

Joline S. Hartheimer (1), Sean M. Santos (2), Mert Icyuz (2), Haley D. Albright (2) John L. Hartman IV, MD (2)

1. Department of Chemical and Biological Engineering, The University of Alabama, Tuscaloosa, AL; 2. Department of Genetics, The University of Alabama at Birmingham, Birmingham, AL

Purpose: Aging is associated with declining function and increased risk of disease. Dietary interventions are known to modulate lifespan and healthspan, yet the cellular mechanisms of nutritional influences on aging are not well understood. Saccharomyces cerevisiae can be used to measure aging by calculating Chronological Life Span (CLS), the amount of time that a non-dividing yeast cell survives. CLS can be measured using Quantitative High Throughput Cell Array Phenotyping (Q-HTCP) which uses robotics and computational analysis. Of great interest to aging in eukaryotes is the fraction of quiescent cells in stationary phase yeast culture that have exited the cell cycle due to nutritional stress, but are still capable of reentering upon rich nutritional conditions. We aim to identify gene-nutrient interactions affecting S. cerevisiae on chronological life span and better characterize their cellular mechanisms.

Methods: CLS was measured by Q-HTCP to identify aging phenotypes resulting from different auxotrophic alleles of S. cerevisiae on human-like media with different concentrations of carbon (dextrose) and nitrogen (ammonium sulfate). Mitochondrial activity and cell cycle distribution (G1/G0 arrest) were assessed with flow cytometry.

Results: Dextrose restriction and methionine auxotrophy protected against aging over 35 days. Ammonium sulfate's effects depended on auxotrophy and dextrose concentration. In two auxotrophic strains, mitochondrial activity decreased over 6 days. Prototrophs had a more stable quiescent (G0) state than auxotrophs over 7-10 days.

Discussion/Conclusion: Complex gene-nutrient interactions exist in yeast and their mechanisms will be further analyzed with multiwell and multiplex flow cytometric assays with respect to apoptosis, bud scars, and lysosomal acidity.

Cytotoxic CD8 T-Cells in Von-Hippel Lindau Renal Tumors

Fares Hosseinzadeh, Caroline S. Jansen, Jennifer W. Carlisle MD, Kevin R. Melnick, Kyu Kim MD, Amir I. Khan, Kristin Larsen, Tiffany Ding, Nataliya Prokhnevska, Yilin Zhang, Viraj A. Master MD PhD, Haydn Kissick PhD

Von-Hippel Lindau (VHL) syndrome predisposes carriers to the production of abundantly vascularized tumors in the retina, cerebellum, spine, kidney, adrenal gland and pancreas. People affected by this disease have a germ-line mutation that inactivates one copy of the VHL gene in all cells. Immunotherapy is a vital therapeutic option for patients because it can suppress tumor growth and terminate or reduce the spread of cancer. Many drugs have been recently discovered that can facilitate the immune response of cancer patients, but none have been proven to work specifically towards VHL tumors. In our experiment, we have compared the number of cytotoxic CD8 T-cells in our VHL renal tumor samples and non-VHL renal tumor samples. We processed samples using the same protocol to create a tumor lysate and then used Fluorescence-activated cell sorting (FACS) flow cytometry to analyze the immune profile of the frozen-fresh tumor samples. This study will help us further understand the immunology of this vaguely understood disease. With our findings, we hope to aid future research towards the creation of new or improved immunotherapeutic regimens for treatment of patients diagnosed with VHL.

Microfluidics for environmental control and quantitative analysis of mouse stem cell aggregate differentiation to motor neurons

Amanda W. Schaefer, Emily L. Jackson-Holmes, Hang Lu, PhD

School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, Georgia

Pluripotent stem cells (PSCs) can be differentiated as three-dimensional aggregates into specific cell and tissue types for therapeutic purposes and to study mechanisms of embryonic development. Current culture methods for culturing cell aggregates include microwells, hanging drops, multiwell plates, petri dishes, and spinning bioreactors; however, these methods are often unable to reproducibly control the culture environment, track individual samples longitudinally, and image samples in situ. To address these challenges, we have developed a microfluidic platform that provides a controlled environment for culture and differentiation of mouse embryonic stem cell (mESCs) aggregates. We use this platform to study how cell culture environmental parameters affect differentiation of mESCs to motor neurons.

Our microfluidic devices are fabricated out of polydimethylsiloxane (PDMS) replica molded from a silicon master mold and plasma bonded to glass coverslips. Devices are loaded with Olig2-GFP mESC aggregates, which are formed by forced centrifugation of cells in microwells in a neural induction media overnight. Devices are connected to a syringe pump in a cell culture incubator, and media is perfused continuously at a set flow rate for up to nine days. Aggregates are cultured in batch stirred suspension culture as a control. Live imaging of aggregates on-chip and in batch on days four through nine indicates that aggregates on-chip are more uniform in size, and we hypothesize that the microfluidic platform can better modulate the cell microenvironment. Controlling the cell culture environment impacts differentiation and can help us study differentiation mechanisms. Initial evidence suggests that microfluidic culture conditions have significant effects on generation of Olig2+ progenitor motor neurons. We will continue to screen additional media perfusion flow rates to investigate whether culturing on-chip has an advantage over culturing in batch such as producing purer populations of motor neurons or reducing heterogeneity in differentiation.

Modulating Inflammasome Activity by using FcyRIIa

Katherine Quintin, Michelle Colbert, Katherine Cascino, Laura Cohen, Michael A. Chattergoon, Andrea L. Cox

Johns Hopkins School of Medicine

When a virus is endocytosed by a cell, a signaling cascade is initiated and results in inflammasome activation and the production of pro-inflammatory cytokines. When antibodies are produced in response to the virus, the antibodies bind to Fc receptors (FcRs), specifically FcyRIIa, and inflammasome activity is suppressed. The mechanism by which this inflammasome suppression occurs is unknown. In order to study how the presence of antibodies suppresses inflammasome activity, a stable THP-1 cell line with over expression of FcyRIIa (WT and mutant) can be made and used as a tool for inflammasome activation experiments. By developing a FcyRIIa mutant lacking a cytoplasmic tail, it will be possible to determine if the signaling part of the FcyRIIa receptor is important for blocking inflammasome activation and signaling, or phagocytosis, or if the antibody/FcR is keeping the virus from entering the cell. To accomplish this, in this study we sought to clone the FcyRIIa WT (and mutant) into pMXs-IRES-GFP plasmids. Then, using the Fc-containing pMXs-IRES-GFP plasmid, we generated a lentiviral gene delivery system to infect THP-1 cells. The same procedure was done with the FcyRIIa Mutant. We were able to clone the FcyRIIa Wild Type successfully, which will be used for the formation of the lentivirus for this ongoing study.

Synthesizing Curcumin Loaded Silver Nanoparticles as a Novel Anti HIV-1 Therapeutic

Elizabeth Quaye, Rakesh Kumar Sharma, Katherine Cwiklinski, Ravikumar Aalinkeel, Jessica L. Reynolds, Donald E . Sykes, Supriya D. Mahajan and Stanley A Schwartz.

Department of Medicine Division of Allergy, Immunology, and Rheumatology State University of New York Clinical Translational Research Center Buffalo, NY 14203, USA.

Silver nanoparticles (AgNPs) have been demonstrated to have antiviral properties and thus could be used as agents against human immunodeficiency virus (HIV). The pathogenic potential of HIV-1 is due to its rapid replication, spread and successful neutralization of host restriction factors which is mediated by its regulatory and accessory proteins. Curcumin has anti-HIV activity as it is an inhibitor of HIV-1 protease, integrase and LTR. Further, it also inhibits NF-κB pathway which is important for HIV-1 gene expression. A nanoformulation of curcumin and AgNPs, (Cur-AgNPs) will allow increased bioavailability of curcumin and should have excellent anti-HIV activity. We synthesized and characterized Cur-AgNPs and found them to be 45nm by dynamic light scattering with a maximum absorbance at 406 nm. The antiretroviral effects of Cur-AgNP were determined in ACH-2 cells latently infected with HIV-1. ACH-2 cells, were treated with Cur-AgNP for 24-48 hr. Expression of HIV-1 LTR and p24, the pro-inflammatory cytokines, IL-1b, TNF-a, and NF-κB were quantitated. Treatment of ACH-2 cells—latently infected with HIV-1—with Cur-AgNP produced no toxic effects but significantly inhibited the expression of: HIV-1 LTR (-73%, $p<0.01$) and p24 (-57%, $p<0.05$), IL-1b (-61%, $p<0.01$), TNF-a (-54%, $p<0.05$), IL-6 (-68%, $p<0.01$), and NF-κB (-79%, $p<0.0001$) as compared to untreated controls. Thus, Cur-AgNP have therapeutic potential as direct antiretroviral agents, as well as immunomodulatory activities inhibiting the expression of pro-inflammatory mediators induced by infection with HIV-1. Experimental controls, such as AgNP alone, curcumin alone and conventional silver nanoparticles capped with citric acid produced no similar biological effects. We conclude that treatment of HIV-1 infected cells with Cur-AgNP, significantly reduced replication of HIV by inhibition of NF-κB nuclear translocation and the downstream expression of the pro-inflammatory cytokines IL-1β, TNF- and IL-6. Subsequent in vivo studies with Cur-AgNP using a humanized mouse model of HIV infection are underway.

Antimicrobial Peptide Resistance Mechanisms in Staphylococcus aureus Infection

Emilie L. Fisher*, Gordon Y. C. Cheung*, PhD, Joshua W. McCausland*+, Justin Choi*%, Seth W. Dickey*, PhD, Michael Otto*, PhD

*Laboratory of Bacteriology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD; +Johns Hopkins University, Baltimore, MD; %Cornell University, Ithaca, NY

Antimicrobial peptides (AMPs) constitute an important part of the innate host defense. Possibly limiting the therapeutic potential of AMPs is the fact that bacteria have developed AMP resistance mechanisms during their co-evolution with humans. However, there is no direct evidence that AMP resistance per se is important during an infection. Here we show that the Staphylococcus aureus Pmt ABC transporter defends the bacteria from killing by important human AMPs and elimination by human neutrophils. By showing that Pmt contributes to virulence during skin infection in an AMP-dependent manner, we provide evidence that AMP resistance plays a key role in bacterial infection.

The Effect of Antibiotics on Non-growing Bacterial Populations

Nilang N. Shah, Ingrid C. McCall, MPH, Adithi Govindan, Bruce R. Levin, PhD.

EcLF Lab, Department of Biology, Emory University, Atlanta, Georgia.

Non-growing populations of bacteria are known to be phenotypically refractory to antibiotics to which they are genetically susceptible. Here, we consider three classes of non-growing planktonic bacteria: (1) Stationary phase, when the concentration of the limiting resource is too low to allow for replication; (2) Minority subpopulations of non- or slowly replicating cells, known as persisters; (3) Populations exposed to bacteriostatic antibiotics that prevent their replication and kill them slowly, if at all. Using experimental populations of Staphylococcus aureus Newman and Escherichia coli K12 and 10 different antibiotics of various classes, we address the quantitative question of how refractory non-growing populations of these different types are susceptible to antibiotics and the rates at which different concentrations of these drugs kill them. Contrary to the dogma that non-growing bacteria are fully refractory to antibiotic-mediated killing, non-growing populations of Gram-positive and Gram-negative bacteria of all three classes are susceptible to killing by some antibiotics. Albeit at a rate substantially less than when they are growing exponentially, the aminoglycocides like gentamicin and tobramycin, are particularly effective at killing non-growing S. aureus and E. coli of all three types; other drugs do so at lower rates. With the aid of mathematical computer simulation models, we consider the potential clinical implications of antibiotic-mediated killing of non-growing bacteria to the course of treatment and the emergence of resistance.

Immune response of the bed bug, Cimex lectularius, to simulated traumatic insemination

Lily M. Brooks and Jonas G. King, PhD.

Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University, Starkville, MS

The bed bug, Cimex lectularius, is an ancient and persistent human pest. Their bites can cause a medically significant reaction in some people and their numbers and range have been expanding recently due to increases in insecticide resistance and international travel. While bed bugs are thought to have evolved several immune-related adaptations to aid their unique lifestyle, the specific mechanisms involved in their innate immune system are poorly understood. Along with hematophagy, bed bugs undergo major exposure to pathogens during the process of traumatic insemination. During this process, male bed bugs pierce the female through their body wall and inseminate the body cavity directly. It has previously been shown that females have evolved physiological adaptations that abate potential fitness costs associated with this process. As the bed bug genome was completed in 2016, we now have the capability of measuring immune gene regulation in response to immune challenges. Here, we used simulated traumatic insemination events followed by correlative microscopy and qRT-PCR-based analyses to take a systemic approach to understanding the Cimex defensive response against hemocoelic inoculation with bacteria native to the Cimex cuticle. Our primary goal is to measure the transcriptomic response of a suite of conserved innate immune-related genes: Prophenoloxidase, Nitric Oxide Synthase, C-type Lectin 8, Argonaute-2, and Defensin. As a secondary goal we are also measuring population changes in the bacterial endosymbionts Wolbachia and Cimex-specific proteobacteria and changes in expression of a Cimex RHS-family protein, which appears to have been obtained via horizontal gene transfer and could potentially serve a role in reducing the threat posed by exposure to pathogenic bacteria. Current results suggest that antimicrobial peptides respond as anticipated to this immune challenge, while ongoing studies examining the responses of RHS proteins and microbial symbiont levels will be presented.

GDNF improves cutaneous wound healing through hair bulge stem cells in mice

Rajkumar, C., Braun, R.E., Lisse, T.

The Jackson Laboratory

Glial Cell-Derived Neurotrophic Factor (GDNF) is a well-studied neuroprotective factor, however its extraneuronal functions remain less characterized. Recent studies made the unexpected finding that application of GDNF can enhance cutaneous wound repair and hair regeneration in mice; however the mode of action is unclear. By using the Krt15-CrePR1:R26R-Confetti reporter mouse model, we show that recombinant GDNF may promote the activation and commitment of hair bulge stem cell (BSC)-derived progenitors to the neo-epidermis seven days after injury (DPI). In vehicle treated wounds, we observed 2.2 (±0.2 SEM; n= 2 wounds) uniquely colored fluorescent labels, 3.4(±0.8 SEM) number of labeled colonies, and 2.1(±0.6 SEM) cells within individual colonies after injury in the neo-epidermis. In contrast, GDNF treatment yielded on average 3.2(±0.4 SEM; n= 4 wounds) uniquely colored labels, 9.1 (±3.2 SEM) number of labeled colonies, and 2.8 (±0.9 SEM) cells within colonies of wounds after injury. Next, we conditionally deleted Ret within hair follicle BSCs and assessed wound healing responses. In both male and female Krt15-CrePR1:Retflox/flox mice, we observed a -1.4 and -1.6mm2 effect on wound closure, respectively, in comparison to control animals at 4 DPI. Histological analysis revealed an expansion and impaired resolution of the granulation matrix in the wound bed of Ret-deficient animals. Additionally, we observed a 15% decrease (p=0.03, n=3 serial sections, Student T test) in proliferative epidermal basal cells within the neo-epidermis upon Ret ablation, suggesting impaired wound re-epithelization. Our data suggests that GDNF-RET signaling may underlie BSC activation and migration of their progenitors to the wound neo-epidermis to improve skin healing outcomes.

Insulin-Like Growth Factor 1 Receptor Expression in Postmortem Brain Tissue of Autism Spectrum Disorder

Brooke E. Beasley*, Aubrey Sciara+, Macy Davis*, Gregory Ordway, PhD^, and Michelle Chandley, PhD*

*Department of Health Sciences, East Tennessee State University, Johnson City, TN; +Department of Biological Sciences, Quillen College of Medicine, Johnson City, TN; ^Department of Biomedical Sciences, Quillen College of Medicine, Johnson City, TN.

Purpose: Autism spectrum disorder (ASD) is a condition that presents in a complex net of developmental, sensory, and social deficits. In the past ten years, the prevalence of autism has grown over 119.4% to affect one 1 out of 62 children (1 out of 48 boys). Despite the significance, there is still no known cause, cure, or objective diagnosis. It is possible that overexpression of growth factors may lead to one of the few pathological findings in ASD of increased brain size. IGF1 binds to the insulin-like growth factor receptor 1 (IGF1R) to signal downstream phosphorylation pathways that ultimately activate the mammalian target of rapamycin (mTOR). The activation of mTOR induces dendritic synapse formation that may lead to increased brain size. Overall, the purpose of this experiment was to investigate expression of IGF1 and IGF1R in ASD.

Methods: Quantitative PCR was used to analyze the gene expression of IGF1 and IGF1R using postmortem anterior cingulate cortical (ACC) brain tissue from subjects diagnosed with ASD and typically developed (TD) age-matched control brain tissue. Immunohistochemistry of paraffin embedded formalin-fixed brain tissue was used to analyze protein expression of IGF1R using area fraction analysis in gray and white matter in ASD compared to control tissue.

Results: Increased gene expression of IGF1 was shown in ASD when compared to control tissue, but gene expression of IGF1R was unchanged. Immunohistochemistry revealed that IGF1R protein was unchanged in ASD versus control tissue.

Discussion/ Conclusion: Although, the expression of IGF1R was unchanged in ASD, the increased gene expression of IGF1 growth factor could indicate potential signaling overlap with other receptors including the insulin receptor and IGF2R. These findings could have important implications on downstream therapeutic treatment with IGF1 in ASD.

Gene-based synaptic inhibition: a proof of principle study for sustained botulinum toxin expression in the treatment of muscle spasticity

Long Di, Orion Paul Keifer, Muhibullah Tora, Hannah Chen, Paavali Hannikainen, Yujin Kim, Anthony Donsante, Nicholas Matthew Boulis

Department of Neurosurgery, School of Medicine, Emory University

Purpose: Spasticity is a debilitating medical condition involving the involuntary contraction of muscles resulting in stiffness and rigidity. Botulinum neurotoxin (BoNT) injections are the current gold-standard of treatment and prevent pre-synaptic acetylcholine (Ach) release at the neuromuscular junction (NMJ). However, high cost and a need for repeated injections renders this therapy sub-optimal. Gene-based neuromodulation (GBNM) offers a promising solution by using transgene delivery to modulate central and/or peripheral nervous system function. The purpose of this study was to test the efficacy of different BoNT gene fragment combinations in inhibiting pre-synaptic Ach release using an adenoviral vector. The neuromodulatory action of BoNT has been well studied and it is generally agreed that the light chain (LC) of BoNT inhibits synaptic vesicle release by cleaving proteins in the SNARE complex. However, previous studies have implicated the receptor binding domain (RBD) and translocation domain (TD) as having a role in the LC's catalytic activity. Here, we tested three combinations of BoNT transgenes encoding different toxin domains: LC, LC and RBD, and LC and TD.

Methods: Adult male Sprague-Dawley rats received injections of adenoviral vectors containing BoNT transgenes combinations into their lumbar spinal cord and behavioral deficits were assessed using motor scoring.

Results: Preliminary behavioral data shows persistent motor deficits apparent seven days after surgery. Rats receiving a combination of either the LC and RBD or LC and TD displayed greater functional deficit than rats that receiving the LC alone, indicating a catalytic role of the TD and RBD.

Conclusion: While our data indicate that GBNM may be a viable method for sustained BoNT expression and synaptic inhibition at the NMJ, the study is still ongoing. We are currently conducting IHC for NeuN, GFAP, and ChAT to ensure motor neuron integrity was not compromised during surgery. Furthermore, we will confirm BoNT expression via Western blot analysis and IHC.

IL-10 Expression Leads to Early Mortality Without Affecting Dopaminergic Neurons in a Mouse Model of Alpha Synucleinopathy

Samuel G Cockey, Emily J Koller, Benoit Giasson, PhD, Paramita Chakrabarty, PhD

Department of Neuroscience, McKnight Brain Institute, University of Florida, Gainesville, FL

Purpose-- Parkinson's disease (PD) is the second most common neurodegenerative proteopathy. The alpha synuclein (aSyn) protein is present in healthy human brains, but misfolded and aggregated aSyn leads to neuronal cell death and initiates an inflammatory immune response in the central nervous system (CNS). PD is a synucleinopathy characterized by the aggregation of aSyn into Lewy bodies (LB) and by the loss of dopaminergic neurons, both of which occur in the substantia nigra compacta (SNc). Immune modulation is one type of therapy for neurodegenerative diseases. Interleukin 10 (IL-10) is an anti-inflammatory cytokine secreted by microglia that promotes neuronal repair. The objective of our study was to investigate the effect of overexpressing IL-10 on a mouse model of alpha synucleinopathy. Methods—Neonatal homozygous mice were injected with recombinant adeno-associated virus (rAAV) expressing IL-10 or a control protein, enhanced green fluorescent protein (EGFP). At 2 months of age, these mice were injected with fibrillar aSyn or normal buffered saline in the hindlimb muscles. Around 4 months post intramuscular injection, these mice display hindlimb paralysis due to induction and spread of aSyn pathology along the neuraxis. Postmortem, we evaluated (1) accumulation of pathological synuclein aggregates in the neuraxis and (2) dopaminergic neuron counts in the SNc with anti-tyrosine hydroxylase (Th) staining. Results-- Both mice groups showed induction of synucleinopathy in the spinal cord and brain stem. IL-10 expressing mice had significantly accelerated mortality compared to control mice. However, there was no significant difference in dopaminergic neuronal counts between mice overexpressing IL-10 and control mice expressing EGFP. Discussion/Conclusion— Our results suggest that IL-10 leads to earlier mortality in a mouse model of alpha synucleinopathy without affecting dopaminergic neurons.

Effects of Dopamine 2 Receptor Agonist Treatment on Microglia Polarization following Cerebral Ischemia in Mice

Solana Liu*, Xiaohuan Gu*, Shan Ping Yu*, and Ling Wei*,+

*Department of Anesthesiology; +Department of Neurology, Emory University School of Medicine, Atlanta, GA, USA 30033

Purpose: The purpose of this study was to investigate the effects of a highly selective dopamine 2 agonist on microglia polarization following cerebral ischemia in mice.

Methods: 8 week old C57 male mice were subjected to transient distal MCA occlusion. Mice were sacrificed 3, 10, and 14 days after stroke. To investigate the effects of a D2 agonist, the D2 agonist Sumanirole was given intranasally at 1.5 mg/kg twice per day for 7 days beginning 3 days after stroke. Matching controls groups received saline. 10 days after stroke, mice were sacrificed, then their brains were perfused, flash frozen, and sliced into 10 um thick sections. Sections were stained for microglia (Iba1), D2 Receptor (D2R), iNOS (M1 marker), and CD206 (M2 marker) to note changes in microglia polarization. Cell counts were obtained from six non-overlapping images taken in the peri-infarct region of each brain section.

Results: Baseline microglia counts indicated that both M1 and M2 were observed up to 14 days after stroke. At 14 days, M1 and M2 microglia make up 27% and 22% of all microglia, respectively. We observed an increase in D2R expression in activated microglia and both M1 and M2 forms expressed D2R 3 days after stroke. Mice that received the D2 agonist treatment had increased number of M1 microglia 10 days after stroke. D2 agonist treatment did not appear to affect microglial distance from the ischemic core.

Discussion/Conclusion:

Our results indicate that prior to ischemia, microglia do not express D2R. After ischemia, both the pro-inflammatory M1 and anti-inflammatory M2 forms of microglia express D2R. D2 agonist treatment significantly increased number of M1 but not M2 microglia. This suggests that dopamine may exacerbate post-stroke inflammation. Future studies will further elucidate the immunomodulatory effects of dopamine after cerebral ischemia.

NIHSS and Variation of Infarct Volume by Hemisphere

Tyler P. Behymer, BS (1), Achala Vagal, MD (2), Heidi Sucharew, PhD (3), Vineeth Yeluru, MBBS (1), Arjun Minhas, BS (4), J. Michael Hazenfield, MD (2), Mahati Reddy, MD (5), Courtney Frey, MD (2), Kathleen Alwell, RN BSN (1), Charles J. Moomaw, PhD (1), Matthew L. Flaherty, MD (1), Simona Ferioli, MD (1), Jason Mackey, MD (6), Felipe De Los Rios La Rosa, MD (1,7), Sharyl R. Martini, MD, PhD (8), Opeolu Adeoye, MD (1), Dawn Kleindorfer, MD (1), Brett M. Kissela, MD, MS (1), Pooja Khatri, MD, MS (1), Daniel Woo, MD, MS (1)

(1) Department of Neurology, University of Cincinnati (UC) Neuroscience Institute, Cincinnati, OH; (2) Department of Radiology, University of Cincinnati Medical Center, Cincinnati, OH; (3) Department of Biostatistics, Cincinnati Children's Hospital, Cincinnati, OH; (4) Georgetown University School of Medicine, Washington, DC; (5) Department of Radiology, Southside Regional Medical Center, Petersburg, VA; (6) Department of Neurology, Indiana University School of Medicine, Indianapolis, IN; (7) Department of Neurology, Baptist Health of Miami, Miami, FL; (8) Department of Neurology, Michael E. DeBakey VA Medical Center, Houston, TX.

Purpose: The National Institute of Health Stroke Scale (NIHSS) is the most widely used measure of neurologic deficits in clinical trials. Using the placebo arm of the NINDS t-PA Stroke Trial, it has been demonstrated that the total volume of cerebral infarction in patients with similar NIHSS scores is greater for right compared to left hemisphere strokes. Our objective was to verify this finding in a non-clinical trial, independent dataset of acute ischemic strokes.

Methods: The Greater Cincinnati/Northern Kentucky Stroke Study (GCNKSS) is a population-based study that tracks the incidence of stroke. A convenient subsample from the 2010 GCNKSS ischemic stroke cohort (N=368) underwent detailed imaging analysis. Research nurses abstracted patient records to include baseline retrospective NIHSS score. The 24 hour infarct volume was segmented using manual tracing. NIHSS was compared between left and right brain using Wilcoxon rank sum test. Spearman rank correlation determined the association between the NIHSS score and infarct volume by hemisphere. Patients were stratified by NIHSS (0 to 5, 6 to 20, and greater than 20).

Results: Among 368 ischemic stroke subjects with imaging data, excluded were 77 brainstem/cerebellar infarcts, 37 undetermined volume, 1 missing NIHSS, and 3 undetermined laterality. For the remaining 250 patients, 132 were left and 118 were right hemisphere strokes, 210 had an MRI and 40 had CT. Median time from onset to imaging was 24 hours. Baseline NIHSS were similar by hemisphere and correlated with stroke volume ($r=0.38$, $p<0.01$). The infarct volume of right hemisphere strokes were greater than left hemisphere (p-value=0.02) (Table).

Discussion/Conclusion: The NIHSS score correlates with volume by hemisphere, but has a larger infarct volume for right than left hemisphere for similar NIHSS. This likely reflects the different weighting of the NIHSS with regard to language. This finding confirms prior results in an independent dataset.

Pre-gestational oxycodone exposure impacts maternal motivation but not maternal caregiving or drug seeking in the postpartum mouse

Amanda B. Watters, Katharine M. Cammack

The University of the South, 735 University Avenue, Sewanee TN 37383

Purpose: Opioid analgesics are effective and widely prescribed painkillers, but the dramatic escalation of misuse, dependence and addiction has become a public health crisis. In humans and animal models, females are uniquely sensitive to drugs of abuse, but it remains unclear how opiates affect maternal (postpartum) females. Females' endogenous opioid system mediates maternally relevant physiological functions and reward processes. While opioids impact maternal caregiving and offspring development, a history of opioid exposure, prior to pregnancy, may also affect subsequent maternal and reward-related behaviors. The present study explores the extent to which a history of oxycodone exposure impact postpartum females' maternal behavior, motivation for her offspring (pups), and drug-seeking behavior.

Methods: Adolescent male and female mice were pseudorandomly assigned to receive oxycodone or saline, twice daily for 12d. Drug-induced behaviors were recorded, and withdrawal scores calculated after drug was discontinued. Females were mated and remained undisturbed for gestation. After parturition, females' maternal behavior and maternal motivation were tested. All mice then underwent a conditioned place preference (CPP) procedure for oxycodone.

Results: Females' maternal behavior was not disrupted by pre-gestational oxycodone exposure. Postpartum females' performance on the maternal motivation test suggested that pre-gestational oxycodone reduced females' approach behavior toward their pups. Mice learned the CPP procedure and most mice expressed a chamber preference. Postpartum females' oxycodone CPP was similar to that of nonmaternal females and males and did not differ based on drug history.

Discussion/Conclusion: Maternal behaviors are relatively stimulus-bound/reflexive and remained robust despite oxycodone history. Pre-gestational oxycodone impacted maternal motivation, suggesting that oxycodone can alter brain circuits involved in motivated, effortful behavior. Postpartum status did not reduce drug seeking. Results have implications for rehabilitation efforts directed toward new mothers, with support/treatment to restore proper function of motivation-related brain circuits that support maternal care but are compromised by abused drugs.

A Combined 7T Stroop fMRI and MRS Study in First Episode Schizophrenia

Gregory Overbeek*; Timothy Gawne+, PhD; David White*, MPH, MPA; Meredith Reid^, PhD; Adrienne Lahti*, MD

*Dept. of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham; Dept. of Vision Sciences, University of Alabama at Birmingham;^Magnetic Resonance Imaging Research Center, Auburn University

Purpose: Antipsychotics fail to treat the cognitive symptoms of schizophrenia (SZ), and much of the neurobiology behind these deficits remains unclear. SZ show abnormal executive network activation and default mode network deactivation during tasks, and their cognitive deficits are also attributed to an imbalance in the excitatory and inhibitory neurotransmitters glutamate and GABA respectively. The present study used fMRI and proton magnetic resonance spectroscopy (H-MRS) to investigate how the neurochemical and functional network abnormalities of SZ were related.

Methods: 21 first-episode SZ were matched with 21 HC for age, gender, and family socioeconomic status. Within and between groups general linear model (GLM) comparisons of the Blood Oxygen Level Dependent (BOLD) response to the Stroop task were performed using 7T fMRI. Neurochemical levels were obtained from the bilateral dorsal anterior cingulate cortex (ACC) using 7T H-MRS. Two multiple-regression whole brain analyses investigated how glutamate, glutamine, and GABA related to the BOLD response in HC and SZ separately. A third investigated how the relationships between BOLD response and each of the three neurochemicals differed between groups.

Results: Glutamate levels were significantly lower in SZ than HC. Compared to HC SZ had increased executive and default mode network activity during the Stroop task. Replicating a previous finding, HC ACC glutamate levels negatively correlated with the posterior default mode network BOLD response, but this relationship was positive in SZ. Glutamine levels negatively correlated with the BOLD response in both task positive and task negative regions, and these relationships were stronger and included more regions in HC. In SZ but not HC GABA correlated with the ACC BOLD response, and two independent measures of cognitive function.

Conclusion: Our results suggest glutamate and GABA imbalances underlie functional network abnormalities in SZ. The developments of treatments that restore this imbalance may successfully treat SZ cognitive symptoms.

Validating the Effect of RBFOX1 Expression on the Regulation of MAPT Splicing in vitro

Derian A. Pugh*, Kathryn Bowles**, PhD, Alison Goate**, DPhil.

Xavier University of Louisiana, New Orleans, LA, Summer Undergraduate Research Program, Icahn School of Medicine at Mount Sinai*.Ronald M. Loeb Center for Alzheimer's Disease, Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, Department of Neuroscience, New York, NY**

The microtubule - associated protein tau (MAPT) gene encodes for the protein Tau, which is abundant in the neurons of the central nervous system. Tau is responsible for stabilizing microtubules, which supply essential nutrients to the cell, and it participates in cell division. Tau can aggregate to form neurofibrillary tangles that ultimately end up disintegrating this vital transport system, resulting in cell death. Diseases that are associated with defective and aggregated Tau are referred to as Tauopathies. Exons 2, 3, and 10 of the MAPT gene are alternatively spliced in human brain. Altered splicing of these exons is hypothesized to be a contributing factor to the development of Tauopathy. In an effort to understand MAPT expression, splicing, and regulation in the human brain, we altered the expression of a candidate splicing factor, RBFOX1, to functionally validate its effect on the regulation of MAPT in vitro. We transfected HEK293T cells with iBac-MAPT, a construct that contains the entire MAPT gene. We evaluated the effect of RBFOX1 overexpression and knockdown on RBFOX1 and total MAPT expression by qRTPCR and changes in MAPT exon 2 and exon 10 splicing by PCR. We validated the overexpression of RBFOX1 in vitro, but were not able to validate its knockdown due to very low endogenous expression in HEK293T cells. Further investigation and optimization of the iBac-MAPT construct is required. We plan to test other candidate splicing factors in the future to better characterize and understand the regulation of alternative splicing, and how this may be associated with Tauopathies such as Alzheimer's disease (AD), frontotemporal dementia (FTD), and progressive supranuclear palsy (PSP).

The Effects of Gestational Psychological Stress on Neonatal Mouse Intestinal Development

Juhi Shah*, Sara B. Deas**, Jon Neilson**, Changchun Ren***, Tamas Jilling***, Kyle Brawner***, Colin Martin***

*Department of Clinical and Diagnostic Sciences, **Department of Medicine, ***Department of Surgery, University of Alabama at Birmingham, Birmingham, AL 35294

Purpose: Psychological stress during pregnancy has shown to cause subsequent harm to the fetus. The purpose of this study was to determine the effect of psychological stress during pregnancy on the newborn's intestinal architecture and growth.

Methods: 8-week-old C57BL/6 littermates underwent timed breeding. Pregnant dams were subjected to one hour of daily psychological stress by using a restraint model during days E7-E14 of the gestational period. The distal ileum of 2-week-old offspring of stressed mothers and non-stressed controls was harvested for histological analysis. Slides were blinded to measure villus height and crypt depth. To determine the effect of excess stress hormones on intestinal stem cell proliferation, an explant model was used. 2 mm biopsies were taken from wild type non-stressed mice and treated with 100 nM of corticosterone for 24 hours. RT-PCR was performed to determine the effect of corticosterone on the intestinal stem cell marker Leucine-rich-repeat-containing G-protein-coupled Receptor 5 (LGR5), growth factor Epidermal Growth Factor Receptor (EGFR), and receptor for growth factor Insulin Growth Factor-1 (IGF-1).

Results: The average villus height was 126.4 ± 5.6 m for control and 100.4 ± 4.5 m for stress mice, p value <0.05. The average crypt depth was 63.9 ± 1.2 m for control and 53.7 ± 2.3 m for the stressed group, p value <0.05. Explants exposed to corticosterone had a 2.1-fold increase in LGR5 compared to controls, p value = 0.04. There was no significant difference in the IGF-1 and EGFR expression between control and treatment groups.

Conclusion: We establish that neonatal mice with stressed mothers during pregnancy have significantly shorter villi and crypts compared to controls. Also, pups from stressed mothers had higher expression levels of the intestinal stem cell marker LGR5, which may suggest a compensatory response to stress. Future studies will further clarify how excess stress hormones affect neonatal intestinal development.

Do Lactic Acid Bacteria Convert Glucosinolates to AHR Agonists during Food Fermentation?

Rachel E. Sutton1, Christopher Graham2†, Ph.D., Poulami Basu Thakur3†, M.S., Casey Morrow4†, Ph.D., Greg Kennedy2†, MD, Ph.D., Michael J. Gray3† Ph.D.

1Martin Methodist College, Pulaski, Tennessee 38478, 2Department of Surgery, 3Department of Cell, Developmental, and Integrative Biology, 4Department of Microbiology. † University of Alabama Birmingham, Birmingham, Alabama 35294.

There has been a long-standing observation that fermented foods provide health benefits in the human gut. The Aryl Hydrocarbon Receptor (AHR) found in the human gut has also been found to promote a healthier gut by reducing inflammation and the risk of cancer. The AHR is activated by a variety of molecules, which are termed AHR agonists. Precursors of AHR agonists found in vegetables, which include glucosinolates, are thought to be converted into AHR agonists by the action of enzymes or bacteria, either in foods or in the microbiome. We hypothesize that the lactic acid bacteria (LAB) in fermented foods might convert glucosinolates to AHR agonists which may promote a healthier gut. In collaboration with Dr. Greg Kennedy, UAB division of Gastrointestinal Surgery, we tested levels of AHR agonists and bacterial populations over the course of a natural sauerkraut fermentation. We prepared sauerkraut and measured AHR agonist activity at different time points using a dioxin response element (DRE) luciferase assay, which allowed bioluminescence to be recorded as an indicator of AHR activity. Results from the assay showed very low levels of AHR activity around 1 (fLUC/rLUC). We will run the assay again with samples that have a neutral pH and a higher concentration to test whether our hypothesis is correct. If the assay is successful, and AHR activity is increased throughout the fermentation process, it may lead to further studies regarding AHR activation and fermented foods.

Development & Prioritization of Resident-Sensitive Quality Measures in Pediatric Emergency Medicine

Abigail I. Parrigan*+, Daniel J. Schumacher+, MD, MEd; Larissa B. Fomum-Mugri+#, MPH; Kartik R. Varadarajan+, MPH

*Department of Integrative Biology, University of South Florida, Tampa, FL; +Department of Emergency Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; #Michigan State University College of Human Medicine, East Lansing, MI

Purpose: This project sought to develop and prioritize resident-sensitive quality measures. These measures are necessary to effectively assess resident performance, drive resident improvement, and influence and improve patient care.

Methods: The research team first utilized a Nominal Group Technique (NGT) with 8 pediatric residents to gather prospective patient care measures for 3 common diagnoses and overall care in the Pediatric Emergency Department (PED). The diagnoses of interest were Asthma, Bronchiolitis, and Closed Head Injury (CHI). Following the NGT, an additional 16 pediatric residents completed three rounds of a Delphi process to prioritize the developed measures. Their prioritization decisions were based on how well the measures represented resident work as well as importance to quality care for the illness.

Results: The NGT produced a total of 151 potential measures, 57 for general care in the PED, 33 for Asthma, 28 for Bronchiolitis, and 33 for CHI. After the prioritization of these measures during the Delphi process a total of 32 measures remained, 15 for Asthma, 10 for Bronchiolitis, and 7 for CHI. Across illnesses, measures often focused on following guidelines, pertinent documentation, correct medication dosing, and ensuring adequate discharge guidance.

Discussion/Conclusion: The NGT helped develop a foundation of resident-sensitive quality measures that could be used to begin prioritizing what measures are most essential to the care provided by residents. Although these measures only represent the thoughts of residents and not other stakeholder groups, these measures could be compared to those brought forth by these other groups. The measures produced in this study could also be directly compared to other metrics of performance for residents, including how much residents are trusted by their supervisors. These results can continue to be applied and assessed for other diagnoses in the PED to further resident improvement.

How does lumbopelvic-hip complex instability affect segmental sequencing amongst collegiate softball athletes?

Gabrielle Gilmer*, Jessica Washington M.Ed.*, and Gretchen Oliver Ph.D.*

*Auburn University, School of Kinesiology, Sports Medicine and Movement Laboratory

In throwing, the body acts as a kinetic chain comprised of the lower extremity (LE), lumbopelvic-hip complex (LPHC), and upper extremity (UE). Previous studies have found athletes who properly engage their LPHC while throwing have higher segmental velocities thus resulting in greater ball velocity. The single leg squat (SLS) is an assessment tool for LPHC stability and LE injury. Thus, the purpose of this study was to examine the effects of LPHC instability, classified via the SLS assessment, on segmental velocities of the pelvis (PV), trunk (TV), humerus (HV), and forearm (FV) amongst collegiate softball athletes. We hypothesized that athletes with LPHC instability in the non-throwing side leg would display significantly slower segmental velocities. Eighteen athletes (165.0 ± 14.0 cm, 69.0 ± 8.0 kg, 20.9 ± 1.8 years) performed three 60 feet overhead throws, then executed bilateral SLS. Kinematic data were collected using an electromagnetic tracking system then averaged across three trials. Participants were classified as "unstable" if they displayed knee valgus greater than 15 at 45 knee flexion in the descending phase of the SLS. Four stability groups were derived: bilateral stability, unstable on the throwing side leg (TS instability), unstable on the non-throwing side leg (NTS instability), and bilateral instability. The throwing motion was analyzed for the throwing events: foot contact (FC), maximal shoulder external rotation (MER), ball release (BR), and maximal shoulder internal rotation (MIR). One-way ANOVAs and Bonferroni post-hoc tests revealed the NTS instability group had significantly higher TV at MER than the bilateral instability group (Mean Difference: 334.8498.58, $p = 0.020$), and the TS instability group had significantly higher FV at MER than the bilateral instability group (Mean Difference: 207.9888.99, $p = 0.012$). These findings suggest the bilateral instability group is less efficient in transferring energy from LE to UE and is not utilizing their LPHC properly. Future studies should consider muscle activation between groups.

Amino Acid PET and Blood Brain Barrier Disruption Due to Radiation Necrosis

Shreya N. Kashyap^, Jonathan McConathy, MD/PhD*

^UAB Medical Scientist Training Program, *UAB Department of Nuclear Medicine

Purpose: Positron emission tomography (PET) may be valuable distinguishing recurrent tumor from radiation necrosis (RN), a late onset complication of radiation therapy (RT). The significant upregulation of amino acid transporter systems A and L, in cancer cells can be targeted with radiolabeled amino acids. Because System A tracers do not traverse the blood brain barrier (BBB), they may be better than System L tracers for detecting early stages of blood brain barrier (BBB) disruption related to RT-induced radiation necrosis. Since System A transport is a unidirectional, concentrative system, lesions may display increased System A tracer retention. The primary objective of the study was to compare the uptake and kinetic parameters of [18F]FAMPe (mixed System L and System A transport), [18F]MeFAMP (System A transport), and [18F]FDG in rodent radiation necrosis models.

Methods: Gamma knife induced cerebral radiation necrosis rodent models were scanned 0-60 minutes after tracer (MeFAMP, FAMPe or FDG) administration, at 2 hour and 4 hour timepoints to test for prolonged tracer retention, and at 1 week, 4 weeks, and 8 weeks after irradiation to study tracer washout and decreases in uptake over time. RN lesion was defined as the segmented contour with greater than 60% of the mean radioactivity of the whole brain contour. Radiation necrosis to brain ratios (RNBRs) were derived by dividing mean radioactivity per tissue volume in the RN lesion by the mean radioactivity per tissue volume in the rest of the brain.

Results: [18F]MeFAMP RNBRs were higher than other tracers at just 1 week after irradiation. [18F]MeFAMP displayed a sustained retention pattern, unlike [18F]FAMPe and [18F]FDG. Average [18F]FAMPe RNBRs were greater than [18F]MeFAMP RNBRs at the 4 week timepoint, and identical to [18F]MeFAMP at the 8 week timepoint.

Conclusion: This illustrates the dynamic nature of RN lesions in vivo—changes in amino acid transporter expression over time since irradiation may inform time based PET strategies to identify and monitor RN.

Discovery of Novel Quinolone Derivatives as Psychotropic Agents

Reid D. Loveless*, Ashley B. Williams*, Vinoth K. Sittaramane*, PhD

*Department of Biology, Georgia Southern University, Statesboro, GA

Psychotropic agents are loosely defined as a group of compounds that lead to changes in consciousness, perception, and behavior through chemical alterations of the nervous system. Current psychotropic agents can be used as antidepressants, anesthetics, hallucinogens, mood stabilizers, and anxiolytics. However, despite their numerous applications, almost all psychoactive drugs have the potential to cause serious side effects including paralysis and death. Thus, the discovery of novel psychotropic agents which exhibit high potency and low toxicity remains a high priority. In this study, we aim to determine the psychotropic potential of quinolone-derived compounds, as well as gain a more comprehensive understanding of their modes of action through the employment a zebrafish model system. The initial screening of 20 quinolone derivatives revealed a varying degree of effects and ultimately lead to the focus and examination of three potential candidates: 6, 16, 24. Preliminary data show that treated zebrafish larvae exhibit a reduction in motor activity, loss of balance, and failure to respond to touch. As the concentration of the compound administered changes, the degree of behavioral differences observed also changes, indicating dose-dependence. Physiological examinations show that (6 and 16) decrease larvae heart rate, though, are capable of being restored upon washing with E3, indicating the potential of recovery from prolonged exposure to the compounds. Based on the reversibility and short reaction time of observed effect, ion modulation was investigated as a potential mechanism of action. Specifically, CoroNa Green, an ionic indicator dye, was used to visualize the effects of these compounds on sodium transport. Further, we would like to investigate other ions such as calcium and potassium as possible facilitators of the observed effects.

Honokiol and Pioglitazone ameliorate Alzheimer's Disease pathologies in vitro and ex vivo

Tyler O. Lynd, Manoj Govindarajulu, Gwyneth Briggs, Murali Dhanasekaran, Vishnu Suppiramanium

Department of Drug Discovery and Development, Harrison School of Pharmacy, Auburn University, Auburn, AL

Amyloid beta (AB) plaques are extracellular, neurotoxic protein aggregations. These plaques have historically been considered the hallmark of Alzheimer's disease due to their ability to reduce long term potentiation (LTP) and promote long term depression (LTD). Peroxisome proliferator-activated receptor gamma (PPARg) agonists have been shown to be a novel and promising class of drugs capable of alleviating AD pathologies. In this study, we observed the neurotoxic effects of amyloid beta on hippocampal field potentials on the Schaffer collateral. In addition, we displayed honokiol, a novel SIRT3 and partial PPARg agonist, and pioglitazone, a full PPARg agonist, are capable of improving mitochondrial function, increasing LTP, and reducing AB aggregation. The hippocampal field potential along the Schaffer collateral was measured by the LTP theta burst protocol, while signaling mechanisms were evaluated using western blot, RT-qPCR, mitochondrial assays, and imaging techniques. We hypothesize and provide evidence that these compounds regulate mitochondria-endoplasmic reticulum communication in order to modify AD pathologies providing evidence for the MAM hypothesis. Both compounds performed comparably suggesting honokiol may be a promising compound for the treatment of AD as its blood brain barrier (BBB) permeability is much higher than pioglitazone.

Hepatic Pannexin-1 Deficiency Reduces Steatosis and Inflammation in Diet-Induced Steatohepatitis in Mice

Nathaniel P. Oberholtzer*, Renata Polanowska-Grabowska*, Suresh Mendu*, Bimal Desai*, Ulrike Lorenz+, Melissa Bevard+ and Norbert Leitinger*

*Department of Pharmacology, University of Virginia, Charlottesville, VA. +Robert M. Berne Cardiovascular Research Center, University of Virginia, Charlottesville, VA

Pannexin-1 (Panx1) channels are thought to contribute to pathophysiological ATP release in lipoapoptosis induced by saturated free fatty acids (FFAs), but their role in hepatic pathologies such as non-alcoholic steatohepatitis (NASH) has not been investigated. To examine the role of Panx1 channels in liver pathologies, we produced a hepatocyte-specific (Albumin-Cre) Panx-1 KO mouse. Excessive accumulation of fat (steatosis) in hepatocytes is a potent stimulus for the subsequent hepatocyte apoptosis, inflammation and liver fibrosis, and circulating saturated FFAs, such as palmitic acid (PA) contribute to the pathogenesis of NASH. We found that overnight incubations with 100 and 500 μM PA induced dose-dependent ATP release in isolated WT but not in Panx1-deficient (KO) hepatocytes, indicating that PA-induced ATP release from murine hepatocytes is Panx1-dependent. Feeding a methionine- and choline-deficient (MCD) diet to rodents is a standard nutritional model replicating some key features of human NASH, including severe liver steatosis and inflammation with subsequent liver fibrosis. Compared with MCD diet-fed WT mice, MCD diet-fed Panx1-KO mice showed significantly reduced liver steatosis as evaluated by H&E staining and confirmed by biochemical analysis of hepatic triglyceride content (15.1 and 8.8 mg TG/g liver, respectively). Furthermore, livers of Panx1-KO mice on MCD diet showed significantly lower mRNA levels of Col1a1, Col4a1 and TGFβ-1 and Picro-Sirius-Red staining. Next, we injected the Panx1 inhibitor spironolactone (50mg/kg/day) into WT mice fed either standard chow or MCD diet for 14 days. Spironolactone treatment significantly inhibited hepatic steatosis, as determined by processing images of Oil-Red O stained frozen liver sections using ImageJ. These results indicate that hepatocyte-specific Panx1-KO mice were protected against MCD-diet induced steatosis and NASH and that the pharmacological inhibition of Panx1 on hepatocytes might improve hepatic steatosis and prevent occurrence of inflammation and fibrosis.

Effects of the cannabinoid-1 receptor agonist WIN 55,212-2 on reversal learning and delay discounting in adolescent mice

Katelyn R. Johnson, Steven R. Boomhower, & M. Christopher Newland, PhD

Department of Psychology, Auburn University, Auburn, AL

Marijuana, a psychoactive drug that activates cannabinoid-1 (CB1) receptors in the brain, is the most prevalently abused illicit drug among American adolescents and young adults. However, the long-term consequences of adolescent exposure to cannabinoids on the brain and behavior remain poorly understood. In both humans and nonhumans, adolescence is characterized by the maturation of the endocannabinoid neurotransmitter system in the prefrontal cortex and striatum—brain regions that underlie choice and decision making and are densely packed with cannabinoid-1 (CB1) receptors. The current study investigated the effects of chronic WIN 55,212-2 (a CB1 agonist) exposure during adolescence on reversal learning and delay discounting in mice. Twenty-four male C57BL/6 mice were equally and randomly assigned to two exposure groups (n = 12 in each): WIN 55,212-2 or vehicle control. Injections of WIN 55,212-2 (i.p.; 3.0 mg/kg/day) and vehicle were administered for twenty-one days from postnatal day 28 to 49. Thirty days after exposure (in adulthood), the mice were trained on a spatial-discrimination-reversal (SDR) task in operant chambers in which the spatial location of a response (e.g., a right lever press) results in reinforcement. Mice were then trained on a delay-discounting procedure, which measures how quickly the value of a reinforcer decreases across time. Results showed there was no difference in errors to criterion or omissions to criterion following a reversal, suggesting activation of CB1 receptors at this dose does not impair reversal learning. However, the mice given WIN 55 in adolescence displayed greater impulsivity in the form of preference for smaller-sooner reinforcers over larger-delayed ones in the delay-discounting procedure. These data suggest the maturing adolescent brain is vulnerable to chronic CB1-receptor activation, and behaviors that underlie impulsivity may be particularly susceptible.

Evaluating the Use of a 1-up 3-down Interleaved Staircase in Perception and Cognition Research in Older Adults

Rachit Kumar, Jenny A. Walker, Kurt Braunlich, Ph.D., Mark E. Wheeler, Ph.D.

Department of Psychology, Georgia Institute of Technology, Atlanta, GA

Studies that explore human cognition and visual perception often make use of images with varying levels of "noise" or static. Accordingly, studies investigating these topics in older adult populations must likewise account for age-related differences in visual acuity. This potential confound can be addressed by identifying each participant's threshold of "noise" and using that value to normalize task difficulty relative to visual ability. Our lab implemented a method for finding this threshold known as a 1-up 3-down double interleaved staircase in MATLAB, and we determined its efficacy compared to two alternative psychophysical techniques. We evaluated the efficacy of the following techniques: 1) the method of constant stimuli, which presents images at each noise level and creates a linear model of overall performance versus noise, 2) a just-noticeable difference manual staircase, where images at gradually changing noise levels are presented until the participant indicates that they can just see the images (these just-noticeable difference levels are then averaged), and 3) a 1-up 3-down double interleaved staircase, which shows images at varying noise levels and automatically adapts to an individual's performance until the difficulty is adjusted to a specified percent accuracy. Results indicate that the just-noticeable difference manual staircase was ineffective, while the method of constant stimuli and the double interleaved staircase were effective at finding each participant's threshold. The latter two methods also indicated the same value for every subject. Moreover, the double interleaved staircase was substantially faster at identifying the threshold, requiring 5 minutes while the method of constant stimuli required 25 minutes. Our results indicate that our implementation of the double interleaved staircase is an effective and more efficient method for identifying accuracy thresholds, and future work will apply this staircase in fMRI studies to explore visual perception and cognitive aging.

ABSTRACTS – POSTER SESSION 2

Impact of Vancomycin Treatment on Human Mesenchymal Stromal Cells during Osteogenic Differentiation

Brian Traub, BA, Emory University School of Medicine
Rishin J. Kadakia, MD, Emory University School of Medicine
Nick Willett, PhD, Emory University School of Medicine
Jason Bariteau, MD, Emory University School of Medicine

Purpose: Vancomycin is frequently applied locally to the surgical site during foot and ankle procedures to help prevent local infection. While the efficacy of locally applied vancomycin has been demonstrated in spine surgery, there is no consensus on dosing and indication within foot and ankle surgery. Unlike the spine, there is a smaller surface area and limited soft tissue envelope in the foot and ankle, which may result in higher concentrations of locally applied vancomycin powder. Osteogenic differentiation of human mesenchymal stem cells (hMSCs) and their functional mineralization is key to healing of both fractures and arthrodesis. The purpose of this research was to determine the impact of vancomycin on hMSCs during osteogenic differentiation.

Methods: Human mesenchymal stromal cells (RoosterBio, Frederick, MD) were cultured in osteogenic differentiation media (Lonza, Walkersville, MD) to promote osteogenic differentiation. Cells were treated with vancomycin at differing concentrations of 0, 50, 500 and 5000 µg/mL. Viability and cell growth was assessed via LIVE/DEAD™ viability/cytotoxicity kit (Invitrogen, Waltham, MA) after 1, 3 and 7 days of treatment. Differentiation and mineralization was assessed via alizarin red staining after 21 days of treatment. Semi-quantification of mineralization in alizarin red stained samples was performed by measuring absorbance at 405 nm using a microplate reader. Mean cell viability, cell number, and absorbance of alizarin red stained samples were compared between treatment groups using one-way ANOVA and the Tukey-Kramer method for post hoc pairwise comparisons. Statistical significance was defined as $P<.05$.

Results: At the highest concentrations of vancomycin, there was a significant reduction in cell viability ($P<.05$) and proliferation ($P<.05$) after 3 days compared to all other treatment groups. Mineralization was also significantly decreased with 5000 µg/mL vancomycin compared to the control ($P<.01$) and 50 µg/mL group ($P<.05$).

Conclusion: At high concentrations, vancomycin may impair hMSC viability and function during the osteogenic differentiation process and decrease local mineralization critical to bone healing. Surgeons should exercise caution and consider the limited soft tissue envelope when applying vancomycin locally during foot and ankle surgery, especially during arthrodesis procedures.

The role of MIF in mitochondrial metabolism and macrophage polarization.

Jordan Noe, Beatriz Rendon, Ashley Wise-Mitchell, Eun Jung Kim, Robert Mitchell, PhD

Department of Biochemistry and Molecular Genetics, University of Louisville, Louisville, KY.

Soluble factors within the tumor microenvironment polarize tumor-associated macrophages (TAMs) to an M2 phenotype which promotes angiogenesis, metastasis, and the suppression of anti-tumor immune responses. We have identified a role of two soluble factors, pyruvate and macrophage migration inhibitory factor (MIF), in promoting M2-polarization. Pyruvate is the metabolic end-product of glycolysis and increases some markers of M2 polarization; an effect independent of glycolysis per se, but requires mitochondrial pyruvate uptake. Mitochondrial pyruvate metabolism can produce mitochondrial ROS which enhances HIF-1a stability and may allow expression of HIF1a-dependent M2 markers. We hypothesize M2 macrophages increase mitochondrial metabolism and oxidize pyruvate to increase mitochondrial ROS leading to HIF1a-dependent M2 polarization. In support of this, pyruvate increases HIF-1a protein and inhibiting the electron transport complex, which produces mitochondrial ROS, decreases mitochondrial oxygen consumption and markers of M2 polarization. Previously, we identified that intra-tumoral macrophage migration inhibitory factor (MIF) is a critical determinant of polarization for M2-TAMs. MIF binds to Jab1, a COP9 signalosome subunit, which regulates E3 ubiquitin ligase complexes that targets proteins for degradation. NRF2 is a transcription factor that increases mitochondrial biogenesis, promotes M2 polarization, and is regulated by the Cul3-E3 ubiquitin ligase. We hypothesize that MIF antagonizes Jab1 during M2 polarization, leading to enhanced NRF2 stability and mitochondrial biogenesis. We have found MIF-deficient M2 macrophages have a significant decrease in nuclear NRF2 and mitochondrial oxygen consumption, as well attenuated M2 markers in response to pyruvate. Taken together, MIF promotes M2 polarization possibly by increasing NRF2 stability, leading to enhanced mitochondrial biogenesis and/or metabolism.

Nedd4-2-catalyzed Polyubiquitination Requires Two E2~ubiquitin Binding Sites

Dustin R. Todaro, Allison C. Augustus-Wallace, PhD, Jennifer M. Klein, and Arthur L. Haas, PhD,

Department of Biochemistry and Molecular Biology, LSU Health Sciences Center, New Orleans, LA

The Hect (Homologous to E6AP Carboxy-Terminus) family of ubiquitin ligases consists of 28 function-specific paralogs in humans which are essential to a variety of cellular processes and their deregulation is implicated in numerous diseases. Each family member contains a highly conserved 350-residue C-terminal catalytic domain responsible for binding their cognate E2~ubiquitin co-substrate, formation of a Hect~ubiquitin thioester intermediate, and subsequent conjugation of the target protein. The Nedd4-2 ligase is best known for targeting a broad range of cell surface receptors for polyubiquitin chain-mediated endocytic uptake and lysosomal degradation, including the amiloride-sensitive epithelial Na+ channel in the distal nephron. Additionally, Nedd4-2 and other Nedd4 family ligases facilitate vesicular trafficking and are exploited in viral budding of Ebola and Marburg viruses. The current studies explore the mechanism of Nedd4-2 by employing biochemically-defined kinetic assays examining rates of 125I-polyubiquitin chain assembly as a functional readout of ligase activity. We demonstrate that Nedd4-2 exhibits hyperbolic Michaelis-Menten kinetics (KM = 44 ± 6 nM; kcat = 0.020 ± 0.007 s-1) and substrate inhibition above 0.5 μM (Ki = 2.5 ± 1.3 μM) tending to zero velocity, the latter requiring two functionally-distinct E2~ubiquitin binding sites. A Ubc5BC85S-ubiquitin substrate analog exhibits competitive inhibition at the high affinity Site 1 (Ki = 720 ± 340 nM) and non-essential activation at the lower affinity Site 2 (Kact = 750 ± 260 nM). In contrast, a Ubc5BC85A product analog non-competitively inhibits Nedd4-2 (Ki = 2.0 ± 0.5 μM), consistent with the two site model. Additional studies utilizing Ubc5BF62A defective in binding the canonical E2 site demonstrates that the cryptic Site 1 is associated with thioester formation, while the canonical site (Site 2) is associated with polyubiquitin chain elongation. These studies demonstrate that Nedd4-2 catalyzes polyubiquitin chain assembly using two E2~ubiquitin binding sites analogous to E6AP, the founding member of the Hect ligase family. [Supported by GM034009]

Na+/H+ Exchange Regulates CHOP Levels in Pulmonary Arterial Smooth Muscle Cells During Pulmonary Hypertension

Micheal C. Munson*, John C. Huetsch+, Haiyang Jiang+, Xin Yun+, and Larissa A. Shimoda+

* = Department of Chemistry and Biochemistry, Baylor University, Waco, TX, + = Department of Pulmonary and Critical Care, Johns Hopkins School of Medicine, Baltimore, MD

Vascular remodeling within the pulmonary arterioles is a key component of pulmonary arterial hypertension (PAH). In a rat model of severe PAH, the SU5416-Hypoxia (SuHx) model, pulmonary arterial smooth muscle cells (PASMCs) demonstrate a resistance to apoptosis and contribute to vascular remodeling. Na+/H+ exchange (NHE) is increased in these PASMCs; however, few studies have elucidated the role of NHE in the signaling pathway responsible for apoptosis within these PASMCs. Previous studies have shown a link between NHE and extracellular receptor kinase 1/2 (ERK1/2). Other studies have linked ERK1/2 to the expression of CHOP, a critical mediator of apoptosis. Therefore, we tested the role of NHE on these components of the apoptotic pathway within PASMCs. PASMCs were isolated from distal pulmonary arteries from control rats and rats injected with SU5416 and exposed to 3 weeks of hypoxia followed by 2 weeks of normoxia. PASMCs were treated with pharmacologic inhibitors of NHE (EIPA) and ERK1/2 (PD98059) and controls were treated with the respective vehicles; protein was then isolated. Immunoblot analysis was performed to determine the effect of inhibiting NHE and ERK1/2 on CHOP protein levels. Inhibition of NHE reduced ERK1/2 phosphorylation/activation. Inhibiting either NHE or ERK1/2 upregulated CHOP. Our findings suggest that NHE plays an important role in regulating apoptosis signaling through ERK and CHOP in PAH PASMCs.

Role of 5-Lipoxygenase in Angiogenesis after Myocardial Infarction in mice

Ogechukwu J. Otiji*, Ganesh V. Halade+,PhD

*Department of Biology, Georgia Gwinnett College, Lawrenceville, Georgia;+Department of Cardiovascular disease, University of Alabama at Birmingham Alabama.

Purpose: Myocardial infarction (MI) also known as a heart attack is a cardiovascular disease characterized by the lack of oxygen supply to the heart muscle leading to necrosis of the cardiomyocytes. If left untreated or unresolved MI could eventually lead to heart failure due to resistance on the left ventricle by the process of fibrosis common after any exaggerated injury. The formation of new blood vessels from preexisting ones, a process also known as angiogenesis play a vital role in preventing as well as healing the damage caused. Angiogenesis expedites healing of damaged heart muscle by allowing reperfusion to the infarcted area, increasing and restoring blood flow to the damaged area and preventing hypoxia. Hours after MI has occurred the healing process takes over, this includes an inflammatory phase of healing. This phase is substantiated by the enzyme 5-lipoxygenase which plays a role in transforming arachidonic acid into leukotrienes and lipoxins. Leukotrienes are the major contributors of inflammation in the healing process and lipoxins facilitate clearance of inflammation. This study will also focus on the role of 5-lipoxygenase in the process of angiogenesis and thus the delay in the progression of heart failure pathology. Progression of angiogenesis will be measured in an ischemic injury mouse model at different stages of acute (1-3), resolving (3-5), and chronic (28-56) days post-MI in wildtype mice samples as well as knockout Lox samples to accurately depict the process of angiogenesis.

Methods: To measure angiogenesis, 3-4 marked areas in left ventricle will be analyzed using histological analysis and if required confocal microscopy. Dr. Halade lab provided the samples for histology after myocardial infarction surgery to mice.

Results: LV was successfully stained and pictures were taken under 40X microscopy. Conclusions: No significant correlation between lipoxygenase enzyme and angiogenesis. Wildtype samples had lower levels of angiogenesis.

Structural and Functional Studies of the Metastatic Factors P-Rex1 and P-Rex2: Small-Molecule Inhibitor Development

Prateek V. Sharma*†, Jennifer N. Cash, PhD*†, and John J.G. Tesmer, PhD†§.

* Department of Biological Chemistry, University of Michigan, Ann Arbor, MI, USA † Life Sciences Institute, University of Michigan, Ann Arbor, MI, USA § Department of Biological Sciences, Purdue University, West Lafayette, IN, USA

Purpose: Metastases are known to cause 90% of human cancer mortalities, and there remain no clear methods for optimal treatment. The phosphatidylinositol 3,4,5-trisphosphate (PIP3)-dependent Rac exchanger (P-Rex) family of RhoGEFs is strongly associated with cancer metastasis. Both P-Rex1 and P-Rex2 are activated by the lipid PIP3, generated by activated receptor tyrosine kinases, and free Gβγ subunits, generated by G-protein coupled receptors. Thus, the P-Rex family is positioned downstream of multiple classes of cell surface receptors that control processes underlying cell migration and is an attractive therapeutic target for the suppression of cancer metastasis. However, development of P-Rex inhibitors has been hindered by the fact that their structure and regulatory mechanisms are poorly understood.

Methods: The focus of this study was to obtain structural information on P-Rex2 and to identify inhibitors of P-Rex activation. An N-terminal fusion of a his-tagged solubilizing protein (MBP) to the P-Rex2 pleckstrin homology (PH) domain was over-expressed in E. coli and purified. Sparse-matrix screening was performed to identify crystallization conditions. Using differential scanning fluorimetry, high-throughput screening was performed for molecules that target the PIP3-binding site.

Results: Here we present the crystal structure of the P-Rex2 PH domain, which contains the regulatory PIP3 binding site. Six compounds were identified that bind to the P-Rex PH domain but the nature of this interaction, specifically the occupancy of the PIP3 binding site, is not yet confirmed. To investigate this, I performed crystal soaks with the compounds in attempts to obtain a co-structure. Resulting electron density maps suggest partial occupancy of some compounds in the PIP3 binding site, and I am performing further studies to resolve this.

Conclusion/Discussion: A co-structure will provide insight into how these small molecules bind the PH domain and facilitate their modification to improve binding specificity and potency, potentially leading to a novel anti-metastatic drug.

Measurement of Cell Viability in Human Breast Cancer Cells following Exposure to Polycyclic Aromatic Hydrocarbons

Rajendra Neupane, Samir Raychoudhury, Ph.D., William McAmis

Department of Biology, Chemistry, and Environmental Health Science, Benedict College, Columbia, SC

Polycyclic Aromatic Hydrocarbons (PAHs) are ubiquitous and persistent environmental contaminants. Some of them are suspected carcinogens and may affect the reproductive systems as potential endocrine disruptors or may have direct toxic effects on human. The objective of this study is to measure and compare cell viability in two different types of human breast cancer cells (MDA-MB-231 and MCF7) by conducting Lactate Dehydrogenase (LDH) and MTT Assays. During the experiments, the human breast cancer cells were first exposed to various concentrations of PAHs for 24 hours and then the two cell viability assays were performed. Cultured cells were incubated in the presence of medium alone, medium containing 1.14% acetonitrile (both as vehicle controls), or in the presence of a mixture of PAHs (500nM. 2μM and 5μM). Both of these assays give colored formazan compound as the final product by the reduction of the substrate. The optical density of formazan compound represents damaged cells in LDH Assay and viable cells in MTT Assay. In both cell types, higher concentrations of PAHs damaged more cells leaving less viable cells in the sample. In addition, MDA-MB-231 cells appeared to be more resistant to PAHs than MCF 7 Cells. These data suggest that the PAHs are toxic to breast cancer cells. However, additional experiments are needed to examine the mechanism of action of this varied toxic effect of PAHs on two different cells.

Synaptotagmin 9 Regulates Tomosyn-2 Protein Abundance to Affect Early Phase of Insulin Secretion

Trung C. Huynh1, Rajesh Gupta1, PhD, Alan D. Attie2, PhD, and Sushant Bhatnagar*1, PhD.

1Division of Endocrinology, Department of Medicine, University of Alabama, Birmingham, AL
2Department of Biochemistry, University of Wisconsin, Madison, WI *Corresponding author

The progression of type two diabetes is marked by a decline in the rate of insulin secretion. Thus, understanding the mechanism of secretion is crucial to understanding T2D. Previously we positionally cloned tomosyn-2 gene underlying a diabetes susceptible locus in an F2 mouse cross. We reported that mice congenic to tomosyn-2 were hyperglycemic, hypoinsulenemic, and have reduced insulin secretion from pancreatic islets. Tomosyn-2 binds to syntaxin1A. However, it is not yet established whether tomosyn-2 binding to syntaxin1A limits the formation of the SNARE complex at the plasma membrane and exocytosis. Our preliminary data show that in addition to syntaxin1A, tomosyn-2 binds and co-fractionates with synaptotagmin-9 (Syt9) in a sucrose density gradient under conditions of high glucose and elevated calcium concentrations. Syt9 protein is a calcium sensor and is known to regulate the formation of the SNARE complex. However, its role in regulating insulin secretion is not yet well characterized. We observed a significant increase in in vivo insulin secretion at 5-minutes and 15-minutes post-glucose challenge in 10-week old male Syt9-/- vs. control mice. Moreover, Syt9-/- mice were glucose tolerant and show no difference in insulin sensitivity - suggesting that Syt9 regulates early phase of insulin secretion from beta cells. Interestingly, islets of Syt9-/- vs. control mice have reduced tomosyn-2 protein abundance by 50% without altering the levels of other key t-SNARE or v-SNARE proteins. Altogether, these results indicate that the reduction in tomosyn-2 protein abundance leads to an increase in insulin secretion observed at early time points in Syt9-/- mice. The results presented here point to an as-yet-undescribed role of Syt9 in chaperoning or localizing tomosyn-2 protein from the cytosol to the SNARE complex to regulate insulin secretion. Herein, unpublished data describe a critical role of tomosyn-2 in regulating the early phase of insulin secretion modulated by Syt9 protein.

Identification of Specific Feed-forward Apoptosis Mechanisms and Associated Higher Survival Rates for Low Grade Glioma and Lung Squamous Cell Carcinoma

Dhiraj Sikaria*, Yaping N. Tu*, Diana Fisler*, James Mauro*, George Blanck*+

*Department of Molecular Medicine, Morsani College of Medicine, University of South Florida;
+Immunology Program, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida

Purpose: The mechanisms of cell proliferation due to the overexpression of certain transcription factors (TFs) are well understood. However, many of these same TFs have pro-apoptotic effects when expressed or activated at high levels, a process referred to as feed-forward apoptosis (FFA). This paper determines whether cancers could be stratified on the basis of specific FFA TFs and pathways.

Basic Methods: RNASeq values were downloaded for genes representing pro-proliferative TFs, for glioma and lung cancer datasets. Survival relationships were then obtained by matching patient survival data with the top and bottom 20% of the RNASeq values for each TF. RNASeq values for apoptosis-effector genes that had binding sites for the four TFs most important in this study were also obtained from the glioma and lung cancer datasets.

Results: Higher MYC and YY1 RNASeq values were statistically, significantly associated with higher survival rates for glioma (LGG) and lung cancer (LUSC), respectively. Higher STAT3 and JUN expression was associated with lower survival rates for LGG and LUSC, respectively. Expression levels of sets of apoptosis-effector genes, with binding sites for the above TFs, correlated with the expression levels of the TFs, respectively. Finally, the expression levels of the apoptosis-effector genes themselves represented independent indicators of survival outcomes, although the apoptosis-effector gene survival-associations were consistent with the TF survival outcome associations, as reflected by the presence of specific TF binding sites in the apoptosis-effector genes.

Discussion: The above analyses indicate that FFA can be associated with specific TFs and specific apoptosis-effector genes in a cancer patient setting, such that there is a consistent pathway for the FFA mechanism to proceed, from TFs to the activation of apoptosis-effector genes. Such specific mechanisms may indicate an approach for exploiting oncoprotein activity to improve patient survival rates.

The Utility of Wearable Technology in Assessing Sedentary Behavior in Patients with Heart Failure

Michael K. Appeadu, Amit Shah, M.D., Candy Wakwe, Alaa Hammoud

Division of Cardiology, Atlanta VA Medical Center, Emory University, Atlanta, GA

Purpose: Low physical activity in patients diagnosed with congestive heart failure (CHF) has been associated with almost twice the risk of both all-cause mortality and cardiac mortality. This exploratory analysis aims to assess the feasibility of utilizing wearable and mobile technology to analyze sedentary behavior in patients with CHF.

Methods: We enrolled 20 patients who were admitted to the Atlanta VA Medical Center for new onset CHF or CHF exacerbation and gave them Jawbone UP3 fitness wristbands upon discharge to measure step counts. Data from the fitness wristbands were available to view via an app on the patient smartphones. Patients who did not have smartphones were provided with one. The mean daily step count over 7 days post-discharge was recorded. We also measured the highest daily total step count within seven days of enrollment as a marker for post-discharge mobility capacity. We classified patients into sedentary (less than 2000 average daily steps), low active (2000-5000 average daily steps), and active (over 5000 average daily steps).

Results: Patients enrolled were ages 49 to 76. 45% were over age 65. 90% were males. Average daily step counts ranged from 132.40 to 11238. Maximum step counts ranged from 336 to 21900. 45% of patients were classified into the sedentary category, 40% were considered low active, and 15% were considered to be active.

Discussion/Conclusion: Our findings suggest that wearable technology can be used to assess sedentary behavior in patients with CHF and may provide valuable data to help prevent mortality in this at-risk population. Compliance issues among more elderly patients stemming from inexperience with the devices may have factored into the wide range of step counts and the majority of patients being classified as sedentary or low active. The framework of this study can be expanded in the future to yield more beneficial data.

Discovery of a Proliferative Gene Signature Predictive of Patient Survival in Renal Cancer

Paul M.H. Tran*, Ashok Sharma*, PhD, Shan Bai*, MS, Jin-Xiong She*, PhD

*Center for Biotechnology and Genomic Medicine, Augusta University, Augusta, GA

Introduction: The role of personalized medicine is to use molecular analyses to identify patient subgroups within a clinically homogeneous patient population. This new classification scheme should improve clinical outcomes for these patients. We herein describe the data mining of The Cancer Genome Atlas database to identify patient subgroups in renal cancer from gene expression data.

Methods: We downloaded TCGA renal cancer gene expression RNASeq data (KIRP, KIRC, and KICH) from the UCSC Xena Public Data Hub. The data downloaded showed gene-level transcript count estimates as log2(x+1) transformed RNASeq by expectation maximization (RSEM) normalized data. We then fit each gene to a Cox regression model using the patient phenotype data. We ranked the genes using the log-rank test from smallest to largest p-value and filtered out genes with low expression level variation in the population. We used the resulting gene list to define our gene signature.

Results: We report the identification of a gene signature which can determine the prognosis of any renal cancer patient regardless of histologic subtype. This subgroup of patients has worse survival characteristics compared to renal cancer patients who do not exhibit this signature. This is demonstrated through Kaplan-Meier survival analysis and Cox proportional hazard models. In all three renal cancer subtypes, the poor prognosis group represents a small portion of all patients. The gene signature is enriched for genes associated with cellular proliferation.

Conclusions: We have shown that renal tumors positive for our proliferation gene signature confer worse patient survival compared to negative renal tumors. The genes used to identify the patient group exhibit a high degree of correlation with each other, with poor prognosis patients exhibiting higher expression levels of all genes compared to the better prognosis patient group. The high degree of correlation suggests the signature is a co-regulated transcriptomic network.

3D Bioprinting the Cardiac Purkinje System Using Human Adipogenic Mesenchymal Stem Cell Derived Purkinje Cells

Evan P. Tracy, B.S.*, Brian C. Gettler, M.S.*, Joseph S. Zakhari, M.S.*, Ravi K. Birla, Ph.D.+, Robert J. Schwartz, Ph.D.+, Stuart K. Williams, Ph.D.*

*Department of Physiology, University of Louisville, Louisville, Kentucky + Department of Stem Cell Engineering, Texas Heart Institute, Houston, Texas

Purpose: Human adipogenic mesenchymal stem cell derived purkinje cells in a collagen matrix were 3D bioprinted into a purkinje-like network. We hypothesize that these purkinje cells will retain structure, cellular identity, conductive function, and appropriate response to external stimuli upon culturing in a) a 3D collagen matrix and b) a 3D bioprinted purkinje-like network.

Methods: An anatomical image of an India Ink injected bovine left ventricular purkinje network was used to program our 3D printer. A pluronic mold of this image was printed and filled with varied density of purkinje cells in varied concentration of collagen. Syncytium formation, viability, cellular identity, and conductive ability were evaluated using phase contrast microscopy, live/dead assay, connexin 40 staining, and simultaneous electrical stimulation and fluorescent imaging with the membrane potential dye DiBAC4(5). To explore whether this printed network could respond to relevant physiological stimuli, connexin 40 relative fluorescence and localization in response to electrical pacing and changes in membrane potential in response to acetylcholine were monitored.

Results: The bioficial purkinje network had a 59% viability and syncytium formed throughout the construct. Connexin 40 staining revealed that the cells retained cellular identity when cultured in a 3D collagen matrix and when 3D bioprinted. Pacing for one hour lowered the relative fluorescence unit count of connexin 40, while localization shifted towards cellular connections between cell aggregates. The mean fluorescence intensity of DiBAC4(5) within purkinje cells in 3D collagen culture decreased with electrical stimulus and treatment with acetylcholine.

Conclusion: The effort to print a Total Bioficial Heart must start with the ability to print its components. The bioprinting method described is a feasible method for the creation of a purkinje network model. Future experiments will recreate a more accurate representation of the purkinje network using a 3D CT or MRI image to directly program our 3D printer.

Deep Convolutional Neural Networks for Classifying Head and Neck Cancer using Hyperspectral Imaging

Martin Halicek1,2, Guolan Lu1, James V. Little3, Xu Wang4, Mihir Patel5,6, Christopher C. Griffith3, Mark W. El-Deiry5,6, Amy Y. Chen5,6, and Baowei Fei 1,6,7

1The Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, USA; 2Medical College of Georgia, Augusta University, Augusta, GA, USA; 3Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA, USA; 4Department of Hematology and Medical Oncology, Emory University School of Medicine, Atlanta, GA, USA; 5Department of Otolaryngology, Emory University School of Medicine, Atlanta, GA, USA, 6Winship Cancer Institute of Emory University, Atlanta, GA, USA; 7Department of Radiology and Imaging Sciences, Emory University, Atlanta, GA, USA

Surgical cancer resection requires an accurate and timely diagnosis of the cancer margins in order to achieve successful patient remission. Hyperspectral imaging (HSI) has emerged as a useful, non-contact technique for acquiring spectral and optical properties of samples. In this study, a convolutional neural network (CNN) classifier is developed to classify excised, squamous-cell carcinoma (SCCa), thyroid cancer, and normal head and neck tissue samples using HSI. The CNN performed on all 50 patients with 81 ± 19% sensitivity, 78 ± 20% specificity, and 80 ± 14% accuracy. Further results are presented when the patients are divided into two groups, i.e. thyroid and SCCa. These preliminary results indicate the potential of hyperspectral imaging and deep learning for automatic tissue-labelling of surgical specimens.

A Novel Near-Infrared (NIR) Dye Can Accurately Measure Human Neuroendocrine Cancer Proliferation

Brendon R. Herring. Jason D. Whitt PhD. Samuel Jang. Renata Jaskula-Sztul, PhD. Herbert Chen, MD

Department Of Surgery, University Of Alabama at Birmingham, Birmingham, Alabama, USA

PURPOSE: Ex vivo patient-derived xenografts have the potential to test personalized therapies prior to patient administration. However, techniques to measure cancer cell proliferation in these models are lacking. In this study, we investigated a novel cancer-specific near-infrared (NIR) fluorescent dye, IR-783. Specifically, we hypothesized that IR-783 accurately measures neuroendocrine (NE) cancer cell proliferation in vitro and in vivo.

METHODS: NE cancer cell lines (TT, H727, UMCII, MZ, and QGP1) and non-cancerous control cells (HEK293, WI-38 and 917) were plated in culture slides coated with fibronectin. Cells were incubated with 20 uM IR-783 before fixation and images acquired with confocal microscopy. Single cell images were then obtained with an Imagestream Flow Cytometer, and their signal intensities were measured. Dye uptake in 2D culture was measured with an In Vivo Imaging System (IVIS) in 12-well plates containing increasing cell number, and intensities compared to the results of the MTT assay. Furthermore, NE cancer cells transfected with Luciferase were subcutaneously injected into Nu/Nu mice, excised after 7wks of growth, and implanted into a 3D Bioreactor system for growth to 20 days. IR-783 was added to the growth medium. The Bioreactor system was exposed to Luciferin before imaging with an IVIS for Luciferase activity and IR-783 uptake.

RESULTS: IR-783 was retained to a higher degree in NE cancer cells compared to non-cancerous cells, detected by confocal microscopy and flow cytometry. NE cancer cells exhibited a mean maximum pixel intensity (mMPI) of 247 while non-cancerous control cells showed an mMPI of 103 (P=.015). In 2D culture, IR-783 signal intensity increased with cell density. This correlation was also shown in the Bioreactor system (R2=0.49 and 0.96 for IR-783 signal and Luciferase activity, respectively)

CONCLUSION: As IR-783 is more avidly internalized by NE cancer cells compared to non-cancerous cells, it is a reliable indicator of changes in NE cancer cell number in both 2D culture and the 3D Bioreactor system. It could serve as a powerful tool for detecting the cytotoxic effects of drug candidates in the 3D Bioreactor system for NE cancer cells derived from patients.

In Vitro 3-Dimensional Modeling Of Tumor Microenvironment In Non-Small Cell Lung carcinoma: Hypoxia and Cell derived Matrices

Saforo Douglas J.1, Shah Parag P.3, Siskind Leah J.1,3, and Beverly Levi J.1,2,3

1Department of Pharmacology and Toxicology, University of Louisville, Louisville, KY, and 2Department of Medicine, University of Louisville, Louisville, KY. 3James Graham Brown Cancer Center, University of Louisville, Louisville, KY.

Purpose: Determine how ECM interactions with lung adenocarcinoma cells can promote hallmarks of cancer metastasis in a physiologically relevant in vitro model.

Methods: Human WI-38 fibroblasts and primary cancer associated fibroblasts patients were maintained for 7-10 days and then decellularized to produce ECM. Plasminogen Activator Inhibitor-1 (PAI-1), a known modulator of cell migration and regulator of ECM organization, was overexpressed and knocked out via CRISPR/Cas9 in the human lung adenocarcinoma cell line A549. These cell lines were then grown on matrices (3D) or plastic (2D) at superoxic (20% O2) and hypoxic (5-2% O2) conditions. ECM to cell interactions were disrupted via inhibitors of 'outside-in' signaling including integrins, focal adhesion kinase, src family kinases, and cytoskeletal remodeling. Statistical analysis was performed using unpaired T test and 2way ANOVA with Tukey's multiple comparison test. Results are expressed as mean ± SEM of multiple experiments.

Results: Relative wound closure of A549 carcinoma cells grown on 3D matrices is significantly increased compared to cells grown on 2D at 6 ($p=0.0089$), 24 ($p=0.0066$), and 48 hours ($p=0.0009$). Saracatinib treatment delayed wound closure of A549 on 3D matrices compared to 2D. Relative cell spreading of A549 spheroids on ECM is increased in a hypoxic environment of 5% O2 compared to 20% O2 ($p=0.0012$). Saracatinib treatment attenuated A549 spheroid spreading on ECM in both hypoxic and normoxic conditions ($p< 0.0001$).

Conclusions: We demonstrate ECM-to-cell interactions in vitro in a way that is not possible for traditional 2D models. These interactions play a pivotal role in determining the fate of cancer cells. Manipulating the cell culture environment to mimic physiological hypoxia enhances cell migration and invasion. An understanding of how remodeling of the tumor microenvironment is involved in late stage disease will allow development of preventative and curative therapeutic strategies to halt tumor progression and metastasis.

The role of ICAM-2 in conferring a non-metastatic phenotype in neuroblastoma

Roxanne M. Lockhart, Joseph M. Feduska, PhD, Aubrey C. Miller, Skyler Hendrix, Karina J. Yoon, PhD

Department of Pharmacology and Toxicology, University of Alabama at Birmingham, Birmingham, AL

Purpose: Neuroblastoma (NB) is a cancer of childhood that originates from neural crest cells. Approximately half of NB patients are classified as high-risk and 5-year survival rates are less than 40% for these patients. The leading cause of death in patients diagnosed with NB is the development of metastatic disease. Currently, there are no therapies targeting inhibition of metastasis, emphasizing the need for identifying molecular interactions that regulate tumor dissemination and target metastasis.

Methods: Experiments will compare the phenotype of SK-N-AS cells expressing either ICAM-2 WT or CD89ΔED. Vector control cells (neo) will be used. Changes in cell migration, invasion, anchorage-independent growth, and adhesion will be assessed in vitro. The SK-N-AS cell line was derived from NB tumor cells in a bone marrow specimen obtained from a pediatric patient with Stage IV NB. The parent cell line used to generate isogenic transfectants, SK-N-AS, is not MYCN-amplified and does not express detectable endogenous ICAM-2.

Results: We observed that in NB cells, intercellular adhesion molecule-2 (ICAM-2) suppressed cell motility in vitro and development of disseminated tumors in vivo in a murine model of metastatic NB. In SK-N-AS NB cells in vivo data with paired transfected cells demonstrated that NB cells expressing ICAM-2 produced no tumors in this model of metastatic disease, whereas NB cells expressing no detectable ICAM-2 produced disseminated tumors.

Discussion: Our lab found that NB cell lines and primary tumor cells express various levels of ICAM-2, a member of the immunoglobulin superfamily. Primary NB tumor cells with ICAM-2 expression are associated with a limited metastatic potential phenotype. We will further investigate if the non-metastatic phenotype conferred by ICAM-2 is due to intracellular or extracellular protein interactions. A more complete understanding of molecular events that mediate the metastatic process would facilitate development of therapeutic agents that target the process of metastasis.

Constitutively Active STAT5 in CD8 T cells Enhances the Antitumor Effect of Adoptive Cell Transfer with Peptide Vaccination

Aaron E. Fan, Esteban Celis, MD, PhD

Cancer Immunology, Inflammation, & Tolerance Program, Georgia Cancer Center, Augusta University, Augusta, GA.

Purpose: Adoptive cell therapy (ACT) of retrovirally transduced (RV) T cells is a powerful technique that has shown promise in tumor eradication in cancer patients. Our laboratory previously demonstrated the use of TriVax, a potent peptide vaccination strategy that dramatically expands ACT cell populations and bypasses harmful and toxic adjunct procedures commonly employed in current methods. Our purpose was to determine the antigen-specific antitumor response of RV T cells to TriVax, and if the responses could be enhanced when transduced with constitutively active STAT5 (CA-STAT5), which has been shown to increase CD8 T cell survival.

Methods: CD8 T cells were purified from B6 mouse splenocytes, activated with CD3/CD28 beads, and transduced with RV encoding mouse gp100 TCR. In some experiments, cells were also co-transduced with CA-STAT5 RV. Transduction efficiency and functional activity was assessed using flow cytometry, cytotoxicity, and EliSpot. Naïve and B16F10 tumor-bearing congenic CD45.1 mice were given ACT (1.0x105 tetramer+ cells) and subsequently vaccinated with TriVax.

Results: TriVax administration selectively expanded the ACT cell population expressing gp100-TCR; this was preferential for ACT populations over endogenous. Cell numbers in spleen indicated a significant fold expansion compared to initially transferred cells 25 days after vaccination. When co-transduced with CA-STAT5, an even higher fold expansion was observed, and CA-STAT5- transduced cells seemed to persist longer in vivo over time. CA-STAT5+ cells also expanded more robustly than CA-STAT5- cells when stimulated with a subsequent vaccine boost, demonstrating a 5000-fold increase in tetramer+ CD8 T cells. ACT of these cell populations into tumor-bearing mice with TriVax administration demonstrates a powerful antitumor effect, leading to tumor regression in treated groups.

Conclusion: RV T cells expressing gp100 TCR are capable of antigen-dependent expansion in response to TriVax. Co-expression of CA-STAT5 greatly enhances the boost effect of TriVax, leading to a dramatic antitumor effect.

Immunofluorescent Staining and Spheroid Culture for Characterization of Cancerous and Non-Cancerous Primary Renal Cells

Lynn K. H. Tran, Sharad Purohit, Ph.D.,and Jin-Xiong She, Ph.D.

Center for Biotechnology and Genomic Medicine, Augusta University, Augusta, GA

Purpose: We have isolated primary renal cancer cell cultures and non-cancer cell cultures from fresh patient tissues and are characterizing these cultures to provide a resource for future studies in cancer biomarkers and therapies. We hypothesized that renal cell carcinoma human primary cell cultures will possess consistent immunostaining profiles that differentiate them from the profile of normal primary renal cell cultures.

Methods: Primary renal cell carcinoma and non-cancerous renal cell cultures were created through overnight collagenase digestion of human tissue specimens. Spheroid culture using low-adherence plates was used to demonstrate the differential tumorigenicity of the cancerous culture as opposed to the normal culture. Then, primary cultures were characterized using an immunofluorescence (IF) stain panel.

Results: Mean normalized intensity of staining for our IF staining panel (comprising 15 markers) were calculated, and a nested 2-way ANOVA analysis was performed, with the dependent variable being each stain, where the factors are culture type (tumor versus normal) and replicate (1, 2, and 3). No significant differences in staining were detected between cancerous cell cultures (BAT514T, CT31T, and CT35T) and non-cancerous cell cultures (all $p > 0.05$). All three cancer cell cultures formed spheroids in low-attachment cell culture plates, whereas normal primary renal cell cultures do not.

Discussion and Conclusion: Our data demonstrates that primary renal cell carcinoma cultures (BAT514T, CT31T, and CT35T) and primary normal renal cell cultures (CT4N and CT6N) demonstrate similar IF staining profiles of 15 markers, but can be differentiated by their ability to form spheroids in low-attachment culture. We conclude that our primary renal cell carcinoma cultures demonstrate a consistent IF staining profile, but that our panel does not differentiate them from the normal primary renal cell cultures.

Investigating the role of lysine specific demethylase 1 (Lsd1) in retinal development and retinoblastoma differentiation

Salma Ferdous1,2, Hans. E. Grossniklaus PhD2,3, John M. Nickerson PhD1,2

1Genetics and Molecular Biology Graduate Program, Emory University, Atlanta, GA; 2Department of Ophthalmology, Emory University, Atlanta, GA; 3Cancer Biology Graduate Program, University, Atlanta, GA

Purpose: The purpose of this study was to determine the role of lysine specific demethylase 1 (Lsd1) in retinal cell differentiation. Lsd1 specifically removes H3K4 and H3K9 methylation. Popova et. al found that late progenitor retinal cells express Lsd1 as they become postmitotic and begin to differentiate. Proper retinal differentiation is important for normal visual function, but also aberrantly occurs in retinoblastoma, the most common primary pediatric intraocular tumor. These tumors display several hallmarks features, namely, Homer Wright (HW) and Flexner - Wintersteiner (FW) rosettes, which mimic retinal differentiation, and fleurettes, which mimic photoreceptor differentiation. Because rosettes and fleurettes mimic general retinal differentiation, we investigated the role of Lsd1 in normal murine retinal development.

Methods: Retinoblastoma affected eyes were enucleated, fixed with paraformaldehyde, and underwent immunohistochemistry for Lsd1. Additionally, immunohistochemistry was conducted on murine retinal sections at various stages during and after development.

Results: In retinoblastoma tumors, Lsd1 shows remarkable expression in highly differentiated areas, but is absent in undifferentiated areas. Murine retinal sections show high expression of Lsd1 in all retinal progenitor cells during development, but cell-type specific expression after development. In mature retinas, Lsd1 is expressed in all three nuclear layers, the retinal ganglion cell layer (RGC), inner nuclear layer (INL), and outer nuclear layer (ONL). The INL shows uniform staining, however, the RGC and ONL show variation. Co-labeling of Lsd1 with short-wavelength cone opsin pigment (S-OPSIN) reveal that Lsd1 expressing cells in the ONL are cone photoreceptors.

Conclusions: These experiments highlight Lsd1 involvement in the differentiation of particular retinal cell subtypes and possible contribution to the differentiation and aggression of retinoblastoma tumors. Currently, Lsd1 inhibitors are in clinical trials for the treatment of various cancers, including acute myeloid leukemia (AML) and lung cancer. Therefore, we hypothesize that these inhibitors may be a potential therapeutic strategy for retinoblastoma.

The Role of Tumor-Derived Exosomes in the Formation of a Pre-Metastatic Niche in Cancer

Samantha M. Morrissey M.S.*, Jun Yan M.D.,PhD.+

*Department of Microbiology & Immunology, University of Louisville, Louisville, Kentucky +James Graham Brown Cancer Center, University of Louisville, Louisville, Kentucky

Purpose: Determine if tumor derived exosomes (TDE), small membrane derived nanoparticles, are capable of suppressing αβ T cell effector functioning through PD-L1 (programmed-death-ligand-1) upregulation on macrophages and γδ T cells. Preliminary data has shown that exosome stimulation can differentially upregulate PD-L1 expression on murine peritoneal macrophages and lung resident γδ T cells while simultaneously upregulating PD-1(programmed death-1) expression on αβ T cells. The ultimate goal of this project is to assess whether elimination of tumor derived exosomes can synergize with current PD-1/PD-L1 treatments by removing antagonizing factors and improve clinical outcomes.

Methods: TDE were isolated using serial ultracentrifugation from Lewis Lung Carcinoma cells. Peritoneal macrophages and γδ T cells were stimulated with 80μg/mL of exosomes for 16 hours prior to co-culturing with ova-transgenic –I (CD8+) T-cells to assay for suppressive function. Anti-PD-1 antibody was added to the culture system to confirm that the PD-1/PDL-1 axis mediates the immune suppression. Peritoneal macrophages were additionally assayed for upregulation of classical M2 markers using RT-PCR. Lastly, human peripheral blood samples from lung cancer and anti-PD-1 treated lung cancer patients were analyzed via flow cytometry for comparison of γδ T cell percentage, and PD-1/PD-L1 surface marker expression on both αβ and γδ T cells.

Results: The results suggest that TDE can potentially play a role in tumor metastasis by priming a distant metastatic niche for tumor implantation by polarizing γδT cells and macrophages to an immunosuppressive PD-L1+ phenotype capable of restricting αβ T cell effector functioning.

Discussion/Conclusion: These data suggest that exosomes could be directly antagonizing the effects of immune checkpoint therapies promoting disease progression. Therefore, future experiments will focus on the effect elimination of exosome secretion from primary tumor cells using siRNARab27 inhibition as well as the small drug compound, cambinol, concurrently with anti-PD-1 or anti-PD-L1 monoclonal antibody has on metastatic burden.

The role of βIGH3 in the TGF-β signaling pathway in the breast cancer tumor microenvironment

Mudit Dutta, Hongyu Tian, Huanrui Hu, Gerard Blobe

Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina

The transforming growth factor-beta (TGF-β) signaling pathway regulates many cellular processes including proliferation, differentiation and apoptosis. TGF-β serves as both a tumor suppressor during early stages of carcinogenesis and a promoter of tumor invasion and metastasis during cancer progression. We conducted a proteomic screen for molecules in the tumor microenvironment that might regulate this switch in TGF-β's role during cancer progression, and identified transforming growth factor-beta-induced (βIGH3) as a potential positive regulator of the TGF-β pathway in breast cancer. We demonstrate that βIGH3 is highly expressed in a metastatic breast cancer cell line (MDA-MB-231-4175) relative to the poorly metastatic parental cell line (MDA-MB-231). βIGH3 mRNA was decreased in MDA-MB-231 cells relative to MDA-MB-231-4175 cells, and treatment with DNA methylation and histone deacetylase inhibitors induced expression in MDA-MB-231-4175 cells, suggesting epigenetic regulation. TGF-β1 further increased expression of βIGH3 in MDA-MB-231-4175 cells, but not in MDA-MB-231 cells. CRISPR knockout (KO) of βIGH3 in MDA-MB-231-4175 decreased cancer cell migration in vitro and tumor growth in vivo relative to non-target control (NTC) MDA-MB-231-4175 cells. Immunostaining of the dissected tumors revealed higher levels of pSmad2 in NTC MDA-MBA-231-4175 tumors, indicating higher activation of TGF-β signaling. These results demonstrate that βIGH3 may contribute to breast cancer tumor progression by promoting TGF-β signaling to increase migration and metastasis.

Phosphorylation of a Linker Region Masks Cdc15's F-BAR Domain to Regulate Its Cytokinetic Ring Scaffolding Activity

Maria Santa Mangione, Nathan A. McDonald, Ph.D., Kathleen L. Gould, Ph.D.

Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN

Plasma membrane remodeling is required for many cell processes including endocytosis, migration, neurite growth, and cytokinesis. The F-BAR family—defined by the membrane-binding F-BAR domain—coordinates actin cytoskeleton rearrangements at membranes. F-BAR protein regulation is one method by which cells orchestrate these vital processes. Here we investigate the regulation of F-BAR protein Cdc15, which is essential for cytokinesis in fission yeast. Cdc15 secures the actin-based cytokinetic ring (CR) at the division site by binding the plasma membrane through its N-terminal F-BAR domain and scaffolding CR components through its C-terminal SH3 domain. A disordered central linker is phosphorylated on >35 sites during interphase, which inhibits Cdc15; dephosphorylation in mitosis is required for Cdc15 to scaffold the CR. We exploit the technical advantages of fission yeast including rapid gene editing and facile live-cell microscopy to determine the molecular mechanism by which phosphorylation inhibits Cdc15. Our data indicate that phosphorylation of the linker enables direct interaction between the phosphorylated linker and the F-BAR domain, which obstructs membrane binding. First, intramolecular fluorescence resonance energy transfer (FRET) assays of Cdc15 with acceptor and donor fluorophores at opposite termini reveals that interphase Cdc15 exhibits a FRET signal indicative of nearby N- and C-termini, or a "closed" conformation. Additionally, a phosphomutant with abolished phosphorylation on 27 sites has reduced FRET signal throughout the cell cycle, indicating an "open" conformation and that phosphorylation controls conformation. Next, Cdc15 F-BAR domain binds directly to the phosphorylated linker; the SH3 domain is neither necessary nor sufficient for this interaction. Finally, when bound by the phosphorylated linker, Cdc15's F-BAR domain cannot interact with synthetic membranes. Together our findings provide a model of F-BAR protein regulation by phosphorylation-dependent intramolecular inhibition. Since many F-BAR proteins are phosphorylated and contain disordered regions, this may be a common mechanism of F-BAR protein regulation.

Energetics and Vibrational Signatures of Nucleobase Argyrophilic Interactions

S. Paul Lee* and Gregory S. Tschumper, PhD

Department of Chemistry and Biochemistry, University of Mississippi, University, MS 38677

This study investigates the interactions of both purine (adenine and guanine) and pyrimidine (cytosine, thymine, and uracil) nucleobases with a pair of silver atoms (Ag2). Full geometry optimizations were performed on several structures of each nucleobase/Ag2 complex and the corresponding isolated monomers using the M06-2X density functional method with a correlation consistent triple-ζ basis set augmented with diffuse functions on all atoms and a relativistic pseudopotential on Ag (aug-cc-pVTZ for H, C, N, and O and aug-cc-pVTZ-PP for Ag; denoted aVTZ). Harmonic vibrational frequencies were computed in order to confirm that each structure corresponds to a minimum on the M06-2X/aVTZ potential energy surface. Relative electronic energies for interactions between Ag2 and each nucleobase were compared to elucidate energetic differences between isomers. Further analysis of the changes in vibrational frequencies, infrared intensities, and Raman scattering activities reveals how different Ag2 binding sites might be identified spectroscopically. These results provide molecular-level insight into the interactions between nucleobases and silver, which may lead to better understanding and interpretation of surface-enhanced Raman spectroscopy (SERS) experiments on nucleobases and related systems.

Chemoenzymatic synthesis of bioorthogonal peptidoglycan derivatives: tools to remodel bacterial cell wall

Zachary Jones(1), Kristen DeMeester(1), Hai Liang(1), Catherine Leimkuhler Grimes(1,2)

(1) Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716; (2) Department of Biological Sciences, University of Delaware, Newark, DE 19716

The cell wall of bacteria is surrounded by a polymer known as peptidoglycan (PG). This material serves as a protective coat in Gram-positive and Gram-negative bacteria alike. The PG consists of alternating sugar monomers of N-acetyl muramic acid (NAM) and N-acetyl glucosamine (NAG) with layers linked by pentapeptide chains. Fragments of PG have been shown to activate the innate immune response in Crohn's disease, and so, by manipulating PG and observing its chemical interactions, it is hypothesized that labelling bacteria on the glycan backbone can be used as a tool to reveal structural features and immune response interactions. Glycan chemical synthesis, particularly of UDP sugar probes, has proven difficult to achieve with a reasonable yield. The objective of these experiments is to synthesize large-scale quantities of UDP-NAM derivatives and to utilize these chemical probes for incorporation into the PG of bacteria. By tracking the dynamics of PG, specifically the breakdown of these chemical probes, innate immune reactions and responses may be better understood. The method developed utilizes the recycling enzymes anomeric NAM/NAG kinase (AmgK) and uridylyltransferase (MurU) in order to chemoenzymatically synthesize UDP-NAM derivatives. The enzymes were expressed and purified from Escherichia coli, and utilized in-vitro to add the UDP moiety. Purification was carried out by high-pressure liquid chromatography (HPLC) coupled to liquid chromatography mass spectrometry (LC/MS). Products were characterized by LC/MS and nuclear magnetic resonance (NMR) imaging. Currently, large scale synthesis, purification, and characterization has been completed of the natural UDP-NAM substrate, along with many derivatives with varying bioorthogonal function. Additionally, the 2-azido-UDP-NAM has successfully been incorporated into the PG of Lactobacillus acidophilus. From these results, it is confirmed that utilizing AmgK and MurU to create UDP-NAM derivatives is viable, and thus the library of PG probes can be expanded for cell wall remodeling. The next step is to attempt to label other biologically relevant bacteria, visualize the digestion of the labeled PG, and continue to explore the utility of these glycan probes.

Grady Healthy Living: A Student-Run, Longitudinal Program to Address Social Determinants of Health at Grady Memorial Hospital

Zoë M. Kopp, Sahil Angelo, Stacie R. Schmidt MD

Department of Medicine, Emory University SOM, Atlanta, Georgia

Purpose: Social determinants of health (SDH) are the base upon which provider decisions and patient outcomes are formed. Grady Healthy Living (GHL), a medical student-run volunteer organization, implemented a program to screen primary care clinic patients for SDH, supply those patients with local, patient-centered resources, and create a long term relationship via health-coaching phone calls.

Methods: GHL created a screening survey, focusing on 7 health determinants, based on the clinically validated screening toolkit from Health Leads. Students approached patients awaiting their appointment, offered to screen them, and provided targeted resource sheets based on question responses. Students invited the patient to participate in a health-coaching relationship, involving regular follow-up phone calls.

Results: Of the 138 patients approached, seventy-two (52%) agreed to be surveyed. Twenty-two (31%) did not complete high school; thirteen patients (18%) achieved an associate's degree or higher. Ninety percent of patients live below the federal poverty line. Of the patients screened, 29% screened positively for prohibitive healthcare costs, 36% for food insecurity, 22% for poor health literacy, 28% for unreliable transportation, 24% for housing instability, 14% for unemployment, 31% for difficulty with stress management, 11% for unsafe home environment, 10% for loss of utilities, and 4% for lack of childcare. Fifty-five patients (76%) expressed interest in and were provided with resources. After follow-up, 13 patients (24%) had used the resource provided, with 11 (85%) citing the resource as helpful.

Conclusions: Grady's primary care patients face a number of barriers to health. The majority are interested in this program, in which they receive tailored resources and longitudinal support. Improving follow-up rates is a key challenge to assessing the program's effectiveness. GHL represents a channel through which SDH can be actively addressed, collecting vital data about Grady's patients, disseminating information about local resources, and filling a void in patient support.

Drug Overdose Deaths among Women Age 30-64 years– United States, 1999-2015

Jacob P. VanHouten, PhD; Rose A. Rudd, MSPH; Michael F. Ballesteros, PhD; Karin A. Mack, PhD

Background: In 2015, drug overdoses accounted for over 52,000 deaths, of which, over 33,000 involved opioids. While the number of drug overdose deaths remains greater among males, the rate of increase has been greater among females. Even among females, however, the increase in overdose death risk is not uniform. The ten-year age groups that had the largest percent change from 1999 to 2010 in the rate of overall drug overdose deaths were 45-54 and 55-64 years. That information neither takes into account secular trends in specific drugs used by women, nor does it consider within age group changes. This work examines trends in drug overdose mortality among cohorts of women in the United States through analyses of 1999-2015 National Vital Statistics System (NVSS) mortality data.

Methods: Mortality data for U.S. residents were from the 1999-2015 NVSS, which is based on information from all death certificates filed in the 50 states and the District of Columbia. Mortality data were coded according to the International Classification of Diseases, 10th Revision (ICD-10). Analyses were restricted to deaths with an underlying cause of death based on the ICD-10 codes for drug overdose (X40-X44, X60-X64, X85, and Y10-Y14). We analyzed all drug overdose deaths among female U.S. residents between the ages of 30-64 years. We identified the type of drug, or drug class, involved based on ICD-10 codes for opioids, heroin, cocaine, benzodiazepines, and antidepressants. Crude rates are reported as deaths per 100,000 population.

Results: In 1999, there were 4,314 deaths from drug overdoses among women ages 30-64 years (crude rate: 6.7 deaths per 100,000 population). In 2015, there had been over a 200% increase in the annual crude rate of drug overdose deaths among this population, with 15,265 deaths (crude rate: 20.6 deaths per 100,000 population). The number and rate of deaths involving opioids, heroin, and benzodiazepines also increased during this time period, with a notable increase in the rate of deaths involving heroin since 2012. Age distribution changes in drug specific overdose deaths are demonstrated with population pyramids.

Conclusions: Between 1999 and 2015, overdose death rates increased more than 200% among women 30-64 years of age, with particularly sharp increases in overdoses involving heroin and benzodiazepines. Interventions to reduce the misuse of drugs should target both illicit drugs and prescribed medications. Public health professionals and clinicians can use these findings to identify specific drug use demographics or age groups in need of targeted interventions.

A Method for Targeted Community Engagement Using Geographic Information Systems (GIS): A Case Study with a Free Primary Care Clinic

John P. Shelley*, Alec Hersh+, Beverly Parker+, Andrea L. Cherrington*

*Division of Preventive Medicine, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL; M-POWER Ministries Health Center, Birmingham, AL

The effects of socioeconomic status on health have been well documented. Access to care can mediate this relationship, but identification of those in need of care can be an expensive and time-consuming endeavor. Given the increasing availability of socioeconomic data at smaller geographies, geographic information systems (GIS) have the potential to make both assessment and outreach more efficient without compromises in accuracy. We describe the development of a GIS-enabled approach to community engagement and the implementation of this approach in a free clinic. We modified a validated health care need index for use at the block group level. We compiled eight socioeconomic measures from the American Community Survey's five-year estimates from the years 2011 to 2015. The measures included percent of individuals living in poverty, uninsured, out of the work force, and without a high school diploma; and the percent of households headed by single parents, renter-occupied, overcrowded, and without a car. Using principal factor analysis, we calculated modified index scores for 519 block groups in Jefferson County, Alabama, the clinic's service area. We then mapped the scores, highlighted high-need clusters, and identified community resources within these clusters using GIS. The index created by the factor analysis weighted the variables, from highest to lowest weight, as following: poverty, uninsured, without a high school diploma, households without a car, renter-occupied households, not in the labor force, single-parent households, and overcrowded households. We identified 15 high-need neighborhood clusters within the county. Community organizations within the clusters were identified and then contacted by clinic staff. We illustrate the utility of using GIS to map health care need at a geographic level conducive to boots-on-the-ground outreach. Being both time- and cost-efficient, this method could be used by small clinics and large health systems alike to generate meaningful observations about the areas they serve.

Examining the role of Dyrk1a in the development and function of inhibitory neurons.

Rachel V. Levy, Department of Psychology, University of Florida, Gainesville, FL; Zhong-Wei Zhang

The Jackson Laboratory, Bar Harbor, ME

Dual-specificity tyrosine phosphorylation-regulated kinase 1A (Dyrk1a) has a crucial role in brain development, and studies have revealed links to Down Syndrome (DS) and autism spectrum disorders (ASDs). The purpose of this study was to determine whether deletion of Dyrk1a alters the number and distribution of parvalbumin (PV) neurons in the cortex and innervation of neurons by PV neurons. The overall goal was to reveal how this genetic mutation plays a role in the development of ASD. Cre-lox technology was used to produce the genetically mutated mice carrying heterozygous deletion of Dyrk1a in inhibitory neurons. Perfusion was performed on both mutant and control mice at 8 weeks old. After perfusion, the mice were dissected, and their brains were sectioned and treated with immunofluorescent staining for PV and GAD67. They were then analyzed through fluorescent and confocal microscopy, with a focus on the cerebral cortex. Data analysis showed that Dyrk1a mutation disrupts the development of PV neurons. These trends were present in the density and size of PV neurons, as well as in the distribution of synaptic terminals. This study could serve as a base for future research into ASD in humans, including potential for treatment and preventative measures.

Immunologic Concordance in Metastatic Renal Cell Carcinoma

Amir I. Khan*, Kevin R. Melnick*, Carey S. Jansen*, Kyu S. Kim, MD+, Jennifer W. Carlisle, MD^, Fares Hosseinzadeh*, Nataliya Prokhnevska*, Viraj A. Master, MD, PhD*$%, Haydn T. Kissick, PhD$@!

*=Emory University School of Medicine, Atlanta, GA; +=Department of Pediatrics, University of California San Diego, La Jolla, CA; ^=Department of Hematology & Oncology, Emory University, Atlanta, GA; $=Department of Urology, Emory University, Atlanta, GA; %=Winship Cancer Institute, Emory University, Atlanta, GA; @=Department of Microbiology & Immunology, Emory University, Atlanta, GA; !=Emory Vaccine Center, Emory University, Atlanta, GA

Purpose: Renal cell carcinoma (RCC) has been shown to be a genetically and morphologically heterogeneous cancer. As the focus grows on tumor-infiltrating lymphocytes (TILs) in cancers such as RCC, it is critical to examine how the phenotype of TILs manifests in metastatic RCC. With the high treatment failure observed with immunotherapy for metastatic RCC, such analysis may help explain the role of the immune landscape in developing such resistance. This study will thus compare the phenotype of primary tumors, venous tumor thrombi (VTT), and metastases to observe whether immune heterogeneity exists within metastatic RCC.

Methods: RCC tissue was collected from patients intraoperatively and digested into single cell suspensions. Suspensions were fluorescently stained for TIL surface markers and processed using fluorescence-activated cell sorting (FACS). TIL populations and their expressed receptors were analyzed using FlowJo to capture %CD8+ and %CD4+ in total tumor. The %CD28+ and %PD1+ of the CD8+ cells was also measured to observe the co-stimulatory and co-inhibitory receptor expression, respectively.

Results: Analysis was performed for 20 total patients, encompassing 20 primary tumors with matched 15 VTT and 6 metastases. Metastases included 3 adrenal, 2 bone, and 1 liver metastasis. Immunological concordance was observed in all measured parameters (%CD8+, %CD4+, %CD28+, and %PD1+) for primary tumors and VTT, as well as for primary tumors and their metastases.

Discussion: Despite the genetic and morphologic heterogeneity seen in RCC, immunologic concordance was observed in the analysis of primary tumors, VTT, and metastases in RCC. This concordance suggests that there may be a more systemic cause affecting the tumors rather than features of the local tumor microenvironment. With preliminary studies showing that blood inflammatory marker levels (BIM) impact immune phenotype, an ongoing study will next examine the role of systemic inflammation in RCC using patient-derived xenograft (PDX) models in immunocompromised mice.

In Vitro and In Vivo Evaluation of Radiolabeled Particulate Beta-Glucan

Anne E. Geller, M.S., Jun Yan M.D. PhD., Haixun Guo, PhD

Department of Microbiology and Immunology, University of Louisville, Louisville, KY.

Purpose: Whole B-glucan particles (WGP) has shown promise as an anti-tumor agent. It converts immunosuppressive M2 macrophages into classically activated, M1 macrophages, which are associated with improved tumoricidal effects. Much has been characterized about its efficacy in reducing tumor burden, but it is not determined how exactly WGP distributes in the body and how it's distribution impacts anti-tumor effects.

Hypothesis / Objective: The aim of this study was to confirm the anti-tumor effects of WGP in an in-vivo model and to characterize the bio-distribution and trafficking of WGP. To study the biodistribution of WGP, it was radiolabeled with 68Ga, and mice were subjected to a PET/CT. A secondary aim was to assess the impact of radiolabeling WGP on its ability to influence macrophage surface receptor and cytokine expression, along with Dectin-1 targeting ability.

Methods: An in-vivo study measuring tumor burden with and without treatment was performed. Additionally, an in-vitro assay was preformed to assess the effect of WGP radiolabeled with 68Ga. The results were generated using an ELISA and BD FACSCaliburTM. Another in-vitro assay was performed to assess whether radiolabeled WGP maintained Dectin-1 specificity. The biodistribution of 68Ga-WGP was evaluated in WT and Dectin-1 KO mice. Finally, the distribution and uptake in tissues of 68Ga-WGP was measured in mice with subcutaneous LLC tumors using PET/CT.

Results: Our study indicated that WGP does have anti-tumor effects. Comparison of non-radiolabeled WGP to 68Ga-WGP indicated that the radiolabel does not significantly influence the expression of surface receptors or cytokine production. Further, it was confirmed that 68Ga-WGP maintains Dectin-1 targeting ability and that after oral administration WGP is delivered to the multiple organs, with marked uptake of WGP by tumor cells 4h post dose administration.

Conclusions: Results indicate that Radiolabeled WGP is not only an important anti-tumor agent, but also that radiolabeling WGP does not impact the effectiveness or interaction profile. Further the enhanced uptake of WGP by a tumor indicates an important interaction with the tumor microenvironment, and further studies will be conducted to better identify the mechanism of this interaction.

Tumor CD8+ T cells in Renal Cell Carcinoma

Carey. S. Jansen, Kevin R. Melnick, Kyu Kim MD, Amir I. Khan, Jennifer W. Carlisle MD, Farres Hosseinzadeh, Nataliya Prokhnevska, Viraj A. Master MD PhD, Haydn T. Kissick PhD

Purpose: The prognostic significance of tumor infiltrating immune cells have been explored in various cancers. In a systematic review across multiple tumor types, CD8+ tumor infiltrating lymphocytes (TILs) were associated with improved overall, disease-specific, and progression-free survival. However, this association is less clear in renal cell carcinoma (RCC), which is a significant cause of oncologic mortality and morbidity in the United States. This study seeks both to clarify this association and to elucidate the biological mechanisms behind it.

Methods Human RCC tumors are surgically resected and processed for analysis via flow cytometry and histology (H&E, immunofluorescence). Matched clinical data is accessed via a secure database.

Results & Conclusions: Human RCC tumors have variable proportions of CD8+ TILs, with some tumors having near 0% CD8+ TILs, while others have more than 10% CD8+ TILs. When CD8+ TIL proportion is compared between patients who do and do not recur, patients with higher CD8+ TILs appear to be less likely to recur. This association may prove to have great clinical significance.

We have identified tertiary lymphoid structures (TLS) in and near the tumor via H&E staining, and we have found that CD8+ T cells and CD11c+ dendritic cells (DCs) exist in aggregates in the tumor via immunofluorescence. We hypothesize that there are characteristic functional differences between DC-aggregate-associated CD8+ T cells and those dispersed throughout the tumor. Ongoing study seeks to determine whether these aggregates represent the TLS seen on H&E and whether these cells are functionally distinct from those dispersed throughout the tumor.

Assessing CD8 Polyfunctionality in HVTN502 Recipients Receiving Pre-Adapted and Non-Adapted Vaccines

Jacob K Files, Sushma Boppana, and Paul Goepfert MD

Department of Medicine, University of Alabama Birmingham, Birmingham, AL.

CD8 T cells play an important role in controlling HIV infection which is partly mitigated by viral adaptation to these responses. Much of this adaptation can be quantified by identifying mutations that accumulate in chronic infection and are associated with a particular HLA-I allele. Recent studies from our lab show that patients infected with a virus pre-adapted to their HLA-I alleles have diminished CD8 T cell responses and poorer clinical outcomes. However, the role of HLA-I adaptation in the context of vaccination remains unknown. We hypothesize that vaccine recipients who are "pre-adapted" to the vaccine insert sequence will have a less immunogenic and less functional CD8 response than those who were not. Using samples from the HVTN502 vaccine efficacy trial, we tested 19 samples from "high adaptation" and "low adaptation" groups. Adaptation scores were generated based on each individual's HLA-I allele in relation to the given HIV vaccine. Through intracellular staining, we measured the effector cytokine response to peptide pools spanning each vaccine-encoded protein (Gag, Pol, and Nef). Surprisingly, we observed that the high adaptation recipients showed stronger CD8 responses, with more individuals producing a given cytokine than the low adaptation group. When comparing polyfunctionality, or the number of cells producing multiple cytokines simultaneously, we saw the high adaptation group produce more polyfunctional responses to Pol1 ($p=0.0247$) while the low adaptation group produced more polyfunctional responses to Nef ($p=0.0308$). Overall, these results suggest that adaptation to the vaccine insert results in a skewed CD8 T cell response. The surprising finding that higher adaptation to the vaccine insert is associated with higher CD8 responses may be explained with single epitope mapping. Ultimately, we hope to definitively illustrate the impact of HLA-I adaptation in generating vaccine responses in order to inform future HIV vaccine design.

IRF8 Regulates the CD44-Osteopontin Axis to Control CD8 T Cell Homeostasis and Proliferation

John D. Klement1,2,3,Amy V. Paschall1,2,3, Mary A. Zimmerman1, Mohammed L. Ibrahim1,2, Priscilla S. Redd1,2,3, Chunwan Lu1,2,3, Hussein Sultan1,2, Esteban Celis1,2, Keiko Ozato4 & Kebin Liu1,2,3

1Department of Biochemistry and Molecular Biology, Medical College of Georgia, Augusta, GA 30912, USA. 2Georgia Cancer Center, Augusta University, Augusta GA 30912, USA. 3Charlie Norwood VA Medical Center, Augusta, GA 30904, USA. 4Division of Developmental Biology, National Institute of Child Health and Human Development, National Institutes of Health, Rockville, MD 20852

Purpose: The transcription factor interferon regulatory factor 8 (IRF8) plays an essential role in lineage differentiation and function of hematopoietic cells. Here, we aimed to test the hypothesis that IRF8 plays a critical role in host cancer immunosurveillance by regulating T cell homeostasis and activation.

Methods: In vitro T cell responses were assayed by stimulation with anti-CD3/CD28 antibodies. To examine the role of IRF8 in T cells, a peptide-based vaccine was utilized to measure in vivo responses. Mixed-chimera mice were generated by lethal irradiation of recipient host followed by reconstitution with CD45.1+ WT and CD45.2+ IRF8-KO bone marrow. Naïve and vaccine-challenged mice were further analyzed by qPCR and flow cytometry.

Results: Loss of hematopoietic IRF8 allowed for engraftment of an allogenic mouse breast tumor and enhanced the growth of a spontaneous sarcoma model, and, similarly, in vivo stimulation of CD8+ T cells demonstrated defective T cell proliferation in IRF8-KO mice. However, isolated T cells from IRF8-KO mice displayed no deficiency following in vitro polyclonal stimulation or an in vivo mixed chimera model. Loss of IRF8 led to the accumulation of a CD44hiCD8+ population that was not present in a mixed-chimera model. This was accompanied by a twelve-fold increase in the expression of the CD44 ligand osteopontin by IRF8-KO splenocytes, which we showed to be a potent inhibitor of T cell proliferation in vitro.

Discussions and Conclusion: Therefore, IRF8 intrinsically regulates CD44 expression to mediate T cell differentiation and homeostasis while extrinsically regulating osteopontin in myeloid cells to mediate T cell activation and expansion. Together, these intrinsic-extrinsic mechanisms of IRF8 control T cell function in the context of the tumor microenvironment.

HIV-specific CD8 T cell cross-reactivity following Ad5-based vaccination is shaped by vaccine regimen and prior Ad5 exposure

Sushma Boppana*, Sarah Sterrett*, Anju Bansal PhD*, and Paul A. Goepfert MD*

*Department of Medicine, Univ. of Alabama at Birmingham, Birmingham, AL

Purpose: The ability of the CD8 T cell response to cross-recognize several variants of a single epitope could be an important factor in optimizing future HIV vaccine design. Some prior work has indicated that CD8 cross-reactivity plays a role in HIV control in individuals with protective HLA-I alleles; however, not much is known about CD8 cross-reactivity in the context of vaccination. The purpose of this project was to evaluate the vaccine-induced CD8 cross-reactivity in two prior HIV-1 vaccine efficacy trials.

Methods: We examined the CD8 responses within two preventative vaccine studies, both of which used adenovirus serotype 5 (Ad5) vectors: HVTN502 (MRKAd5) and HVTN505 (VRC DNA prime, Ad5 boost). We measured the responses to vaccine-encoded epitopes and their common variants using IFNγ ELISpot assays. We also quantified the antigen sensitivities by measuring the IFNγ responses to log fold serial peptide dilutions.

Results: Overall, CD8 responses to variant epitopes had a lower magnitude and decreased antigen sensitivity than those targeting vaccine-encoded epitopes ($p<0.0001$ and $p=0.014$). A greater number of mutations, less conservative amino acid substitutions, and HLA-I driven mutations negatively affect the immunogenicity of cross-reactive responses ($p<0.0001$, $p=0.0003$, and $p<0.0001$, respectively). Additionally, cross-reactive responses had a higher magnitude in MRKAd5 recipients with low pre-existing Ad5 titers than those with high pre-existing titers ($p=0.0230$). In comparing only Ad5-naïve recipients, MRKAd5-generated cross-reactive responses were decreased in magnitude and proportion in comparison with cross-reactive responses of VRC DNA/Ad5 recipients ($p=0.0052$, $p=0.0488$), despite similar magnitudes towards vaccine-encoded epitopes.

Discussion/Conclusions: Our data shows that cross-reactive responses are frequently elicited by vaccination and that this cross-reactivity is affected by both the vaccine regimen and pre-existing Ad5 titers. In future work, we plan to investigate the TCR clonotypes mobilized by cross-reactivity and determine the biologic and clinical significance of these vaccine-induced cross-reactive CD8 T cells.

Induced MHCII expression on breast cancer cells impairs tumor growth by broadening the responding T cell repertoire and delaying tumor-specific T cell exhaustion

Tyler R. McCaw1, Mei Li, PhD2; Dmytro Starenki, PhD3; Sara Cooper, PhD3; Selene Meza-Perez, PhD1; Rebecca C. Arend, MD4; Donald J. Buchsbaum, PhD2; Andres Forero, MD5; Troy D. Randall, PhD1

1. Department of Medicine, Division of Clinical Immunology and Rheumatology, University of Alabama at Birmingham, Birmingham, AL. 2. Department of Radiation Oncology, University of Alabama at Birmingham, Birmingham, AL. 3. HudsonAlpha Institute for Biotechnology, Huntsville, AL. 4. Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, University of Alabama at Birmingham, Birmingham, AL. 5. Department of Medicine, Division of Hematology and Oncology, University of Alabama at Birmingham, Birmingham, AL

Purpose: We recently reported that aberrant expression of Major Histocompatibility Class II (MHCII) on human triple negative breast cancer cells correlates with increased tumor infiltrating lymphocytes and prolonged progression free survival. This led us to hypothesize that tumor cell expression of MHCII enhanced the intratumoral CD4+ T cell response, thereby bolstering the tumor-specific CD8+ T cell response, leading to impaired tumor growth.

Methods: To test this, we transfected the murine breast cancer lines TS/A and 4T1 with the human class II transcriptional activator (hCIITA) or empty vector, creating MHCII-expressing and MHCII-negative cell lines, respectively. Next we used the histone deacetylase inhibitor Entinostat to induce MHCII on non-transfected cancer cells in vitro and in vivo.

Results: We found that MHCII-expressing tumors grew slower than controls in immunocompetent recipients, but this difference was abrogated in CD4-depleted and lost in SCID mice. Within hCIITA-transfected tumors, CD4+ T cells produced more IFNγ and CD8+ T cells produced more IFNγ and granzyme B for longer times, but both eventually became exhausted regardless tumor MHCII expression. We then demonstrated the ability of Entinostat to substantially upregulate MHCII on tumors in vivo, which correlated with reduced tumor burden. This effect was lost when treating tumor-bearing SCID mice or depleting IFNγ from WT mice, suggesting HDACis potentiate adaptive immunity, as reflected by enhanced effector T cell functions. Finally, TCR repertoire analysis demonstrated increased breadth and magnitude of T cell responses to MHCII-expressing tumors.

Discussion/Conclusions: Inducing MHCII on tumors, through transfection or HDACis, can potentially avail an augmented T cell response to all patients for enhanced tumor control. Since progressive loss of T cell functionality through exhaustion is a common mechanism by which tumors escape immune control, the strategies described herein may be broadly applicable to reversal of T cell exhaustion and control of tumor growth in many cancers.

Commensal Propionibacterium strain UF1 mitigates intestinal inflammation: Synthetic Biology Approaches to Elucidate Mechanism

Max R Van Belkum, Yong Ge PhD

University of Florida, Department of Infectious Disease and Immunology

Purpose: P.UF1 (a novel probiotic strain of Propionibacterium freudenreichii), isolated from breastfed human preterm infants, is being studied in mice to determine its potential as a lifesaving probiotic for infants fed formula. We seek to mechanistically understand P.UF1 cross talk with the immune system and gut microbiota in vivo by inserting an anaerobic fluorescent gene into the P.UF1 genome regulated by an inducible promoter system.

Methods: The PpFbFP gene (FMN based anaerobic fluorescence gene) was transformed into P. UF1 via being cloned into P. UF1-E. coli shutter vector pYMZ, generating plasmid pYMZ-P4-PpFbFP which expressed His6-tagged PpFbFP gene under control of P4 promoter.

Results: P.UF1 was found to produce propionate, eliciting a decrease in inflammatory cytokines, sustaining regulatory T cells (Treg cells), and markedly mitigating necrotizing enterocolitis (NEC) in neonatal mice. We developed a method to label P. UF1 bacterium by incorporating fluorescence gene, FMN-based fluorescence gene (FbFP). Expression of PpFbFP fluorescent gene was confirmed by qRT-PCR and Western blot. Microscopy indicated that P. UF1 were successfully labeled but only 5-10% of them had bright fluorescence.

Discussion/Conclusion: To gain insight on the function of the bacterium, the next step is to develop an inducible system for P. UF1 by using FbFP as a reporter gene. I am currently working on applying the tetO/TetR anhydrous Tetracycline inducible system to P.UF1 be able to control fluorescence and eventually propionate production in vivo. My project focuses on locating and then modifying the P4 promoter in order to place tetO. Mutations in putative -35 and -10 promoter regions are being performed to accurately locate the promoter region. Our lab's plans include Phase I clinical trials to assess the ability of P.UF1 to improve the immune responses of human infants to NEC and other diseases.

HIV Strains Exhibiting High Replicative Capacity are Associated with Increased Viral Evolution in BLT Humanized Mice

Kimberly Herard, B.S.*; Karen Power*; Meredith Phelps*; Katharine Krupp*; Abigail Bisesi*; Melissa Pack*; Daniel T. Claiborne*, PhD; Vladimir Vrbanac+; Andrew Tager+; Christian L. Boutwell* PhD; Eric Hunter- PhD; Todd M. Allen* PhD

*Ragon Institute of MGH, MIT and Harvard, Boston, USA. +Center for Immunology and Inflammatory Diseases, MGH, Boston, USA, -Emory Vaccine Center, Emory University, Atlanta, GA, USA.

The pathogenesis of HIV infection has been characterized by an increase in cellular immune activation along with a gradual loss of CD4+ T cells leading to overt immunodeficiency. Investigations into viral characteristics of human HIV infections have shown that increased viral replicative capacity (vRC) has an impact on HIV disease progression and leads to increased immune activation and CD4+ T cell loss. Importantly, these associations are independent of plasma viral load. Despite these findings, the mechanism attributable to the association between vRC and accelerated pathogenesis has not been fully defined. Bone marrow-liver-thymus (BLT) humanized mice serve as a potential small animal model to investigate this. They generate functional immune responses restricted by human MHC and are able to drive escape in relevant viral epitopes. To assess the role of viral replicative capacity on in vivo viral evolution we infected BLT mice with HIV strains that have large differences in vRC. We hypothesize that high vRC will be associated with accelerated viral evolution, which may lead to rapid escape from immune responses and increased cellular immune activation and CD4+ T cell loss. To test this hypothesis, 5 groups of 4 BLT mice each were infected with HIV isolates known to have dramatically different in vitro vRC. Viral RNA was isolated from plasma at 3, 6, 12, and 21 weeks post-infection, and used to amplify whole HIV genomes through a nested PCR approach. Amplicons were barcoded and sequenced on an Illumina Miseq to assess viral diversity. We observed de novo escape in autologous HLA-restricted epitopes as well as reversion to consensus. Furthermore, our results demonstrate that high vRC is associated with more rapid viral evolution at early time points. These results support the BLT model for assessing HIV evolution and implicate vRC as a determinant of the kinetics of viral diversification.

Does Oxidative Stress Affect Susceptibility to Bacillus anthracis surrogates?

Jelonia Rumph+, Lou Ann Brown, Phd^

+Florida Memorial University, ^Emory University Children's Center

Purpose: Anthrax is an infection caused by Bacillus anthracis. The clinical forms include: cutaneous, ingestion, injection and inhalation. There have been 24 documented cases of inhalation anthrax in the United States since the mid-20th century. 15 of 24 patients died; 10 of 15 fatalities but only one of nine survivors had an underlying chronic disease associated with oxidative stress. During inhalation anthrax, alveolar macrophages (AM) coordinate the immune responses. However, recent studies have shown that AM phagocytic index (PI), an indicator of microbe clearance, is decreased when they are exposed to oxidative stress. We investigated whether oxidative stress impairs the PI of alveolar macrophages for a Bacillus anthracis surrogate-Bacillus cereus. We hypothesized: ethanol-exposed AM will have a significantly decreased PI compared to control AM. Treatment of ethanol-exposed AM with arginine improves PI.

Methods/Results: Using alcohol abuse as a model of oxidant stress, MH-S cell lines were cultured for 3 days in MH-S media, MH-S media/ethanol, MH-S media/arginine, or MH-S media/ethanol/arginine. Samples were exposed to Bacillus cereus spores and the PI was calculated and statistically analyzed. There was a significant difference between the MH-S media and the MH-S media/ethanol group. The P value calculated from the difference between these groups was 0.036.

Conclusion: Oxidative stress decreases the PI of AM, and could possibly increase susceptibility to Bacillus cereus and Bacillus anthracis. Arginine treatment provides a supply of precursors for antioxidant synthesis and improves the PI of oxidatively stressed AM.

Characterization and visualization of MrgprC11+ neurons

Haley R. Steele*, Yuyan Zhu*, PhD., Claire E. Hanson*, Liang Han*, PhD

*Department of Biology, Georgia Institute of Technology, Atlanta, Georgia.

Purpose: Pruritus (chronic itch) is the development of debilitating itching symptoms that arise from a variety of medical conditions and medication, resulting in pain, distress, scaring, and infection. Previously, Mass-Related G-Protein Receptors (Mrgprs) have been found to mediate itch. However the MrgprC11 family, which functions similar to the human orthologue MrgprX1, has not yet been characterized. Therefore, the objective of this study is the molecular and morphological characterization and visualization of MrgprC11+ neurons within the Dorsal Root Ganglia (DRG).

Methods: We first characterized the MrgprC11+ neuronal population by performing immune-histochemical staining on WT mice DRG to co-label MrgprC11+ neurons with the neuronal markers NF-200, TRPV1, subP, IB4, and CGRP. Furthermore, we stained the DRG of our MrgprA3-Tomato mice with an MrgprC11 antibody to quantify the relationship between these neuronal sub-populations. Next, we established a genetically inducible MrgprC11-Cre-ER-PLAP line, which we used to perform placental alkaline phosphatase (PLAP) staining with sparse labelling to visualize the single sensory arborization of MrgprC11+ neurons in the spinal cord and hairy skin.

Results: MrgprC11+ neurons make up approximately 17% of the total neuronal population of the WT DRG. Additionally, MrgprC11+ does not co-stain with NF-200 and does co-stains with small diameter, c-fiber neuron markers CGRP, IB4, and subP, and with the pain-receptor TRPV1. Furthermore, nearly all MrprA3+ neurons are found within the MrgprC11+ population (93% co-labeling). PLAP staining of the dorsal spinal cord reveals typical central innervation, while the arborization reveals that the MrgprC11+ neurons are primarily free nerve endings.

Discussion: As the MrgprA3+ neurons account for 6-7% of the neuronal population of the DRG and MrgprC11+ neurons account for 17%, this indicates that ~10% of the MrgprC11+ may have unique function separate from the MrgprA3+ neurons. In conclusion, the characterization and visualization of MrgprC11+ neurons offers important insights for future developments in anti-pruritic therapies.

Assessment of Combinational Metal Induced Neurotoxicity In Vitro

Jasmin A. Eatman, Kennie R. Shepherd, Ph.D.

Department of Pharmacology & Toxicology, Neuroscience Institute, Morehouse School of Medicine, Atlanta, Georgia

Purpose: The purpose of this study is to understand the threat of combinational toxicity, in addition to identifying the molecular basis of neurotoxicity as a result of cross exposure to two environmental contaminants, Lead (Pb) and Tellurium (Te).

Methods: We hypothesized that Te would exacerbate Pb induced neurotoxicity. To test this hypothesis, we 1) quantified the production of reactive oxidative species (ROS) in differentiated human neuroblastoma (SH-SY5Y) cells after exposure to Pb and Te, 2) assessed the effects of Pb and Te on mitochondrial function/cell viability, and 3) determined the presence of cell death cascades through immunohistochemistry assays using cleaved caspase-3 antibody.

Results: The results show that Pb significantly increased ROS levels after 24 hours; Te also increased ROS levels after this time period. Pb at 1uM and 10 uM significantly decreased mitochondrial function/cell viability after 48hrs. The reduction in mitochondrial function/cell viability was associated with increased labeling of caspase-3 in SH-SY5Y cells, further demonstrating the cytotoxic effects of Pb. In addition, 10 uM Te caused labeling of caspase-3 in SH-SY5Y cells, suggesting that this dose caused injury/cytotoxicity. Combined effects on mitochondrial functioning/cell viability and caspase-3 labeling was also assessed.

Discussion/Conclusion: Combinational toxicity is the aggregate effect of multiple environmental contaminants, including metals, insecticides, and pesticides, on living systems. Many recent studies have demonstrated that metals cause degeneration of the central nervous system, however, the mechanisms underlying toxicity resulting from the effect of multiple metal types, or metals in conjunction with other classes of environmental contaminants, has yet to be identified. Taken together, the findings from the present study suggest that the presence of trace elements such as Te may exacerbate Pb induced neurotoxicity.

Feasibility Study: fMRI Evaluation of Auricular Percutaneous Electrical Neural Field Stimulation for Fibromyalgia

Melat Gebre, BS, Anna Woodbury, MD, *Vitaly Napadow, PhD, Venkatagiri Krishnamurthy, PhD, Lisa Krishnamurthy, PhD, +Roman Sniecinski, MD, Bruce Crosson, PhD

Center for Visual and Neurocognitive Rehabilitation, Atlanta Veterans Affairs Medical Center, Atlanta, GA; *Martinos Imaging Center, Massachusetts General Hospital, Harvard University, Boston, Massachusetts, +Division of Cardiothoracic Anesthesiology, Emory University School of Medicine, Atlanta, GA

Purpose: Current therapies for fibromyalgia often fall short and in light of the opioid epidemic, a better treatment option is needed. Percutaneous electrical neural field stimulation (PENFS) is a non-pharmacologic therapy that is utilized within the military/VA system, but sufficient evidence regarding its outcomes and neural mechanisms have not been adequately investigated. Auricular PENFS has not been studied with functional magnetic resonance imaging (fMRI). Understanding the underlying neural mechanisms of auricular PENFS could assist in developing targeted treatments for fibromyalgia and lead to improvements in quality of life and cost-savings.

Methods: 20 veterans from the Atlanta VA with fibromyalgia will be randomized into 2 groups: 10 control subjects (standard therapy) and 10 PENFS subjects. Standard therapy will include treatments such as anticonvulsants, NSAIDs, and physical therapy; all individualized to patient co-morbidities. The PENFS group, in addition to standard therapy, will have the Neuro-Stim System (NSS) placed on the ear for 5 days then removed and replaced once per week for 4 weeks. MRI studies will occur within 2 weeks prior to initiation of treatment and repeated within 2 weeks after the final treatment. The bio-behavioral assessments will occur once before treatment and repeated at 2, 8, and 12 weeks after the final treatment.

Results: Since June 2017, we have recruited and conducted bio-behavioral assessments for 13 patients and have conducted 4 pre-treatment MRIs. The primary outcome will be resting fMRI connectivity between the default mode network (DMN) and insula, which will also be correlated with patient-reported analgesic improvements and functional improvements.

Conclusion: This feasibility study is meant to demonstrate the practicality of using fMRI to study neural correlates of PENFS outcomes and provide power calculations in order to design a larger randomized controlled clinical trial to determine the efficacy of PENFS with regards to subjective pain measurements and functional improvements.

Characterizing the Consequences of DEPDC5 Deficiency in Neurons and Astrocytes Generated from Patient-Derived Induced Pluripotent Stem Cells

Lindsay K. Klofas (a), Robert S. Carson (a,b)

(a) Department of Neuroscience, Vanderbilt University, Nashville, Tennessee; (b) Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, Tennessee

PURPOSE: Pathogenic mutations in DEP domain-containing protein 5 (DEPDC5) have recently been identified as a significant cause of focal epilepsies. DEPDC5 is a component of the GATOR1 complex, a protein complex that inhibits mTORC1 signaling in conditions of low nutrient availability to enable autophagy. Loss of function mutations in DEPDC5 lead to hyperactivation of mTORC1 and defective autophagy, however, the mechanistic link between DEPDC5 mutations, impaired autophagy, and epileptogenesis has yet to be defined. We hypothesize that impaired autophagy during neuronal development causes abnormal neuronal function and cortical structure, resulting in a seizure-prone brain.

METHODS: We created a model using neurons generated from patient-derived induced pluripotent stem cells (iPSCs) to investigate the connection between DEPDC5 deficiency, defects in autophagy, and epilepsy. iPSCs were created using Sendai reprogramming of fibroblasts provided by a patient with familial focal epilepsy and a confirmed DEPDC5 mutation. iPSCs were differentiated into neurons using small molecule SMAD inhibitors and FGF2 supplementation.

RESULTS: Patient-derived iPSCs retained the DEPDC5 mutation, expressed markers of pluripotency, and differentiated into all three developmental lineages. Mature neurons generated from iPSCs expressed pan-neuronal markers MAP2, beta-tubulin III, and synapsin I, as well as markers of excitatory neurons, Homer1 and VGluT1. GFAP-expressing astrocytes were identified after post-induction day 100. DEPDC5-mutant neurons exhibited altered neurite morphology.

DISCUSSION/CONCLUSION: Using patient-derived iPSCs, we created excitatory neurons and astrocytes with a truncation mutation in DEPDC5. Neurons expressed markers of excitatory neurons while astrocytes expressed GFAP. We will use this model to investigate the differences in development, morphology, and cell signaling between wild-type and DEPDC5-mutant neurons and astrocytes. Improving our understanding of how DEPDC5 haploinsufficiency contributes to epileptogenesis may suggest new drug targets and provide fundamental knowledge about the pathogenic mechanisms driving the development of epilepsy.

Novel PPAR-gamma agonist improve pathology and memory deficits in a 3xTg-Ad mouse model of Alzheimer's disease.

Manoj Govindarajulu, Tyler Lynd, Jenna Bloemer, Danielle Adamek, Gwyneth Briggs, Muralikrishnan Dhanasekaran, Miranda Reed, Raj Amin and Vishnu Suppiramaniam

Epidemiological and research evidence suggest a possible shared pathophysiology between type 2 diabetes mellitus (T2DM) and Alzheimer's disease (AD) thus establishing the disease as a form of 'type 3 diabetes'.Thiazolidinediones (TZDs) are insulin sensitizing peroxisomal proliferator activating receptor gamma (PPARγ) agonists and have been recognized as promising agents for memory deficits in patients with AD. Although currently available PPARγ agonists show promise for improving memory deficits in AD, poor blood brain barrier permeability results in inadequate bio-availabilit yin the brain requiring high dosing with chronic time frames that are associated with increased incidences of adverse cardiovascular events. Therefore we have developed novel selective PPARγ modulators with high blood brain barrier permeability and less unwanted deleterious effects. We hypothesize that our lead compound(Compound 9) a PPARγ modulator, improves cognitive deficits and pathologies associated from Alzheimer's disease better than current TZDs (pioglitazone) in a triple transgenic 3x Tg-AD mouse model. Triple transgenic 3xTg-AD and C57BL/6J mice were utilized. Two month aged mice representing mild to moderate AD were treated with either Compound 9 or Pioglitazone until six months of age. Six month age group represents advanced stage of AD and the mice were treated for 4 weeks. Behavioral analysis was done using novel-object recognition, Y-maze and contextual fear conditioning tests. Long-term potentiation (LTP) theta-burst protocol was utilized to measure hippocampal field potentials in Schaffer collateral pathway in the hippocampus. Our initial data indicate that Compound 9 decreases Beta amyloid levels and reduced Beta secretase activity when compared to Pioglitazone in an in vitro model. Y maze, novel-object recognition and contextual fear conditioning showed improvement in cognitive deficits. In addition these mice restored memory deficits in transgenic mice similar to control group in electrophysiological studies. Further work by biochemical and electrophysiological evaluation will determine and validate the nature of synaptic deficits and pharmacokinetic studies to test the brain bioavailability of compound 9 compared to pioglitazone.

The Neuroprotective Role of G-Protein Coupled Receptor GPR37 in Ischemic Brain Injury

Myles R. McCrary*, Michael Q. Jiang*, Xin Huang*, Xiaohuan Gu*, Michelle M. Giddens+, Randy A. Hall+, Shan Ping Yu*, and Ling Wei*

*Department of Anesthesiology, Emory University, Atlanta, GA; +Department of Pharmacology, Emory University, Atlanta, GA.

Purpose: Stroke is a leading cause of mortality and morbidity worldwide and there are few effective treatments. There is a desperate need to better understand the pathogenesis of ischemic stroke. Previous work has demonstrated that the G-protein coupled receptor GPR37 is a receptor for the neuroprotective factor prosaposin. However, the mechanisms by which GPR37 elicits protection from ischemia is unknown.

Methods: We induced focal ischemia directed to the right sensorimotor cortex in adult wild-type (WT) and GPR37-knockout (GPR37-KO) mice by surgically occluding distal branches of the right middle cerebral artery and temporarily blocking the common carotid arteries. Sticky dot and rotarod tests were used to measure sensorimotor function. Animals were sacrificed at various timepoints after stroke, and brain tissue was collected for Western blotting, RT-PCR, and immunohistochemical studies. Tetrazolium chloride (TTC) staining was used to measure infarct size.

Results: Our findings indicate that GPR37-KO animals incur significantly enlarged infarct volumes and suffer greater sensorimotor behavior deficits compared to WT controls. Concordant with these data, GPR37-KO mice exhibit elevated cell death and autophagy markers such as Cleaved caspase 3, Beclin-1, and LC3-II. Moreover, these effects are likely mediated by the repression of phosphorylated m-TOR, a critical regulator in the cellular response to hypoxia. Astrocyte proliferation and migration to the stroke region were reduced, suggesting impaired reactive astrocytosis or astrogliosis. The GPR37-KO mice also show increased inflammatory markers and enhanced microglial activation after stroke.

Discussion/Conclusion: GPR37 deficient mice suffer numerous dysfunctional responses to focal cortical ischemia. Our work illustrates a novel and crucial role of GPR37 in regulating neuronal autophagy and apoptosis via mTOR signaling. These results identify GPR37 as a critical player in the modulation of brain damage.

Sirt3 activator Honokiol attenuates Amyloid Beta in an in vitro model of Alzheimer's disease

Sindhu Ramesh, Manoj Govindarajulu, Tyler Lynd, Gwyneth Briggs, Danielle Adamek, Ellery Jones, Jake Heiner, Mohammed Majrashi, Rajesh Amin, Vishnu Suppiramaniam, Muralikrishnan Dhanasekaran

Modern evidence suggests the critical role of Sirtuin 3 (Sirt3)- a mitochondrial protein in the progression of several metabolic diseases, but the role of Sirt3 in neuronal protection remains mostly undefined. Honokiol, a 3activator has been shown to exhibit antioxidant and neuroprotective effects in several experimental models. Amyloid beta (Aβ), the precursor to extracellular senile plaques, collects in the brains of patients with Alzheimer's disease and is related to the development of cognitive impairment and neuronal cell death. The purpose of this study was to determine the neuroprotective and Amyloid beta ameliorating effects of Honokiol- a Sirt3 activator by preserving mitochondrial function in an in vitro model. Chinese Hamster Ovarian (CHO) cells, carrying the APPse and Presenilin 1 mutation, and inheritably secreting high Aβ were treated with these different concentrations of Honokiol. Mitochondrial assays, reactive oxygen species, antioxidant levels and signaling mechanisms were evaluated and established respectively for decreasing Aβ levels. Honokiol enhanced Sirt3 expression, thereby reducing ROS and lipid peroxidation, improving antioxidant enzymatic activity, and mitochondrial function. Moreover, Honokiol treatment increased the expression of AMPK, CREB, and PGC-1a, thereby reducing β-ecretase enzyme which in turn reduced Aβ levels. These results suggest that Honokiol is an activator of Sirt3 capable of improving antioxidant activity, mitochondrial energy regulation, while decreasing Aβ, thereby offering neuroprotective response.

Design, Synthesis, and Structural Identification of Hypochlorite-Oxidized Cysteinyl Dopamine (HOCD)- Implications in Parkinson's Pathogenesis

Gijong Paik, David Njus, Ph.D.

Department of Biological Sciences, Wayne State University, Detroit, MI

Purpose: Dopamine is known to undergo nonenzymatic autoxidation at physiological pH where it can be further metabolized into various metabolites in the brain. One of the metabolites, 5-S cysteinyl dopamine (5SCD), has garnered significant attention due to its cytotoxicity and discovery in dopaminergic neurons in the brain. It is unclear, however, as to how 5SCD is neurotoxic to dopaminergic cells. We propose that 5SCD becomes oxidized by endogenously available hypochlorite to form hypochlorite oxidized cysteinyl dopamine (HOCD), which has significant redox cycling activity. The redox cycling capability of HOCD may elucidate an important link between oxidative stress and Parkinson's disease. Previous studies by the Njus lab has demonstrated that HOCD is more cytotoxic to catecholaminergic PC12 cells at lower concentrations and at faster rates compared to 5SCD. The purpose of this study was to synthesize, extract and purify HOCD in order to identify its chemical structure and properties.

Methods/Results: In order to help elucidate the structure and redox cycling activity of HOCD, a structural analog was chemically synthesized in vitro using 3-methylcatechol and cysteamine to form a benzothiazine derivative (BZT). HOCD was chemically synthesized in vitro using dopamine and L-cysteine, to create 5SCD which was then treated with hypochlorite. Redox cycling activity was assayed by measuring oxygen consumption using a Clark type oxygen electrode. BZT showed strong redox cycling activity and its structure and reduction potential was determined through proton nuclear magnetic resonance (H1NMR) and cyclic voltammetry. The results from BZT are being used to progress efforts in structural identification and redox activity of HOCD.

Discussion/Conclusion: The knowledge gained in this project will be crucial for future studies in pathophysiological mechanisms involving HOCD, its identification in post-mortem analyses of patients, and a potential answer to the molecular basis for the motor symptoms presented in Parkinson's Disease.

NMDA Receptors Are Required for Sensory Driven Neural Activation

Samuel Parkins*, Gabriela Rodriguez+, Hey-Kyoung Lee+, PhD

*Department of Biology, Oakwood University, Huntsville, AL; +Department of Neuroscience, Johns Hopkins University.

The brain is able to adjust to changes in sensory experience by modifying synaptic strength in the primary sensory cortices. In the visual system, dark exposure results in the strengthening of synapses, while light re-exposure leads to weakening of the same inputs. Although NMDA receptors (NMDAR) have been found to play a critical role in this type of synaptic plasticity, their involvement in neural activation due to sensory input is yet to be established. To investigate this question, two studies were conducted. In the first, NMDAR activity was first blocked with the antagonist CPP and in the second, NMDAR was knocked out with the AAV9-CAMKII-CreGFP virus. Subsequently, neuronal activity was assessed by the histological analysis of the expression levels of the immediate early gene c-fos. Concurrently, visual experience was manipulated by placing the animals in three different experimental conditions: standard light-dark conditions, dark exposed and dark exposed with thirty-minute light re-exposure. The results show a significant reduction in c-fos expression levels when NMDAR are blocked or knocked out specifically following the light re-exposure condition indicating NMDARs are required for visually driven neural activation.

Using Microfluidic Electrophysiology to Assess Clozapine Sensitivity In Natural Isolates of C. elegans.

Carey. S. Jansen, Kevin R. Melnick, Kyu Kim MD, Amir I. Khan, Jennifer W. Carlisle MD, Farres Hosseinzadeh, Nataliya Prokhnevska, Viraj A. Master MD PhD, Haydn T. Kissick PhD

Clozapine is one of the most effective antipsychotic drug used in treating schizophrenic patients. However, clozapine's efficacy is compromised by a litany of severe side effects (agranulocytosis, myocarditis…). Clozapine affects multiple aminergic systems (DA, 5-HT, Histamine), but many of its mechanisms of action remain unknown. We plan to find genetic loci involved in clozapine sensitivity by comparing the response to clozapine of genetically distinct natural isolates of C. elegans. We recorded the pharyngeal pumping of 7 distinct natural isolates (N2, CB4856, JT11398, JU775, CX11314, MY16, and LKC34) in response to clozapine using a microfluidic screen chip system. Clozapine induced a decrease in mean pumping frequency across all but one strain (LKC34) and an increase in mean pump duration in all but one strain (LKC34). Different strains also expressed variable sensitivities to clozapine. It was found that clozapine significantly decreased mean pumping frequency among all but one strain experimented, but the degree by which clozapine impacted pumping frequency differed based on strain. Data from the project can be used for a Genome Wide Association Study to pinpoint loci in the genome of C.elegans responsible for the effects of clozapine. These results can be used in the future to better understand how clozapine interacts with the nervous system and modify the drug to reduce its side effects and potentially develop alternatives to clozapine that maintain its efficacy while reducing its side effects.

c-Abl and PARIS (ZNF746) as a-synuclein Targets in Dopaminergic Neurodegeneration: Validation strategies using animal models of Parkinson's Disease (PD)

Stephan Quintin1,2,6, Saurav Brahmachari1,2,6, Preston Ge1,2,6, Esther Kim1,2,6, Rosa Shi1,2,6,Senthilkumar S. Karuppagounder1,2,6,Han Soek Ko1,2,7, Valina L. Dawson1,2,3,4,6, Ted M. Dawson1,3,4,5,6.

1Neuroregeneration and 2Stem Cell Programs, Institute for Cell Engineering, Departments of 3Neurology, 4Physiology, and 5Solomon H. Snyder Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205. USA.

Oxidative stress contributes to the pathogenesis of Parkinson's disease (PD) in both familial and sporadic cases. In both cases, c-Abl kinase activation is a key indicator of oxidative stress. In a study of familial PD, it has been shown that c-Abl activation resulting from a mutant (A53T) a-synuclein-related oxidative stress, critically regulates a-synuclein toxicity. c-Abl mediated inactivation of parkin and accumulation of parkin substrates have been suggested in PD. In this study, we validate the role of c-Abl in the accumulation of parkin substrates PARIS and AIMP2 through the use of conditional TetP-humanA53T transgenic model of PD. In a sporadic PD model study, the transmission of pathological a-synuclein has been shown to be important in the progression of PD pathogenesis. To induce pre-formed fibril (PFF)-based model of a-synuclein transmission, mice were Injected intrastriatal with PFF. This study a confirmed accumulation of PARIS in the a-syn-PFF transmission model.

Prospective validation of a rapid bone density screening method that is applicable to routine abdominal CT images.

Elliot T. Varney BS*, David Joyner MD*, Ellen E. Parker MD*, Todd S. Nichols MD*, Majid Khan MD*, Andrew Smith MD/PhD+

*Department of Radiology, University of Mississippi Medical Center, Jackson, MS; +Department of Radiology, University of Alabama Birmingham, Birmingham, Alabama

Purpose: To prospectively validate an opportunistic screening method utilizing color to detect abnormal bone density on abdominal CT images to improve osteoporosis screening efforts

Methods: For this IRB-approved, HIPAA-compliant study, 200 asymptomatic women >50 years of age presenting for screening mammograms were recruited. Patients underwent nonenhanced CT imaging of the abdomen. The CT images were processed with software designed to color the vertebral bodies green if bone density was normal and red if abnormal. Four radiologists were timed as they interpreted L1/L2 bone density using various methods: quantitative CT (QCT), visual assessment of grayscale (Grayscale) and colored (Color) images and measurement of vertebral attenuation (Attenuation). The mean bone density values at L1/L2 using QCT served as the reference standard. The average accuracy, sensitivity, and specificity were calculated. Inter-observer agreement was assessed using intraclass correlation coefficient (ICC).

Results: Mean attenuation at L1/L2 was highly correlated with mean bone density ($r=0.96$, $p<0.001$). The optimal mean attenuation cut point for differentiating normal from abnormal bone density was 145 HU. The average accuracy, sensitivity, and specificity were higher with the Color method (Accuracy:92, Sensitivity:92, Specificity:93) than with the Attenuation (Accuracy:88, Sensitivity:89, Specificity:89) or Grayscale method (Accuracy:66, Sensitivity:69, Specificity:64). Mean time of assessment of 2.8 seconds using the Color method was significantly faster than 6.0 seconds for the Grayscale method or 15.2 seconds for the Attenuation method ($p<0.001$). Finally, inter-observer agreement was higher with the Color method (ICC:0.90) than with the QCT method (ICC:0.82), Attenuation method (ICC:0.73), or Grayscale method (ICC:0.31).

Conclusion: Using post-processing and colorization of CT images, it is possible to accurately screen for abnormal bone density in 2 seconds, with higher inter-observer agreement than other methods. This form of opportunistic screening could be widely utilized and would have minimal impact on physician time and no additional cost or radiation exposure for patients.

The Prevalence of Klippel-Feil Syndrome in Pediatric Patients: Analysis of 831 CT Scans

Jalea T. Moses MS*, Devin M. Williams MPH**, Bonnie Mason MD***, Addisu Mesfin MD**

*Frank H. Netter MD School of Medicine New Haven, CT; **University of Rochester Medical Center, Department of Orthopaedics, Rochester, NY; ***Nth Dimensions Chicago, IL

Purpose: Klippel-Feil Syndrome (KFS) is characterized by congenital cervical fusion caused by failure of proper cervical segmentation during embryonic development. The incidence of KFS has been reported as 1 in 42000, with females being more impacted than males. Other research suggests the incidence to be as high as 0.5%. Clinically, patients with KFS may be predisposed to degenerative changes of the cervical spine including disc herniation, and cervical spondylotic myelopathy. Our objective was to evaluate the prevalence of KFS in pediatric patients.

Methods: We evaluated CT scans of the cervical spine of pediatric patients treated in the Emergency Room of the Level I Trauma Center at Strong Memorial Hospital from January 2013 to December 2015. CT scans were analyzed for KFS, and the following demographics were also collected: age, sex, race and ethnicity, along with the patients' mechanism of injury. If KFS was present, it was classified using Samartzis Classification as Type I (single level fusion), Type II (multiple, noncontiguous fused segments), or Type III (multiple, contiguous fused segments).

Results: Of the 848 cervical CTs that were taken for pediatric ER patients over the study period, 831 were included. Of these patients, 10 had KFS or a prevalence of 1.2%. According to the Samartzis classification, 9 were Type I and 1- Type III. The average age was 16.02 (10-18), with 8 males (80%) and 2 females (20%). Three had congenital fusions at vertebral levels C2-C3, two at C3-C4, three at C5-C6, 1 at C6-C7, and one with multiple levels of cervical fusion.

Conclusion: The prevalence of KFS amongst 831 pediatric patients, who received cervical CT imaging over a three-year period, was 1.2% (approximately 1 in 83). The two most common levels fused were C2-C3 and C5-C6. The prevalence of KFS is much higher than previously described, and thus warrants monitoring.

Autologous Hypertrophic Chondrocyte Grafting as an Alternative to Iliac Crest Bone Grafts in Posterolateral Spinal Fusion

Juan M. Colazo, h, j, BS; John A. Sielatycki, a, MD; Masanori Saito, a, b, MD; Masato Yuasa, a, b; Stephanie N. Moore-Lotridge, a, g, BS; Sasidhar Uppuganti, a; Alexander A. Hysong, h; Patton J. Robinette, h; Atsushi Okawa, b; Toshitaka Yoshii, b; Herbert S. Schwartz, a, MD; Jeffry S. Nyman, a ,c ,d ,e , PhD; Jonathan G. Schoenecker, a, f, g, I, MD PhD.

a Department of Orthopaedics and Rehabilitation, Vanderbilt University Medical Center 1215 21st 6 Ave. South, Suite 4200 MCE, South Tower, Nashville, TN, 37232, USA; b Department of Orthopaedic Surgery, Tokyo Medical and Dental University Yushima Bunkyo Ward, Tokyo, 113-8519; c Department of Biomedical Engineering, Vanderbilt University 10 PMB 351631, 2301 Vanderbilt Place, Nashville, TN, 37235, USA; d Center for Bone Biology, Vanderbilt University Medical Center 1235 Medical Research Building IV, 2215B Garland Avenue, Nashville, TN 37232, USA; e Department of Veterans Affairs, Tennessee Valley Health Care System F-519 VA Acre Building, 1210 24th 14 Ave. South, Nashville, TN, 37232, USA; f Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center 1161 21st 16 Ave. South, Nashville, TN, 37232, USA; g Department of Pharmacology, Vanderbilt University 18 2200 Pierce Ave, Robinson Research Building, Nashville, TN, 37232, USA; h Vanderbilt University School of Medicine 1161 21st 20 Ave S, #D3300, Nashville, TN, 37232, USA; i Department of Pediatrics, Vanderbilt University Medical Center 4202 Doctor's Office Tower, 2200 Children's Way, Nashville, TN 37232, USA; j Medical Scientist Training Program, Vanderbilt University School of Medicine 201 Light Hall, 2215 Garland Avenue, Nashville, TN 37232, USA

Purpose: Spinal fusion procedures have increased substantially over the past ten years; yet 5-35% of all spinal fusions experience complications such as pseudoarthrosis. Pseudoarthrosis can develop for multiple reasons including, but not limited to, biomechanical failure of an implant, infection of the fusion site, or biological failure of the bone. Thus, innovative surgical implants and graft advancements are being actively investigated. Here, hypertrophic chondrocytes are being investigated as a potential biological graft alternative.

Methods: Using a validated murine fracture model, hypertrophic chondrocytes were harvested from fracture calluses (FCCG) and transplanted into the posterolateral gutters of a genetically identical recipient mouse. Following implantation, posterolateral bone formation was assessed by radiographic analysis, microcomputed tomography (μCT), and histologic analysis. Results were compared with either a standard iliac crest bone graft (ICBG) or a sham surgery control group.

Results: μCT revealed that like standard iliac crest bone grafts, implantation of hypertrophic chondrocytes increased the amount of bone surrounding the posterolateral spine at 6 weeks post implantation. While, bone from iliac crest bone graft was consistent over time, hypertrophic chondrocyte implantation resulted in bone formation over time, resulting in comparable levels at 3 weeks post implantation. Finally, while this study was not designed to test spinal fusion, μCT analysis demonstrated bony union of the laminae/transverse processes as well as a longitudinal bony bridge across the fused levels. This was indicative of successful spinal fusion in a subset of mice from both the iliac crest and hypertrophic chondrocyte groups.

Discussion/Conclusion: Taken together, these results support the hypothesis that hypertrophic chondrocytes can promote bone formation at comparable rates to ICBGs. Currently, ICBG remains the gold standard for augmenting spinal fusion, but its use is hampered by donor site morbidity and limited supply. Thus, hypertrophic chondrocytes may serve as a potential graft alternative for promoting spinal fusion.

Corroboration of the Emory Filter Retrieval Assessment Score (eFRAS) in a County Hospital Population.

Kapil Chandora*, Zachary L Bercu MD+, Janice Newsome MD+, Jonathan G. Martin MD+

*Morehouse School of Medicine, Atlanta, GA +Department of Radiology and Imaging Sciences, Emory University, Atlanta, GA

Purpose: Retrievable inferior vena cava filters (rIVCF) remain within a patient for a variable length of time. The longer a filter remains, the more intimal hyperplasia incorporates the filter legs and filter hook in to the caval wall. Filter retrieval becomes more difficult, requiring more complex and riskier retrieval techniques. A prior study, in an academic hospital system, demonstrated utility of a filter retrieval scoring system to determine the need for advanced techniques in recovering rIVCFs. We seek to corroborate those findings in a different patient population, that of a large county hospital.

Methods: Four years of interventional radiology procedures at Grady Hospital were reviewed in this IRB approved retrospective study. A total of 16 cases of rIVCF retrievals were performed during this period. Procedures were analyzed for IVC filter type, fluoroscopy time, size of sheath used, and use of advanced retrieval techniques. Procedural data were compared to eFRAS score.

Results: There were 15 successful retrievals and one failure. Of 7 cases with filter dwell times of ≥8 months, 57% required advanced techniques. Of 9 cases with dwell time <8 months, 0% required advanced techniques. By eFRAS scoring: 11 cases were level 1; 2 were level 2; 0 were level 3; and 3 were level 4. Of the filters in level 1: Success rate (SR) 100%, average dwell time (ADT) 6.9 months average fluoroscopy time (AFT) 9.3 min; Level 2: SR 50%, ADT 8 months, AFT 9.1 min; Level 4: SR 100%, ADT 10 months, AFT 35.8 min

Discussion/Conclusion Our findings corroborate the eFRAS scoring system, with a significant increase in advanced techniques with increased dwell times, and increased fluoroscopy times for eFRAS level 4. This indicates the need for future research, with prospective studies to evaluate the benefit of early adoption of advanced techniques in eFRAS level 4 cases.

Pre-eclampsia Among Women with Sickle Cell Disease and HIV

Javon Prophet 1*, Kalifa Kelly 1*, Julian Domingo 1*, Helen Ayeni 1*, Xaviera Djoko 1*, Breana Dockery 1*, Farida Allam 1*, Manvir Kaur, BDS, MHA 1*, Javon Artis 1*, Kiara K. Spooner, DrPH, MPH 2, Jason L. Salemi, PhD, MPH 2, Omonike A. Olaleye, PhD 1*,Hamisu M. Salihu, MD, PhD 2.

Affiliations 1* Texas Southern University, 3100 Cleburne Street, Houston, TX, 77004. 2* Baylor College of Medicine, Department of Family & Community Medicine, 3701 Kirby Drive, Suit 600, Houston, TX 77098

The relationship between sickle cell disease (SCD) and severe pre-eclampsia is poorly established. It is also unknown whether the occurrence of HIV infection among women with SCD modifies their risk level for severe pre-eclampsia. We hypothesized that: (1) pregnant women with SCD are at an elevated risk for severe pre-eclampsia as a result of heightened endothelial damage; and (2) the combination of SCD-HIV augments the inflammatory processes of endothelial damage leading to amplified risk for severe preeclampsia. We analyzed more than 57 million pregnancy-related hospitalizations and births in the United States from January 1, 2002 through December 31, 2014. We applied multivariable survey logistic regression to generate odds ratios for the association between SCD, HIV and SCD-HIV status and severe pre-eclampsia with adjustment for potential confounders. Of the total 57,326,459 pregnant women, 57,198,505 (99.78%) did not have SCD or HIV, 73,064 (12.7 per 10,000) had HIV only, 54,890 (9.58 per 10,000) had SCD only and 222 (0.39 per 100,000) had both SCD and HIV. Mothers with SCD and HIVSCD experienced a significant elevation in risk for severe pre-eclampsia of about 60% (OR = 1.61; 95% CI = 1.44, 1.79) and of more than 300% (OR = 4.28; 95% CI = 1.35, 13.62) respectively. In the largest study on SCD and pre-eclampsia in the world, we established SCD to be strongly associated with severe pre-eclampsia. Another unique finding reported for the first time is the synergistic effect of amplified risk for severe pre-eclampsia among mothers with the combined SCD-HIV status.

Identifying Personalized Anti-MHC Class II Antibody Targets for Xenotransplant Recipients

Joseph M. Ladowski, MS, Greg Martens, MD, Luz Reyes, PhD, Zheng-Yu Wang, MD, PhD, Matthew Tector, PhD, A. Joseph Tector, MD, PhD

Xenotransplant Program, University of Alabama-Birmingham, Birmingham, Alabama

Purpose: Xenotransplantation, using organs from genetically-modified pigs, is a potential solution to the organ shortage. Antibody binding to carbohydrates on the pig cell surface was a previous barrier to clinical application, but the elimination of the aGal, Neu5Gc, and Sda glycan antigens provided a negative crossmatch for ~60% of individuals tested. Some crossmatch positive individuals possess anti-human leukocyte antigen (HLA) antibodies that bind the swine leukocyte antigen (SLA). Identification and mutation of these antibody binding sites would provide personalized crossmatch negative organs for these individuals, expanding the pool of potential xenograft recipients.

Methods: SLA class II single antigen cells were developed through calcium phosphate transfection of the heavy chains in the pBUDCE4.1 plasmid into HEK293T cells. 64 individual sera samples from the UAB transplant waitlist were screened via a flow cytometry crossmatch. 13 sera samples were bound to a SLA-DQ cell line, eluted with a low pH buffer, and the SLA reactive antibodies were tested on a panel of single antigen HLA class II beads.

Results: The screen revealed specific antibody binding to each of the cell lines, with the most immunogenic cell expressing SLA-DQA*0101-DQB1*0601 (19 out of 64 samples binding, 29.69%). Of the 13 elution samples, 5 demonstrated epitope-restricted HLA crossreactivity: 4 bound HLA class II beads corresponding to DQ4,5,6 and 1 bound HLA class II beads corresponding to DQ6,7,8,9 cells. Sequence comparison of the SLA class II sequences to all known HLA class II epitopes revealed a conserved arginine in HLA-DQ4,5,6 at the 55th position, and a conserved threonine at the 71st position of DQ6,7,8,9.

Conclusions: SLA-DQ may be a target of cross-reactive antibodies, specifically for individuals with a sensitization to HLA-DQ. The target of these antibodies, 55Arg and 71Thr, is a potential genetic engineering target that could result in personalized pig organs for the highly sensitized.

Aspirin Protects Heart Against Ischemia-Reperfusion Injury via LKB1-Sestrn2-AMPK Signaling Cascade

Shaoxin Lu, MS3, Yanping Bi, MD, Ji Li PhD

Department of Cardiovascular Physiology and Pharmacology, University of Mississippi Medical Center, Jackson, MS

Introduction: AMP-activated protein kinase (AMPK) is a stress signaling enzyme that orchestrates the regulation of energy pathways. Intrinsic AMPK activation protects the heart against ischemic injury, but whether the pharmacologic AMPK stimulation by aspirin mitigates ischemia-reperfusion (I/R) damage is unknown.

Hypothesis: Aspirin as an emerging AMPK agonist could stimulate the cardiac AMPK signaling pathway that attenuates myocardial ischemia-reperfusion injury.

Methods: The cardioprotective activity of aspirin was evaluated in an in vivo regional I/R (45 min/24 hours) injury model in which the left anterior descending coronary artery (LAD) was occluded and released. The Langendroff perfused heart system was used to approach an ex vivo global ischemia and reperfusion model.

Results: Isolated mouse hearts ex vivo pre-treated with aspirin had better recovery of left ventricular contractile function (55% vs. 29% of baseline heart rate-pressure product; $p<0.05$) and less myocardial necrosis (56% reduction in infarct size; $p<0.01$) during post-ischemic reperfusion. Pre-treatment with aspirin in vivo attenuated myocardial infarction in C57BL/6J mice undergoing left coronary artery occlusion and reperfusion compared to vehicle (36% vs. 18%, $p<0.05$). Mouse hearts with genetically inactivated AMPK catalytic subunit were not protected by aspirin treatment, indicating the critical role of cardiac AMPK activation by aspirin in cardioprotection against ischemic injury. Moreover, pre-treatment with aspirin in vivo increased the AMPK downstream phosphorylation and inactivation of eukaryotic elongation factor 2 (eEF2), preserved energy charge during ischemia and delayed the development of ischemic contracture. Aspirin augmented the interaction between AMPK upstream LKB1 and Sestrin2-AMPK complex, also enhanced activation of AMPK downstream endothelial nitric oxide synthase (eNOS) during ischemia, which partially attenuated myocardial stunning.

Conclusions: AMPK is a therapeutic target that can be stimulated by a direct-acting small molecule to prevent injury during I/R. The use of aspirin may represent a novel strategy to protect the heart and other solid organs against ischemia.

A direct and robust assay of G protein-coupled receptor (GPCR)-G protein coupling in cells

Najeah Okashah 1,2 Qingwen Wan Ph.D 2, and Nevin A. Lambert, Ph.D. 2

1 Augusta University MD/PhD Program, 2 Department of Pharmacology and Toxicology
Medical College of Georgia at Augusta University, Augusta, GA

Ligand activated G protein-coupled receptors (GPCRs) interact with heterotrimeric G proteins to mediate important physiological downstream pathways. Compounds that interact at GPCRs represent approximately one third of approved pharmacological agents. Approximately 44% of GPCRs are known interact with multiple G protein subtypes. However, there has been no systematic testing of secondary G protein-GPCR interactions, and there are no set criteria to define primary vs. secondary pathways. Most previous studies relied on measurements of second messenger activity to define coupling, and these measurements can be influenced by crosstalk between pathways.

Here we study GPCR-G protein coupling using a direct bio-luminescence resonance energy transfer (BRET) assay. We compared receptors with previously reported secondary coupling activity and receptors reported to have only one binding partner. We assessed coupling of each receptor to a representative panel that included a G protein from each of the four families (Gs, Gi, Gq/11, and G12/13). In order to stabilize receptor-G protein interactions cells were permeabilized with digitonin, and residual nucleotides were depleted with apyrase.

We found cases where it was possible to detect an agonist-dependent interaction between a receptor and a non-canonical G protein partner (ex. b2AR to Gq). Other interactions were less robust than expected based on prior reports (ex. a2AR to Gs). Rank order of EC50 values for the tested receptors was measured via dose response curves. Non-canonical G protein-GPCR pairs generally had higher EC 50 values (where measurable) than canonical pairings. The ability to detect non-canonical interactions reinforces the notion that sensitive assays may detect coupling events that may or may not be physiologically relevant.

Further work with this assay could increase our understanding of the mechanisms of GPCR selectivity for G proteins, ligand-dependent G protein biased signaling, and help resolve controversies of the G-protein coupling status of receptors such as the Frizzled family.

Discovery and Development of Tools to Study the Role of G Protein-gated Inwardly-rectifying Potassium (GIRK) Channels in Addiction

Krystian A. Kozek*, Emily L. Days**, Yu Du*, and C. David Weaver*

*Department of Pharmacology, Vanderbilt University, Nashville, TN; **High-throughput Screening Facility, Vanderbilt University, Nashville, TN

Purpose: Prescription opioid abuse is a nationwide epidemic. Opioids induce a rewarding release of dopamine from dopaminergic (DA) neurons in the ventral tegmental area (VTA) of the brain. These neurons express G protein-gated inwardly-rectifying potassium channels (GIRKs), and, while GIRK channels are composed of GIRK1, 2, 3, and/or 4 subunits that assemble into homo- and heterotetramers, the VTA DA neurons express only non-GIRK1-containing GIRK channels. These GIRK channels are implicated in the regulation of substance abuse. To further investigate this role of GIRKs, we sought to determine if pharmacological modulation of GIRKs in the VTA can affect drug-seeking behavior. However, non-GIRK1-subunit-containing selective GIRK channel modulators do not exist, and I aim to discover and characterize such compounds.

Methods: We screened a library of >110,000 compounds using GIRK2-overexpressing HEK293 cells in a high-throughput thallium-flux assay. Active compounds discovered from the screen were further characterized using a variety of GIRK channel overexpressing cell lines to determine channel specificity using variations of the thallium-flux assay.

Results: From among the most active compounds of the screen, we discovered that the natural product ivermectin activated GIRK2 channels. We found that ivermectin also activated GIRK1/2 and GIRK1/4 channels. The potency of ivermectin on these channels was >10 μM, and the maximum efficacy on GIRK2 channels was the greatest observed to date in thallium flux.

Conclusion: While not exhibiting the desired non-GIRK1-containing channel specificity, ivermectin will enable the pharmacological probing of DA neurons in in vitro experiments. Future studies will explore analogs of ivermectin in search of compounds that are increasingly potent, efficacious, and selectively activate non-GIRK1-containing channels. Further, active compounds will be studied using in vitro and ex vivo electrophysiology, and we will assess the efficacy of non-GIRK1-containing channel selective compounds in rodent behavioral models of craving and addiction.

CXL146: A Novel Therapeutic Agent That Selectively Targets Multidrug Resistant Leukemia

Jordy F. Botello (1,2), Tengfei Bian(2), Haifeng Sun(2), Kavitha C.(2) and Chengguo Xing(2).

(1) Department of Chemistry, University of Florida; (2) Department of Medicinal Chemistry, College of Pharmacy. University of Florida

Multidrug resistance (MDR), in which administration of chemotherapeutic agents results in cross-resistance of cancer cells to many different therapies, is a major challenge in modern treatments of cancers. There has been an urgent need for new therapies to selectively target MDR malignancies. Recently we have developed CXL146, a derivative of the dual inhibitor of Bcl-2 and SERCA proteins sHA 14-1, which shows selective cytotoxicity towards MDR cancer cell lines in vitro. Here we present cell-based evidence for its therapeutic potential and offer inside toward its mechanism of action. HL60/MX2 is a MDR cell line derived from HL60. Interestingly, cytotoxicity studies in the HL60 and HL60/MX2 cell lines show an increased sensitivity of HL60/MX2 towards HL60. Additionally, our calcium-based assays show that CXL146 exposure to HL60 and HL60/MX2 cell lines causes significant reduction in endoplasmic reticulum (ER) calcium content, where the effect was significantly more pronounced on HL60/MX2 cells. Ongoing research will address the potential of calcium regulation to CXL146's preferential cytotoxicity towards MDR cancer cells. Taken together, our study has led to the discovery of a novel therapeutic agent that selectively targets drug-resistant cancer cells with the potential to treat drug-resistant cancers.

A Little to the Left: Association between Trypophobic Image Characteristics and Discomfort Levels

O'lisa Yaa Waithe and Janelle S. Peifer, Ph.D.

Anxiety is a normal human emotion thought to be a response to perceived threatening stimuli that can also times be a byproduct of an extreme and often debilitating fear, commonly known as a phobia. In a study regarding threat perception and behavioral responses, it was found that the contrast of visual perception was increased, implying that during stressful situations, the magnitude of visuals is increased as well. This note of activity is further supported by findings of hyperactivity in certain areas of the brain in PTSD patients, making new connections between visual stimuli and anxiety/stress. Trypophobia, or the fear of closely arranged holes and like geometric patterns, is a fairly recent (2013) discovery and internet phenomenon. In this study, we aimed to examine the relationship between discomfort and visual (trypophobic) stimuli. The goal was to partially replicate an initial trypophobia study done in 2013, focusing on the key image manipulation properties (contrast/spatial frequency). We hoped to observe changes in image preference in accordance with level and magnitude of discomfort for a given sample of individuals. This study explores the relationship between spatial proportions and visual contrast levels against changes in discomfort. Sixty participants completed a web application where they were randomly assigned to one of two groups. Here, they selected and reported their baseline discomfort levels using a numerical scale with a either a control, or trypophobic image, after which they are presented with three different contrast levels of the same image. The results indicate a possible correlation between specific image properties and discomfort levels, but further testing with larger groups would be optimal for confirmation and more accurate analysis. The knowledge gained from this study is expected to further the understanding of the role of visual processing in discomfort levels and anxiety.

Keywords: Trypophobia, Phobia, Visual stimuli, Discomfort, Anxiety

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University of Georgia, Georgia Institute of Technology (Georgia Tech), and Mercer University.

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