OFFICIAL PROGRAM

Generously Sponsored by:

APSA
American Physician Scientists Association

JCI The Journal of Clinical Investigation
www.jci.org

JCI insight
insight.jci.org

EMORY UNIVERSITY SCHOOL OF MEDICINE

VANDERBILT School of Medicine

MUSC MEDICAL UNIVERSITY of SOUTH CAROLINA

UAB MEDICINE
Knowledge that will change your world

MEHARRY MEDICAL COLLEGE

TABLE OF CONTENTS

SEMSS encourages open and honest intellectual debate as part of a welcoming and inclusive atmosphere at every conference. SEMSS asks each participant to foster rigorous analysis of all science presented or discussed in a manner respectful to all conferees. To help maintain an open and respectful community of scientists, SEMSS does not tolerate illegal or inappropriate behavior at any conference site, including violations of applicable laws pertaining to sale or consumption of alcohol, destruction of property, or harassment of any kind, including sexual harassment. SEMSS condemns inappropriate or suggestive acts or comments that demean another person by reason of his or her gender, gender identity or expression, race, religion, ethnicity, age or disability or that are unwelcome or offensive to other members of the community or their guests. *

*Adapted from the language of Gordon Research Conferences

SEMSS 2018 SCHEDULE

SATURDAY, NOVEMEBER 10, 2018

REGISTRATION — 12:30 – 1:00 PM

Location: Light Hall, North Lobby

WELCOME AND OPENING REMARKS — 1:00 – 1:30 PM

Speakers: Welcome from Chris Williams, Vanderbilt MSTP Director
American Physician Scientists Association
SEMSS Committee

Location: Light Hall, Room 208

KEYNOTE SPEAKER 1 — 1:30 – 2:20 PM

Speaker: Juanita Merchant, MD, PhD
University of Arizona College of Medicine

Location: Light Hall, Room 208

BREAKOUT SESSION 1 — 2:30 – 3:15 PM

- Career Speed Rounds

  Location: Light Hall, Room 410
- MD/PhD Admissions: Putting Your Best Self Forward

  Location: Eskind Biomedical Library, Room 238

POSTER SESSION 1 — 3:20 – 4:20 PM

Location: Light Hall, North Lobby

BREAKOUT SESSION 2 — 4:30 – 5:15 PM

- The Subtleties of Purposeful Networking

  Location: Light Hall, Room 202
- The Art of Making it Work: How to Create Balance as a Physician Scientist

  Location: Light Hall, 4th Floor

POSTER SESSION 2 — 5:20 – 6:20 PM

Location: Light Hall, North Lobby

KEYNOTE SPEAKER 2 // DINNER — 6:30 – 7:20 PM

Speaker: Josh Denny, MD, MS, FACMI
Vanderbilt University Medical Center

Location: Light Hall, Room 208

Dinner will be served in Light Hall, South Lobby prior to the Keynote

---

RECEPTION — 7:30 PM

Location: Light Hall, North Lobby

Dessert, alcoholic and non-alcoholic beverages will be served

SUNDAY, NOVEMBER 11, 2018

ORAL PRESENTATIONS, PART 1 — 8:00 – 9:00 AM

Location: Light Hall, 4th Floor

Continental breakfast will be served starting at 7:30am in the Light Hall, North Lobby

---

BREAKOUT SESSION 3 — 9:05 – 9:50 AM

- #SciMedTwitter: Why and How

  Location: Light Hall, Room 214
- Staying Socially Engaged as a Scientist in 2018

  Location: Light Hall, Room 202

ORAL PRESENTIONS, PART 2 — 10:00 – 11:00 AM

Location: Light Hall, 4th Floor

LIFE AS AN MD/PHD — 11:10 AM – 12:00 PM

Location: Light Hall, 4th Floor

Professional/Career Development: 419 *Media: 412* *Outreach: 410* *MD/PhD Community: 410*

KEYNOTE SPEAKER 3 // LUNCH — 12:15 – 1:00 PM

Speaker: Lorraine B. Ware, MD
Vanderbilt University Medical Center

Location: Light Hall, Room 208

Lunch buffet will be set up in the Light Hall, North Lobby

AWARDS AND CONCLUDING REMARKS — 1:00 PM

Location: Light Hall, Room 208

KEYNOTE SPEAKERS

DR. JUANITA MERCHANT, MD, PHD – UNIVERSITY OF ARIZONA COLLEGE OF MEDICINE

Juanita Merchant, M.D., Ph.D., is the Chief of Gastroenterology and Professor of Cancer Biology at the University of Arizona Cancer Center. Dr. Merchant has made paradigm-shifting contributions to our understanding of the gastric response to chronic inflammation.

Dr. Merchant grew up in Los Angeles and attended Stanford for her B.S. in biology. She then set her sights on medical school and discovered how much she loved working in the laboratory. One of her early mentors offered some life-changing advice by recommending that she get an M.D. *and* a Ph.D. degree. She did just that by enrolling in Yale University where she received both her M.D. and Ph.D. degrees. She completed her internal medicine residency and a research fellowship in gastroenterology at the Massachusetts General Hospital in Boston and her clinical fellowship in gastroenterology at UCLA. She was then recruited to the University of Michigan where she developed her research in transcriptional control mechanisms in the gastrointestinal tract. She has recently moved to the University of Arizona.

Dr. Merchant is a reviewer for multiple journals and has served on the editorial boards of *Gastroenterology*, *American Journal of Physiology—GI*, *Journal of Clinical Investigation*, *Digestive Disease Sciences* and *Physiological Reviews*. She currently serves on several national advisory boards including the Robert Wood Johnson-sponsored Harold Amos Medical Faculty Development Program, where she was a fellow; the advisory boards for NIH-sponsored P30 Digestive Disease Centers for UCLA/CURE, Washington and Vanderbilt Universities. She was one of the inaugural members of the Council of Councils, which directly advises the current NIH director, Dr. Francis Collins, on future trends for NIH.

In addition, during the course of her education and throughout her faculty tenure, Dr. Merchant has received numerous prestigious awards and honors. She is a member of nine professional associations. She was a 2008 inductee into the Institute of Medicine of the National Academy of Sciences. As Associate Director of the University of Michigan Medical Scientist Training (MD-PhD) Program, she has developed a clinical preceptorship for MSTP students and a summer pre-MSTP experience for under-represented groups interested in pursuing both M.D. and Ph.D. degrees in the biomedical fields.

Dr. Merchant's primary research interests include transcriptional control mechanisms regulating cell growth and differentiation and microbial-host interactions in the upper GI tract. She has authored or co-authored over 90 peer-reviewed research publications and is the editor or co-editor of two books and several book chapters. Several press releases on her work related to the role of gastritis in the development of ulcers and gastric cancer have been published nationally.

DR. JOSHUA DENNY, MD, MS, FACMI – VANDERBILT UNIVERISTY MEDICAL CENTER

Joshua Denny, MD, MS, FACMI, is a Professor of Biomedical Informatics and Medicine, Director of the Center for Precision Medicine, and Vice President for Personalized Medicine at Vanderbilt University Medical Center. He completed an internal medicine residency as a Tinsley Harrison Scholar at Vanderbilt. His research interests include natural language processing, accurate phenotype identification from electronic medical record data, and using the electronic medical record to discover genome-phenome associations to better understand disease and drug response, including the development of the EMR-based phenome-wide association (PheWAS).

At Vanderbilt, Dr. Denny is part of the PREDICT (Pharmacogenomic Resource for Enhanced Decisions in Care and Treatment) program, which prospectively genotypes patients to tailor drug response. He is PI of the Data and Research Center of the All of Us Research Program (previously called the Precision Medicine Initiative Cohort Program), which will eventually enroll at least 1 million Americans in an effort to understand the genetic, environmental, and behavioral factors that influence human health and disease. He is also PI for Vanderbilt sites in the Electronic Medical Records and Genomics (eMERGE) Network, Pharmacogenomics Research Network (PGRN), and the Implementing Genomics Into Practice (IGNITE) Network.

Dr. Denny received the Homer Warner award from the American Medical Informatics Association (AMIA) in 2008 and 2009. He received the AMIA New Investigator Award in 2012 and was elected into the American College of Medical Informatics in 2013. He is a member of the National Academy of Medicine, a fellow in the American College of Medical Informatics, and a diplomat of the American Board of Internal Medicine. He serves on several local committees and remains active in teaching medical students and clinical roles.

DR. LORRAINE B WARE, MD – VANDERBILT UNIVERSITY MEDICAL CENTER

Lorraine Ware, MD, received her undergraduate degree from Claremont McKenna College in 1988 and her MD from Johns Hopkins University in 1992. She trained in Internal Medicine at Johns Hopkins Hospital from 1992-1995. She completed a Pulmonary and Critical Care Fellowship at the University of California - San Francisco in 1999 and a postdoctoral research fellowship in the Cardiovascular Research Institute at the University of California - San Francisco in 2001. She joined the faculty in Allergy, Pulmonary and Critical Care Medicine at Vanderbilt in 2002. Currently, she is an attending physician on the pulmonary and medical ICU services at the Vanderbilt University Medical Center. Dr. Ware's lab does basic, translational and clinical research on the role of the alveolar epithelium in the pathogenesis and resolution of acute lung injury and the acute respiratory distress syndrome. In addition to her clinical and research responsibilities she enjoys teaching in both the research and clinical settings and has mentored numerous medical students, residents and postdoctoral fellows.

BREAKOUT SESSIONS

BREAKOUT SESSION 1: SATURDAY, 2:30 – 3:15 PM

MD/PHD ADMISSIONS: PUTTING YOUR BEST SELF FORWARD

Location: Eskind Biomedical Library, Room 238

Panelists:

Ann Chahroudi, MD, PhD – Emory University, MSTP Associate Director

Chris Williams, MD, PhD – Vanderbilt University, MSTP Director

Lynn Tran – Augusta University, G3

Nour Hijazi – Medical University of South Carolina, G2

Sam Morrisey – University of Louisville, G3

William Geisler, MD, MPH – University of Alabama at Birmingham, Clinical MSTP Director

CAREER SPEED ROUNDS

Location: Light Hall, Room 410

Panelists:

Aaron Shaver, MD, PhD – Vanderbilt University, Assistant Professor of Pathology, Microbiology, and Immunology

Elizabeth Townsley, MD, PhD – Vanderbilt University, 3rd Year Resident, Med-Peds

Gretchen Purcell-Jackson, MD, PhD – Vanderbilt University, Associate Professor of Biomedical Informatics and Pediatric Surgery

Jim Cassat, MD, PhD – Vanderbilt University, Assistant Professor, Division of Pediatric Infectious Diseases

Jim Goldenring, MD, PhD – Vanderbilt University, Professor, Department of Surgery; Vice Chair for Surgical Research

John McPherson, MD – Vanderbilt University, Professor of Medicine; Director, Internal Medicine Residency Program

Lori Jordan, MD, PhD – Vanderbilt University, Assistant Professor of Neurology and Pediatrics; Associate Director, Child Neurology Residency Program

Milam Brantley, MD, PhD – Vanderbilt University, Associate Professor of Ophthalmology & Visual Sciences; Director, Eye Institute Initiative for Ocular Pharmacogenomics

Patrick Hu, MD, PhD – Vanderbilt University, Associate Professor of Medicine, Vanderbilt Division of Hematology and Oncology; Director, Physician-Scientist Training Program

Reid Thompson, MD – Vanderbilt University, Chairman, Department of Neurological Surgery

Rolanda Lister, MD – Vanderbilt University, Assistant Professor, Obstetrics and Gynecology

Ryan Muller, MD – Vanderbilt University, Assistant Professor of Radiology and Radiological Sciences; Associate Program Director, Interventional Radiology Residency Program

Sachin Patel, MD, PhD – Vanderbilt University, Associate Professor of Psychiatry & Behavioral Sciences, Vanderbilt Kennedy Center; Director, Division of Addiction Psychiatry

Sonya Heath, MD – University of Alabama - Birmingham, Director, UAB Tinsley Harrison Internal Medicine Residency Program

Stokes Peebles, MD – Vanderbilt University, Professor, Division of Allergy, Pulmonary and Critical Care Medicine; Program Director, Allergy Immunology Fellowship Program

Vance Albaugh, MD, PhD – Vanderbilt University, Chief Resident, Department of Surgery

BREAKOUT SESSION 2: SATURDAY, 4:30 – 5:15 PM

THE SUBTLETIES OF PURPOSEFUL NETWORKING

Location: Light Hall, Room 202

Panelists: **Ashley Brady, PhD** – Vanderbilt University, Assistant Professor of Medical Education and Administration, Director of Career Engagement and Strategic Partnerships in the Office of Career Development

Brent Ferrell, MD – Vanderbilt University, Assistant Professor of Medicine

Nick Markham, MD, PhD – Vanderbilt University, Fellow, Division of Gastroenterology, Hepatology and Nutrition

---

THE ART OF MAKING IT WORK: HOW TO CREATE BALANCE AS A PHYSICIAN SCIENTIST

Location: Light Hall, 4th Floor

Panelists:

Ann Chahroudi, MD, PhD – Emory University, MSTP Associate Director, Associate Professor of Pediatrics in the Division of Pediatric Infectious Diseases, Faculty Advisor for MD/PhD Women's Association

Brian Grieb, MD, PhD – Vanderbilt University, Clinical Fellow, Internal Medicine, Oncology

Chris Williams, MD, PhD – Vanderbilt University, MSTP Director, Associate Director, Physician Scientist Training Program, Professor of Medicine and Cancer Biology

Curtis Gabriel, MD, PhD – Vanderbilt University, Research Fellow, Division of Gastroenterology, Hepatology, and Nutrition

Jim Goldenring, MD, PhD – Vanderbilt University, Professor, Department of Surgery; Vice Chair for Surgical Research

John Stafford, MD, PhD – Vanderbilt University, Assistant Professor of Medicine, Division of Diabetes, Endocrinology, and Metabolism, and Molecular Physiology and Biophysics.

Jordan Wright, MD, PhD – Vanderbilt University, Research Fellow, Internal Medicine, Division of Diabetes, Endocrinology, and Metabolism

Kim Lee, MD, PhD – Vanderbilt University, Neurology Resident

Lauren Prusinski – Augusta University, M3

Lori Jordan, MD, PhD – Vanderbilt University, Assistant Professor of Neurology and Pediatrics; Associate Director, Child Neurology Residency Program

Mark Pepin – University of Alabama, G4

Meena Madhur, MD, PhD – Vanderbilt University, Assistant Professor of Medicine; Associate Director, Institute for Infection, Immunology and Inflammation

Melissa Bloodworth, PhD – Vanderbilt University, M4

Milam Brantley, MD, PhD – Vanderbilt University, Associate Professor of Ophthalmology & Visual Sciences; Director, Eye Institute Initiative for Ocular Pharmacogenomics

Natalia Bilchuk – University of Louisville, G2

Nichelle Winters, MD, PhD – Vanderbilt University, Fellow, Pulmonary and Critical Care

Nour Hijazi – Medical University of South Carolina, G2

Patrick Hu, MD, PhD – Vanderbilt University, Associate Professor of Medicine, Vanderbilt Division of Hematology and Oncology; Director, Physician-Scientist Training Program

Sachin Patel, MD, PhD – Vanderbilt University, Associate Professor of Psychiatry & Behavioral Sciences, Vanderbilt Kennedy Center; Director, Division of Addiction Psychiatry

Sonya Heath, MD – University of Alabama, Director, UAB Tinsley Harrison Internal Medicine Residency Program

Tyler Beck – Medical University of South Carolina, G1

Vance Albaugh, MD, PhD – Vanderbilt University, Chief Resident, Department of Surgery

William Geisler, MD, MPH – University of Alabama at Birmingham, Clinical MSTP Director, Professor of Infectious Disease

BREAKOUT SESSION 3: SUNDAY, 9:05 – 9:50 AM

#SCIMEDTWITTER: WHY AND HOW

Location: Light Hall, 2nd Floor: Room 214

Panelists: **Carey Jansen** – Emory University, G2

@careyjans

Jacelyn Peabody – University of Alabama at Birmingham, G2

@jacepeabody

Jerome Arceneaux – Meharry Medical College, G2

@JSArceneaux91

STAYING SOCIALLY ENGAGED AS A SCIENTIST IN 2018

Location: Light Hall – 2nd Floor: Room 202

Panelists: **David Plazas, MBA** – Opinion and Engagement Director, The Tennessean

Jacy Warrell, MPA – Executive Director, Tennessee Health Care Campaign

Sheryl Fleisch, MD – Vanderbilt University, Assistant Professor of Psychiatry and Behavioral Sciences

ORAL PRESENTATIONS

LIGHT HALL ROOM 407

8:00AM Joe Luchsinger, Vanderbilt University– *Identification and characterization of a control network for BNST-projecting insular neurons*

8:15 AM Morgan Zipperly, University of Alabama at Birmingham – *Dynamic Signaling in the Nucleus Accumbens Contributes to Reward-Seeking and Motivated Behavior*

8:30 AM Tina Tian, Emory University–*The role of the proteasomal deubiquitinating enzyme USP14 in the degradation of aggregate prone proteins*

8:45 AM Mackenzie Lemieux, Massachusetts Institute of Technology – *Neurotensin Gates Valence-specific Plasticity Underlying Associative Learning*

10:00AM Cody Stothers, Vanderbilt University– *Dissecting the Mechanisms of β-glucan Trained Innate Immunity*

10:15 AM Adriana Hernandez, University of Puerto Rico at Ponce – *Methamphetamine reduces the expression of T cell receptors on human T cell-like lymphocytes*

10:30AM John Walker, Vanderbilt University–*Pancreatic islets in short-duration type 2 diabetes have secretory dysfunction, increased inflammatory signaling, and altered islet-enriched transcription factor expression*

10:45 AM Brian Tirado, Mercer University – *The Effect of a Human LNK Polymorphism on Erythropoietin Receptor Signal Transduction*

LIGHT HALL ROOM 411

8:00 AM Adam Jorgensen, Wake Forest University – *In vitro Maturation of 3D Bioprinted Human Skin*

8:15AM Joseph Grech,Wake Forest University–*Optimization of dECM Based Bioink for 3d Bioprinting of Renal Construct*

8:30 AM Valentyna Trull, Judson College – *Expression of Recombinant Human Tamm-Horsfall Protein Analogs in Kidney Cells for Large Scale Isolation and Purification*

8:45 AM Taylor Bono, University of Alabama at Birmingham – *Evaluation of Apremilast as a novel pharmacologic approach in Cystic Fibrosis*

10:15 AM Hernan Gonzalez, Vanderbilt University

10:30AM Malika Nimmagadda, National Eye Institute –*Investigating the Underlying Mechanisms of Late-Onset Retinal Degeneration (L-ORD) Using Induced Pluripotent Stem Cell (iPSC) Technology*

10:45 AM Tyler Beck, Medical University of South Carolina – *Non-Addictive Peptide-Derived Opioid Analgesic for Peripheral Pain*

LIGHT HALL ROOM 415

8:00 AM Alexandra Sundermann, Vanderbilt University– *Alcohol Use in the First Trimester and Miscarriage*

8:15 AM Alexandra Llovet, Emory University– *Leprosy: Stigma Continuity in Brazil*

8:30 AM Schyler Said and Melissa Allison, University of California Santa Barbara–*Improvements in Behavioral Skills in Teens with ASD Through Social Intervention*

10:00 AM Lynn Tran, Augusta University– *Novel Prognostic and Therapeutic Gene Signature Upregulated in Uterine Serous Carcinoma Patients with Poor Survival*

10:15 AM Corey Duke, University of Alabama at Birmingham – *A novel light-inducible CRISPR/dCas9 system for controlling gene expression.*

10:30 AM Eliot Forster-Benson, Vanderbilt University–*Interactions between the HIV-1 Glycoprotein 41 Cytoplasmic Tail and the Gag Matrix Protein*

10:45 AM Nazary Nebeluk, LSUHSC-New Orleans – *Mechanistic Insights on the Broad Spectrum Antiviral Potential of Disrupting Host-Cell Arginine Pathways*

LIGHT HALL ROOM 419

8:00 AM Armando Javier Ruiz-Justiz, University of Puerto Rico at Ponce – *DAMPs-coated Prussian blue nanoparticles as photothermal-nanoimmunotherapy agents for cancer.*

8:15 AM Aaron Fan, Augusta University–*Adoptive cell transfer in combination with peptide vaccination enhances the anti-tumor response of genetically engineered T cells*

8:30 AM Anastasia Hale, Pennsylvania State University – *TIP60 Depletion as a Mechanism of PARP Inhibitor Resistance in BRCA2 deficient cells*

8:45 AM John Klement, Augusta University– *An Osteopontin immune checkpoint controls CD8+ T cell activation and tumor immune evasion*

POSTER SESSION 1

	NAME	ABSTRACT TITLE	SCHOOL
1	Lamario Williams	Key clock effector molecule E4BP4 modulates cardiac fatty acid metabolism	University of Alabama, Birmingham
2	Brittany Foret	Regulation of Central Nervous System Endogenous Opioid mRNA in Chronic Binge Alcohol (CBA) Treated Male Rhesus Macaques Infected with Simian Immunodeficiency Virus (SIV)	Louisiana State University
3	Shelby Harris	Rac1-Induced Mitochondrial ROS mediates Mitochondrial Biogenesis and Pro-fibrotic Polarization of Macrophages in Pulmonary Fibrosis	Jacksonville State University
4	Muntathar Alshimary	A functional module for a response to low nutrients in Arabidopsis	Berea College
5	Katherine Pinkerton	Development of biomarker assays to measure urinary proteins.	Augusta University
6	Favour Akabogu	RECOMBINANT EXPRESSION OF COLLAGEN IV FROM MINISTERIA VIBRANS: THE ANCESTRAL COLLAGEN OF THE ANIMAL KINGDOM	Berea College
7	Anita Qualls	Elucidating the Relationship Between Skeletal Muscle Injury-Induced Inflammation and Mitochondria	University of Georgia
8	Brenda Montanez	SP Molecular Surveyor- a tool to track resistance markers for sulphadoxine-pyrimethamine	Johnson C. Smith University
9	Angela Wei	Enrichment of SNP-heritability of psychiatric disorders in gene and isoform modules	University of Kentucky
10	Hannah Huth	Health Management in the Home: A Qualitative Study of Pregnant Women and their Caregivers	Indiana University
11	Myles McCrary	Sulfated chondroitin sulfate hydrogel cell carriers improve angiogenesis and arteriogenesis after ischemic stroke in mice.	Emory University
12	Sumeeth Jonathan	Targeting ultrasonic neuromodulation in non-human primates with optical tracking-guided MR-ARFI	Vanderbilt University
13	Zishuai Chou	DECELLULARIZED HUMAN SKIN-DERIVED ECM AS A SUPPLEMENT TO FIBRIN HYDROGEL FOR SKIN BIOPRINTING APPLICATIONS	University of California, Berkeley
14	Rachel Miles	Stabilization of siRNA Polyplexes for Improved Systemic Circulation and Enhanced Shelf-Life	Vanderbilt University
15	Ouniol Aklilu	A Computational and Experimental Investigation of Relationships between Magnetic Resonance Diffusion Tensor Imaging Fractional Anisotropy and Applied Mechanical Strain	The Pennsylvania State University
16	Muhan Hu	Tissue specificity of the DAF-7/ TGFbeta pathway in affecting sperm guidance	University of Alabama, Birmingham
17	Micheal Munson	Role of the Na+/H+ Exchanger in Modulating Resistance to Apoptosis in Pulmonary Arterial Smooth Muscle Cells from Rats with Pulmonary Hypertension	Baylor University

	NAME	ABSTRACT TITLE	SCHOOL
18	Brandon Sanders	Expressed miR-17~92 cluster in endothelial cells confers protection against kidney ischemia-reperfusion injury	Claflin University
19	Melina Frantzeskakis	The Influence of Nato3 on Genes Involved in Dopamine Neurogenesis and Maturation	Grand Valley State University
20	Aaron Sandoval	Perfect muscle regeneration in the African spiny mouse (Acomys) after chronic injury	University of Florida
21	Lauren Scott	Polymeric Silicone Material Design and Utility in Medical Devices	Lipscomb University
22	Guillermo Pereda	Regiospecific Surface Encoding of Nanoparticles for Programmable Self-Assembly	The University of Chicago
23	Bryce Forry	Determination of Ochratoxin A in Kentucky Black Walnuts	Asbury University
24	Madeline Dunfee	Supporting Rural Grandparent Caregivers through Building on Appalachian Cultural Traditions	University of Kentucky
25	Joseph Deshan	The Health Impact of Housing Factors among People Living With HIV	University of Alabama, Tuscaloosa
26	Jira White	Uncovering Genetic Interactions in Glia-Mediated Formation of The C. Elegans Brain Neuropil	Claflin University
27	Jeremie Lever	Self-Renewing Kidney Resident Macrophages Downregulate MHC class II and Promote Resolution After Acute Kidney Injury (AKI)	University of Alabama, Birmingham
28	Allison Cummins	Involvement of the Croquemort Receptor in Bacterial Phagocytosis by Glial Cells in the Brain of Drosophila melanogaster	The University of Alabama - Tuscaloosa
29	Sangami Pugazenthi	The NLRP3 inflammasome is a critical mediator of cell-free hemoglobin-induced acute lung inflammation	Vanderbilt University
30	Akenda Walker	Screening of harmful toxins in Cosmetics and personal care products.	Voorhees College
31	Dana Oakes	Evaluation of hNRF2 as a Neuroprotective Gene Therapy Approach in a Transgenic Pig Model Expressing Human P23H Mutant Rhodopsin	University of Louisville
32	Hailey Kresge	Subclinical Compromise in Cardiac Strain Relates to Lower Cognitive Performances in Older Adults	Vanderbilt University
33	Jordan Galbraith	Dopamine Regulation via Allosteric Modulation of the M1 Receptor: Implications for the Negative Symptoms of Schizophrenia	Vanderbilt University
34	Marlisa Shaw	Identifying and Localizing Novel Genes in Zebrafish Spinal Cord Controlling Locomotion	The Pennsylvania State University
35	SaDazia Driffin	Effect of D-Limonene on Developing Human Neurons	Claflin University
36	Alexander Yue	The Role of Prelimbic Cortex Neuronal Ensembles in Fear vs. Reward	Columbus State University
37	Emily Stephens	The effects of peripheral amyloid beta on spermatogenesis in APPswe/PS1dE9 mouse model for Alzheimer's disease	Texas Technology University
38	Shyam Desai	Calcification and Risk Stratification in Femoral Artery Stenosis	University of South Carolina

	NAME	ABSTRACT TITLE	SCHOOL
39	Kristen Williams	Optimizing Imaging of Headaches in the Pediatric Emergency Department	Quinnipiac University
40	Kimberly Gillens	Preoperative administration of L-Arginine, Omega-3 Fatty Acids and Ascorbic Acid effects on postoperative complications in diabetic orthopedic patients	University of South Carolina School of Medicine
41	Gabrielle Gilmer	The risk for anterior cruciate ligament injury is higher when serum relaxin concentrations peak	Auburn University
42	Jacelyn Peabody	MUCUS MATTERS: FERRETS DEMONSTRATE SUSTAINED FIBROSIS, MUCOCILIARY DECREMENT, AND ABERRANT LUNG REPAIR FOLLOWING BLEOMYCIN-INDUCED PULMONARY FIBROSIS	University of Alabama, Birmingham
43	Evan Tracy	Injection of Adipose-derived Stromal Vascular Fraction Restores Adrenergic Function and Alters Reactive Oxygen Species Signaling in Coronary Arterioles from Aged Females	University of Louisville
44	Jake Doiron	Investigation of 1,2,3-triazoles as amide bioisosteres in modulators of the cystic fibrosis transmembrane conductance regulator.	Berry College
45	Katelyn Gurley	Two open source designs for a low-cost operant chamber using Raspberry Pi(TM)	Louisiana State University
46	Emily Vernet	Effects of a Nature Walk with Canopy on Cortisol and Cognition in Children with Autism Spectrum Disorder	University of Central Florida

POSTER SESSION 2

	NAME	ABSTRACT TITLE	SCHOOL
1	Clare Edwards	Uncovering novel inhibitors of Type III CRISPR-Cas systems in Streptococcus thermophilus	University of Georgia
2	Gunnar Eastep	Biochemical studies of HIV-1 MA mutants affecting Envelope glycoprotein incorporation	University of Alabama in Birmingham
3	Akira Nishii	Temperature Regulation of Lipid Desaturation and Lipolysis in Adipocytes	University of Michigan, Ann Arbor
4	Greta Trogen	CTRP3 Overexpression Attenuates Ethanol-Induced Changes to the Liver Fatty Acid Profile	East Tennessee State University
5	Jasmine Carter	Exploring the Role of Nuclear Lamin A in the Regulation of Stress Response Using a Cell Culture Model of Aging	Claflin University
6	Kevan English	Synthesis of a Macrocyclic Triamine for Metal Ion Binding	University of West Florida
7	Alexander Condoroteanu-Oroveanu	Synthesis of a Polyamine Ligand as Metalloenzyme Mimic	University of West Florida
8	Dakota Booth	Molecular mapping and subtyping of gliomas from The Cancer Genome Atlas	Augusta University
9	Eileen Kim	Discovery Pipeline of Cancer Transcriptomes Using Machine Learning	Augusta University
10	Emily Myers	Identifying genetic drivers of poor prognosis in uterine serous carcinoma using regulatory network analysis	Augusta University
11	Steven Yarmoska	Perfluorocarbon Nanodroplets for Extravascular Contrast-Enhanced Ultrasound in Cancer	Emory University
12	Saumya Gurbani	Assessing treatment response of GBM to an HDAC inhibitor, belinostat (PXD101)	Emory University
13	Aliasger Ezzi	The Effect of Epigenetic Pharmacological Agents on Abnormal Nuclear Morphologies in Cancer	University of Florida
14	Stephanie Dudzinski	The Effects of Diet-Induced Obesity on Tumor Microenvironment and Immune Checkpoint Blockade Efficacy	Vanderbilt University
15	Samantha Morrissey	Tumor-derived exosomes drive tumor metastasis through metabolic reprogramming of macrophages in a pre-metastatic niche	University of Louisville
16	Paul Tran	Applications of a Cellular Proliferation Gene Signature in Precision Oncology	Augusta University
17	Nicholas Eustace	Use of a phospholipid binding MARCKS mimetic for the targeted killing of glioblastoma cells	University of Alabama at Birmingham
18	Dewey Brooke	Surrounding the enemy: investigating the influence of cervical microbiome and tumor microenvironment on the prognosis of cervical cancer patients	University of Alabama at Birmingham
19	Adeiye Pilgrim	Defining the Role of the RNA-binding Protein MSI2 in Neuroblastoma	Emory University
20	Rebekah Robinson	IL-6 trans-signaling regulates cell migration in ovarian cancer cell lines	Augusta University

	NAME	ABSTRACT TITLE	SCHOOL
21	Rachel Brown	MTG16 is a transcriptional corepressor that regulates intestinal stem cell differentiation and lineage specification	Vanderbilt University
22	Desmond Harrell-Stewart	RASSF1A forms a direct complex with DAB2IP	University of Louisville
23	Jordy Botello	Adrenergic signaling promotes cancer cell resistance to standard chemotherapies through a mechanism that is calcium-mediated	University of Florida
24	Chimsom Agbim	Exosomes as Therapeutic Biomarkers in Prostate Cancer	Vanderbilt University
25	Berenice Vazquez	Assessing the effects of oncohistone mutations in H3.3 on cell proliferation in Drosophila melanogaster	Johnson C. Smith University
26	Edie Osuma	PD-L1 and PD-L2 Differ in their Molecular Mechanisms of Regulation Mediated via PD-1 Engagement in T cells	Wesleyan College
27	Lauren Prusinski Fernung	Dysregulated DNA Double-Strand Break Repair in Stem Cells from Uterine Fibroids Promotes Mutagenesis and Propagates Uterine Tumor Development	Augusta University
28	Kristin Ates	Genetic modeling and pathophysiological analysis of a putative human disease gene, FAM109A	Augusta University
29	Amara Ejikemeuwa	The Effect of Aripiprazole on Neutrophil Adhesion	University of West Florida
30	Holly McKee	Compound library analysis identifies a combination of small molecules that increase the terminal differentiation of human induced pluripotent stem cells into myotubes and their maturation	University of Minnesota
31	Win Hon	Novel Crystal Forms of the Antibiotic Cefixime	Grand Valley State University
32	Rachel Levy	Examining the Effects of Activating SNORD116 in a CRISPR/Cas9 Mouse Model	University of Florida
33	Tyler McCaw	Appropriately Timed Histone Deacetylase Inhibition Empowers T Cell-Mediated Immunity to Reject Established Breast Tumors in Pre-Clinical Models	University of Alabama at Birmingham
34	Matthew Madden	Glutaminase inhibition and CD8 T cell fate in cancer immunotherapy	Vanderbilt University
35	Jordan Noe	Mitochondrial Metabolism in M2 Macrophage Polarization: The Role of MIF and Lactate	University of Louisville
36	Jacob Files	CD4 T Cell Restricted HIV Cryptic Epitopes, an Implication of Novel Antisense Proteins	University of Alabama at Birmingham
37	Cassandra Woolley	Human neutrophils depend on accessory cells for their survival response to LPS	University of Louisville
38	Spencer Richardson	Influence of Immune System on Osteochondromas	University of Tennessee
39	Adriana Reyes	Localization of Immunological Niches Associated in Human Renal Cell Carcinoma	Emory University
40	Thomas Stovall	Peripheral Blood Markers and Intraoperative Pathology Are Helpful in Predicting Non-Cutibacterium Acnes Shoulder Prosthetic Joint Infections	Meharry Medical College

	NAME	ABSTRACT TITLE	SCHOOL
41	Aum Patel	Characterizing human target cells infection by three geographically distinct isolates of Mayaro virus	University of Florida
42	Mahesh Krishna	Stochastic Microbiome Development Associates With Acute Mammalian Colitis Severity	Rice University
43	Jesse Mangold	Autologous virus neutralizing monoclonal antibodies in HIV-infected, vertically transmitting and non-transmitting U.S. and Malawian women	Duke University
44	Alberto Williams-Medina	Expression and Purification of Full-length Recombinant Plasmodium falciparum PfMC-2TM Maurer's cleft Protein	Cleveland State University
45	Arsany Abouda	Metabolism of Sunitinib in Genotyped Primary Human Hepatocytes	Lipscomb University
46	Clorissa Campbell	The Visual Opsin Genes of Diurnal and Nocturnal Frogs	Middle Tennessee State University

ABSTRACTS – ORAL PRESENTATIONS

Identification and characterization of a control network for BNST-projecting insular neurons

Luchsinger, J., Fetterly, T., Centanni, S. and Winder, D.

While substance abuse is a chronic, relapsing disease with significant economic and personal costs, the biological basis remains elusive. Anxiety disorders and alcohol use disorders are often comorbid conditions, and stress can exacerbate the propensity for relapse. The insula and bed nucleus of the stria terminalis (BNST) have garnered attention for their suspected roles in stress responses and addiction. Our lab has recently obtained data suggesting a key role for insular inputs to the BNST in alcohol abstinence-induced negative affective disturbances. To develop a better understanding of the control of this circuit, we here utilized a combination of viral-based anatomical and functional studies in mice to assess the insula-BNST control network. Using the rabies-based method, tracing the relationships of inputs and outputs (TRIO), which transsynaptically labels cells synapsing on a target population, we have identified several afferent populations upstream of the insula?BNST cells. Populations were identified in both ipsilateral and contralateral cortices while subcortical regions were only found ipsilaterally. Specific thalamic nuclei (i.e. parafascicular and paraventricular) known to project to the insula were also labeled, increasing our confidence in the technique. Likewise, somatosensory cortex is known to send significant projections to the insula and was found to be the region with the highest number of afferent cells, again with ipsilateral outnumbering contralateral inputs. AAV1 anterograde transsynaptic tracing was then combined with channelrhodopsin assisted mapping and electrophysiology as a convergent strategy to both confirm monosynaptic connectivity and initiate functional studies of the circuit. AAV1-Cre (anterogradely crosses the synapse) and AAV5-ChR2-eYFP were injected into a TRIO-identified region while AAVRetro-DIO-TdTomato was injected into the BNST. Cells that receive afferents from the TRIO-identified region and project to the BNST are subsequently fluorescently tagged. TdTomato positive cell bodies were readily observed in the insula using this approach. Excitatory connectivity was confirmed using this strategy in 15 whole-cell patch-clamped cells in ex vivo slices of the insula from 5 mice using optogenetics. Identified cells were then electrophysiologically characterized. This work demonstrates a useful method for efficiently confirming rabies tracing results and provides new insight into potential mechanisms for control of a circuit involved in alcohol abstinence and negative affect.

Dynamic Signaling in the Nucleus Accumbens Contributes to Reward-Seeking and Motivated Behavior

Morgan E. Zipperly, Natalie A. Simpkins, John J. O'Malley, Deja S. Murray, Kendra D. Bunner, PhD, & Jeremy J. Day, PhD.

Department of Neurobiology, University of Alabama at Birmingham, Birmingham, AL.

Purpose: Exposure to drugs of abuse alters neuronal firing patterns, leading to changes in brain circuitry which outlive the direct effects of the drug and may contribute to addiction. The nucleus accumbens (NAc) has a significant role in motivation, reward, and reward-related learning, and has been identified as a key area in the development and maintenance of addiction. However, the contribution of specific NAc neuronal populations and the influence of NAc afferents in drug reward and motivated behavior is still poorly understood. The present study aims to determine how neuronal activity in the NAc is altered in response to acute cocaine reward, and how these changes in activity influence reward-seeking and goal-motivated behavior.

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 and optic fibers were surgically implanted targeting the NAc. Ongoing studies utilize chronically implanted optrodes, combining in vivo optogenetics and electrophysiological recordings to investigate how PFC inputs to the NAc influence medium spiny neuron (MSN) activity.

Results: Acute cocaine exposure resulted in rapid but prolonged increases in neuronal activity in a subpopulation of MSNs in the NAc. This activity pattern is similar to in vitro stimulation of rat primary striatal neurons treated with the dopamine receptor type 1 agonist SKF38393 (1mM). Real-time place preference was generated by photostimulation of ChR2-expressing MSNs in the NAc core.

Conclusion: These findings suggest that acute cocaine experience significantly increases MSN firing in a subpopulation of cells in the NAc, and that this activity is sufficient to drive reward-related behaviors.

Neurotensin Gates Valence-specific Plasticity Underlying Associative Learning

Mackenzie E. Lemieux, Praneeth Namburi, Jacob M. Olson, Natsuko Hitora-Imamura, Anna Beyeler, Gwendolyn G. Calhoon, Sourav R. Choudhury, Xi Shi, Ada C. Felix-Ortiz, Romy Wichmann, Vanessa Page, Hunter O. King, Matilde Borio, Ehsan M. Izadmehr, Margaux Silvestre, Cody A. Siciliano, Kenneth M. McCullogh, Kerry J. Ressler, Feng Zhang, Kay M. Tye

Purpose: The ability to rapidly learn that environmental cues predict rewards or punishments is of paramount importance for survival. Disruptions in the processing of positive and negative valence may be at play in neuropsychiatric diseases including schizophrenia, anxiety, depression, bipolar disorder, and addiction. Circuits emerging from the basolateral amygdala (BLA) have been implicated in the acquisition of associative memories and the routing of positive and negative valence through divergent pathways. BLA projections to the nucleus accumbens (NAc) mediate positive valence (approach) while BLA projections to the centromedial amygdala (CeM) mediate negative valence (avoidance). However, the neural mechanisms that direct transmission and plasticity to the appropriate pathway remain a mystery. Our work demonstrates that a peptidergic neuromodulator, neurotensin (NT), exerts valence-specific effects on the acquisition of associative learning tasks.

Methods: Ex vivo patch-clamp electrophysiology was performed in mouse brain slices to determine the effects of neurotensin 1 receptor (NTSR1) antagonism on long term potentiation in the BLA, CeM, and NAc. The sources of NT to the BLA were then identified through retrograde tracing of BLA circuits with Fast Blue tracer. CRISPR was then used to inactivate the NT gene thereby allowing for selective interrogation of the function of NT while preserving glutamate transmission. To reveal the natural dynamics of NT-expressing neurons, multi-site fiber photometry was performed in NT positive (NT+) populations of the paraventricular thalamus (PVT), medial geniculate nucleus (MGN), and ventral hippocampus (vHPC).

Results: Antagonism of NTSR1 selectively enhances reward learning but not fear learning. NT dose-dependently alters glutamatergic transmission and synaptic plasticity in an opposing manner for BLA□NAc and BLA□CeM neurons. Retrograde tracing revealed NT+ populations in the MGN, vHPC, and PVT projecting to the BLA. NT inactivation in the PVT, but not the MGN nor vHPC, selectively enhanced reward conditioning. Bulk fluorescence of NT+ neurons expressing a genetically-encoded calcium indicator in the PVT decreased after mice learned the reward association, and increased after mice learned the aversive association.

Discussion/Conclusion: The neuropeptide, Neurotensin, gates plasticity in a valence-specific manner through NTSR1. Not only does our circuit-based investigation highlight the role of NT as a critical valence modulator through its input to the amygdala, but it also implicates NTSR1 as a potential target for therapeutic interventions of psychiatric diseases such as depression, anxiety, and addiction. Given the lack of mechanistically-novel targets for mood disorders in the past 50 years, this represents an exciting breakthrough in treating diseases that disrupt the appropriate assignment of emotional or motivational significance.

Dissecting the Mechanisms of β-glucan Trained Innate Immunity

Cody L. Stothers*, Liming Luan, PhD#, Julia K. Bohannon, PhD#, Benjamin A. Fensterheim, PhD*, David L. Williams, PhD&, and Edward R. Sherwood, MD, PhD*,#.

* - Department of Microbiology, Immunology, and Pathology, Vanderbilt University Medical Center, Nashville, TN.
# - Department of Anesthesiology, Vanderbilt University Medical Center, Nashville TN
& - Department of Surgery, East Tennessee University State University, Johnson City, TN

Purpose: Critically ill patients suffer from immunoparalysis that prevents innate leukocytes from combating invading pathogens. Novel treatments that bolster immune responses to infections are required to decrease mortality from antibiotic-resistant organisms. In vitro treatment with the fungal cell wall component β-glucan rewires immune cell metabolism to boost effector function for several days. Leukocytes recognize β-glucan by the pattern recognition receptors Dectin-1 and Toll-like receptor 2 (TLR2). We sought to identify the signaling mechanisms responsible for β-glucan activity in vitro and to characterize the protective effects of β-glucan in vivo.

Methods: Wildtype, Dectin-1-/- and TLR2-/- mice were given 1mg β-glucan or saline intraperitoneally (IP) for 2 days. On day 3, mice were injected IP with 108 CFU/mL P. aeruginosa. Six hours after infection, mice were assessed for core temperature. Subsequently, IP bacterial counts and leukocyte recruitment were quantified by culture and flow cytometry, respectively. Bone-marrow derived macrophages (BMDM) from the same mice were primed with β-glucan and assessed for markers for priming, including phagocytosis and cellular metabolism.

Results: Beta-glucan treated mice sustained near-normal body temperature and had a decreased bacterial burden in the peritoneal cavity. Surprisingly, protection was found in all genotypes. Significantly more neutrophils and macrophages were recruited to the peritoneal cavity in β-glucan-treated mice of all genotypes. In contrast, β-glucan treatment enhanced phagocytosis in wildtype BMDM, but not Dectin-1-/- nor TLR2-/- macrophages. Similarly, only wildtype BMDM showed sustained metabolic alterations after β-glucan treatment.

Conclusion: β-glucan improves survival and antimicrobial clearance in a murine model of P. aeruginosa sepsis, even in the absence of Decitn-1 and TLR2. Surprisingly, Dectin-1-/- and TLR2-/-macrophages do not show significant priming by β-glucan in vitro. Thus, despite prevailing theories of “trained innate immunity

Methamphetamine reduces the expression of T cell receptors on human T cell-like lymphocytes

Adriana Hernández-Santini, Luis R. Martínez, Ph.D.

Department of Biology, University of Puerto Rico at Ponce, PR; Department of Biological Sciences, The Border Biomedical Research Center, The University of Texas at El Paso, TX

Methamphetamine (METH) is a central nervous system (CNS) stimulant used by an estimated 10 million people in the United States and an immunosuppressive agent of both innate and adaptive immunity. METH enhances HIV infection, making users with AIDS more susceptible to opportunistic infections. T cell exposure to METH causes cell dysfunction via the induction of oxidative stress pathways preceded by elevated levels of mitochondrial calcium after T-cell receptor (TCR)-CD3/CD28 stimulation. We have shown that METH reduces the number and activation of T lymphocytes in mice. Therefore, we hypothesized that METH alters the expression of TCR in lymphocytes. Utilizing an immortalized cell line of T lymphocytes (Jurkat), we investigated the impact of METH on the expression and distribution of TCRs upon stimulation by T-cell mitogens, phytohaemagglutinin (PHA) and phorbol 12-myristate 13-acetate (PMA). By using flow cytometry, western blot, and confocal microscopy analyses, we demonstrated that physiological concentrations of METH reduce the number of TCR on Jurkat cells after stimulation with PHA, PMA, or combination. We analyzed the expression of specific intermediates of the TCR signaling pathway such as ZAP 70, CARD1, IKKs, JNK, p38, and NFκB. Our results suggest that METH compromises the TCR signaling cascade resulting in reduced production of IL-2. Surprisingly, we did not observe changes in the expression of CD28, one of the proteins expressed on T cells that provide co-stimulatory signals required for T cell activation and survival. Our findings demonstrate the direct negative effects of METH on T cells, which is likely to increase the susceptibility of users to serious medical conditions, including the acquisition of infectious diseases. Understanding how METH impairs the effector functions of cells of the innate and adaptive immunity may lead to new prophylactic or treatment strategies that enhance the capacity of the host to respond to diverse insults, including invading pathogens.

Pancreatic islets in short-duration type 2 diabetes have secretory dysfunction, increased inflammatory signaling, and altered islet-enriched transcription factor expression

John T. Walker[1], Rachana Haliyur[1], Erick Spears[2], Diane Saunders[2], Shristi Shrestha[3], Nripesh Prasad[3], Shawn E. Levy[3], Radhika Aramandla[2], Greg Poffenberger[2], Rita Bottino[4], Roland Stein[1], Marcela Brissova[2], Alvin C. Powers[1,2,5]

[1]Department of Molecular Physiology and Biophysics and [2]Department of Medicine, Vanderbilt University, Nashville, TN, [3]HudsonAlpha Institute for Biotechnology, Huntsville, AL, [4]Allegheny-Singer Research Institute, Pittsburgh, PA, [5]Veterans Affairs Tennessee Valley Healthcare System, Nashville, TN

Pancreatic islet dysfunction underlies the progressive nature of type 2 diabetes (T2D); however, dissecting mechanisms of this in humans is challenging. To address this, our group is using an integrated approach to functionally and molecularly study the native pancreas and isolated islets from clinically phenotyped human donors. To identify early pathologic mechanisms, we focused on T2D donors with short disease duration (n=13, age 37-66 years, 2-6 years duration, oral medications) and age-matched controls. We determined that T2D islets had reduced stimulated insulin and glucagon secretion in dynamic islet perifusion assays despite no difference in insulin or glucagon content. Pancreatic sections showed no difference in islet morphology or endocrine cellular composition. To assess for changes in the islet microenvironment, we analyzed macrophages and endothelial cells and found a 2-fold increase in intraislet macrophages and no change in intraislet endothelial cell area. RNA-sequencing analysis of FACS-purified α and β cells from T2D islets showed increased inflammatory signaling pathways in both cell types, including cytokine receptors and downstream signaling components. Our transcriptome analysis also showed reductions in several islet-enriched transcription factors known to play crucial roles in maintenance of murine islet cell identity and function, including NKX2.2 and PAX6. In tissue sections, the percentage of NKX2.2+ and PAX6+ α and β cells in T2D islets was approximately half that of controls. To test the role of these transcription factors in human islet cells, we adapted a pseudoislet model to efficiently transduce human islet cells and have found that knockdown of PAX6 or NKX2.2 in pseudoislets leads to impaired glucose-stimulated insulin secretion. Together, these data suggest that increased macrophages in the islet microenvironment may lead to elevated α and β cell inflammatory signaling, and that intrinsic defects in α and β cell transcriptional regulation contribute to islet dysfunction early in the course of T2D.

The Effect of a Human LNK Polymorphism on Erythropoietin Receptor Signal Transduction

Brian A. Tirado, Matthew P. Alexander, MD/PhD, Meenakshi S. Madhur, MD/PhD

Department of Medicine, Vanderbilt University, Nashville, TN.

Hypertension affects approximately 50% of adults in the United States, rising to 75% by the age of 65. Despite its prevalence, current therapeutics are effective in only 50% of patients with hypertension. Ongoing research indicates immune cells play a key role in regulating blood pressure, suggesting novel therapies targeting the immune system could produce more effective outcomes. One potential target is LNK, a lymphocyte adaptor protein encoded by the gene SH2B3 which negatively regulates erythropoiesis and inflammatory pathways. Analysis of multiple genome wide association studies found a significant correlation between single nucleotide polymorphisms of SH2B3 and elevated blood pressure levels. Specifically, the R262W mutation within the plekstrin homology (PH) domain was found to be associated with hypertension in humans, yet the effect of this single nucleotide polymorphism on LNK regulatory function remains unknown. The goal of this study was to develop a system of transfections to model the erythropoiesis pathway in HEK 293T fibroblasts. This model would answer broad questions about LNK function and determine the effect of the R262W mutation on downstream erythropoietin signaling. The PH domain localizes LNK to the plasma membrane, therefore we hypothesize mutated LNK lacks the ability to negatively regulate downstream erythropoietin signaling. To quantify LNK regulation, we monitored activation of a downstream transcription factor called STAT5 using a STAT5 luciferase assay. Cells transfected with major allele LNK displayed attenuated STAT5 luciferase luminescence in response to erythropoietin stimulation, demonstrating that wild type LNK negatively regulates downstream erythropoietin signaling. Overall, the results of these studies demonstrate an important role for LNK in erythropoietin signaling and lay the foundation to determine whether the human single nucleotide polymorphism alters regulation of erythropoietin signaling, and ultimately blood pressure.

In vitro Maturation of 3D Bioprinted Human Skin

Adam M. Jorgensen*, Mathew Varkey*, Lei Xu1, Shay Soker*, Anthony Atala*

*Wake Forest Institute for Regenerative Medicine, Wake Forest University Health Sciences, Winston Salem, NC, USA.

Purpose: Advances in wound treatment and skin regeneration have revolutionized burn and scar revision surgeries. However, currently available products fail to meet the need for full thickness replacement. Bioprinting is a rapidly progressing field poised to address the need for a complete full-thickness skin substitute. We propose the development of full-thickness skin organized into three biomimetic layers (epidermis, dermis, and hypodermis) through 3D bioprinting.

Methods: Human primary keratinocytes, fibroblasts, and adipocytes were isolated from human donor skin, cultured, and expanded in vitro. Cells were characterized by immunocytochemistry with pan-cytokeratin (keratinocytes), vimentin (fibroblasts), and adiponectin (adipocytes). Cells were trypsinized, and resuspended in a fibrin based bioink at varying levels of cellularity (Adipocytes: 10x106 cells/mL, Fibroblasts: 15x106 cells/mL, Keratinocytes 20x106 cells/mL) and bioprinted to form a tri-layer structure using the Integrated Tissue-Organ Printer (ITOP). Bioprinted skin then matured over 21 days in submerged culture, with samples taken for histological processing, staining, and imaging at days 1, 7, 14, and 21.

Results: Primary isolated cells were successfully identified by immune staining, and displayed cell specific morphology. Bioprinted skin matured over 21 days retained a tri-layer structure. Evidence suggestive of extracellular matrix remodeling was evident at 21 days. Results from Masson's trichrome, picrosirus-red, and immunohistochemical stains are still forthcoming.

Discussion: 3D bioprinted skin provides a promising alternative to conventional skin autografts and tissue engineered skin products for burn reconstruction, as it allows for layer specific placement of cells with 3D complexity. Implementing an air-liquid interface during graft maturation will be crucial for future epidermal maturation. Additional work must be done to fully regenerate accessory skin functions, including pigmentation, hair growth, and thermoregulation.

OPTIMIZATION OF dECM BASED BIOINK FOR 3D BIOPRINTING OF RENAL CONSTRUCT

Joseph A. Grech*+, Mohamed Ali*, Sang Jin Lee*, PhD, James Yoo*, MD, PhD;

*Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC; +Michigan State University, East Lansing, MI

Purpose: Kidney diseases remain a formidable health problem in the United States. A potential future treatment approach to kidney disease is to utilize the tissue engineering approach of 3D bioprinting to form a structure that ultimately restores the function of the kidney in human patients. The major prerequisite for the 3D bioprinting process is to form a suitable bioink capable of supporting cell growth and functions. We previously developed a decellularized extracellular matrix (dECM) bioink composed of the whole kidney that could provide a kidney-specific microenvironment for renal tissue regeneration. The objective of this study was to create a printable dECM bioink that is optimal to the whole kidney dECM bioink by analyzing the ECM components and functionality of dECM bioinks composed of the renal cortex, the renal medulla, and the whole kidney, respectively.

Methods: Our dECM bioink formulation process included: decellularization of porcine kidneys, dissection to separate the renal cortex from the renal medulla, enzymatic digestion, and methacrylation. Bioinks made of both cortical and medullary dECM were then printed with human kidney cells.

Results: Histological stains confirmed decellularization and presence of ECM proteins, and quantitative assays confirmed respective amounts of ECM proteins preserved. Printability tests showed both medullary and cortical dECM bioinks to have good printability. Post printing evaluation of the bioprinted constructs by cell viability staining and renal functionality assays indicated survival of human kidney cells seeded in both dECM bioinks one hour after printing and retention of kidney cell function in both dECM groups six days after printing.

Discussion/Conclusion: The kidney dECM bioink formulation process utilized in this study is successful in preserving vital extracellular components and retaining kidney cell function. Future work is needed to determine the optimal renal dECM bioink for tissue specificity for renal tissue regeneration.

Expression of Recombinant Human Tamm-Horsfall Protein Analogs in Kidney Cells for Large Scale Isolation and Purification

Valentyna Trull*; Kaice LaFavers+, PhD; Radmila Micanovic+, PhD; and Tarek El-Achkar+, MD.

*Department of Biology and Chemistry, Judson College, Marion, AL; +Department of Medicine, Nephrology Division, IU School of Medicine, Indianapolis, IN

Tamm-Horsfall Protein (THP), also known as Uromodulin, (product of the UMOD gene) is the most abundant protein found in the urine, and is produced in the thick ascending limb (TAL) of the loop of Henle part of the nephron. Although predominantly secreted apically into the urine, THP is also released basolaterally, toward the interstitium and circulation. THP is a glycoprotein that regulates ion transport in the TAL, protects against urinary tract infections and kidney stones and serves as a marker for kidney function. THP is mainly present in the urine as a high MW polymer (up to 10 X 106 Da). Polymerization of THP in vivo is regulated by its Zona Pellucida domain but our lab has isolated a truncated form of hTHP that does not polymerize. This form has shown promising use as therapeutic treatment to protect against kidney injury in a mouse model. The goal of my project was to develop a system to express this truncated form of hTHP and its other variants recombinantly in mammalian cells and enable their downstream production. To do this, we first determined the optimal conditions for transfection of canine kidney cell line MDCK with recombinant plasmid engineered to express secreted hTHP analogs. We then used these conditions to establish and validate stable cell lines expressing hTHP analogs. Ongoing work includes optimizing conditions for large scale protein production and purification. These findings will be used to reproducibly produce soluble hTHP analogs in cell culture, thereby alleviating the need to extract the protein from human urine. These recombinantly produced hTHP analogs will be used as a therapeutic agent in mouse studies, with an end goal of translating these findings to humans with kidney injury.

Investigating the Underlying Mechanisms of Late-Onset Retinal Degeneration (L-ORD) Using Induced Pluripotent Stem Cell (iPSC) Technology

Malika Nimmagadda*, Kiyoharu Miyagishima* Ph.D., Ruchi Sharma* Ph.D., Mitra Farnoodian* Ph.D., Devika Bose*, Karla Barbosa Sabanero* Ph.D., Aman George* Ph.D., Bokkyoo Jun+ Ph.D., William Gordon+ Ph.D., Rachel Sharp#, Katharina Clore-Gronenborn*, Zoya Qureshy*, Congxiao Zhang* Ph.D., Davide Ortolan* Ph.D., Bin Guan* Ph.D., Yuri Sergeev* Ph.D., Mones Abu-Asab* Ph.D, Catherine Cukras* M.D.,Ph.D., Paul Sieving* M.D.,Ph.D., Nicholas Bazan+ Ph.D., Kathleen Boesze-Battaglia# Ph.D., Sheldon Miller* Ph.D., Kapil Bharti* Ph.D.

*National Eye Institute, National Institutes of Health, Bethesda, MD.;+Neuroscience Center of Excellence, Louisiana State University Health, New Orleans, LA;#Department of Biochemistry, University of Pennsylvania, Philadelphia, PA

Purpose: L-ORD is caused by a single missense mutation (S163R) in CTRP5, an adiponectin paralog highly expressed in the retinal pigment epithelium (RPE). Our aim is to determine how this mutation leads to retinal disease pathogenesis providing insight into how age-induced metabolic dysregulation may contribute to more common forms of retinal disease.

Methods: Induced pluripotent stem cells were derived from fibroblasts from a family with four siblings: two L-ORD patients and two unaffected siblings and differentiated into RPE. Sanger sequencing confirmed retention of the point mutation in patient lines. CTRP5, VEGF, and PEDF secretion were examined by ELISA. AMPK and EMT pathways were assessed by PrimePCR gene expression arrays. Fluorescence and electron microcopy were used to characterize structural changes and identify CTRP5 localization. CTRP5 association with ADIPOR1 was corroborated by shRNA knockdown. ADIPOR1-stimulated pAMPK levels were measured by ELISA at baseline and upon altering AMP or ATP levels. Neuroprotectin D1 (NPD1) and Phospholipase A2(PLA2) activity were measured by mass spectrometry and by enzymatic activity respectively.

Results: L-ORD patient RPE fed photoreceptor outersegments exhibited 15% increased cell area, sub-RPE deposits, and increased EMT gene expression. The missense mutation did not affect CTRP5 transcription or its apical secretion. CTRP5 co-stained with Adiponectin receptor 1 (AdipoR1) and in silico analysis of mutant CTRP5 complexes exhibited reduced binding affinity for ADIPOR1. VEGF secretion was mispolarized in L-ORD patients, but partially restored by shRNA knockdown of AdipoR1. Elevated pAMPK levels in L-ORD patients at baseline were insensitive to changes in the AMP/ATP ratio despite decreased expression of pAMPK-related genes. NPD1 secretion was significantly lower in patients consistent with 40% reduction in PLA2 activity.

Discussion/Conclusion: Native CTRP5 partially inhibits ADIPOR1 signaling. The reduced affinity of mutant CTRP5 for ADIPOR1 results in aberrant pAMPK levels and lipolytic imbalance contributing to lipid deposits and retinal degeneration.

Non-Addictive Peptide-Derived Opioid Analgesic for Peripheral Pain

Tyler C. Beck*, Carmela M. Reichel**, Kristi L. Helke***, Thomas A. Dix*

* Medical University of South Carolina, Department of Drug Discovery & Biomedical Sciences
** Medical University of South Carolina, Department of Neuroscience
*** Medical University of South Carolina, Department of Comparative Medicine

Purpose:
Kappa-opioid receptor agonists are efficacious against peripheral pain but suffer from CNS-mediated effects that have limited their development. Derivatives of the tetrapeptide D-Phe-D-Phe-D-Nle-D-Arg-NH2 such as CR665 exhibit high peripheral to CNS selectivity when administered IV, and are clinically proven to benefit patients experiencing peripheral pain. However, CR665 is inactive when administered orally, hence the goal of this program is to engineer orally-available derivatives.

Methods:
Application of the proprietary JT Pharmaceutical non-natural amino acid technology to CR665 produced orally-active derivatives. Derivatives were screened in rat models of pain to assess analgesic activity. Lead compound JT09 was further screened in behavioral assays, including a self-administration procedure; conditioned place preference procedure; forced-swim assay; and a locomotor activity assay. JT09 was screened in a multiple dose study to assess for toxicity.

Results:
Lead compound, JT09, is a competitive agonist of the kappa-opioid receptor (EC50 = 29.9 nM), while agonist selectivity for kappa versus mu or delta opioid receptors was >33,400-fold. To assess peripheral versus CNS pain modulation, a rat writhing model of peripheral pain and a hotplate model of CNS-mediated pain were performed. Orally administered JT09 is as efficacious as morphine in alleviating peripheral pain, while failing to produce undesired CNS-mediated activity. In an operant self-administration procedure, where rats pressed a lever to receive an IV drug infusion, JT09 failed to maintain lever responding, indicating no abuse liability versus highly salient rewards (e.g., cocaine). Additionally, JT09 did not promote gross or histological signs of toxicity, as well as other effects associated with CNS activity, including sedation, dysphoria, tolerance and addiction.

Conclusion:
Application of the JT Pharma technology to CR665 enabled identification of derivatives that exhibit peripheral analgesic activity when dosed orally but do not promote CNS-based effects. Our lead, JT09, is under further evaluation as an orally available kappa-opioid agonist for peripheral pain.

Alcohol Use in the First Trimester and Miscarriage

Alexandra C. Sundermann*, Digna R. Velez Edwards, PhD *+^, Sarah H. Jones*, Eric S. Torstenson*, Katherine E. Hartmann, MD, PhD *+

* Vanderbilt Epidemiology Center, Institute of Medicine and Public Health, Vanderbilt University, Nashville, TN; + Department of Obstetrics and Gynecology, Vanderbilt University Medical Center, Nashville, TN; ^ Department of Biomedical Informatics, Vanderbilt University, Nashville, TN

Purpose: More than 50% of women report exposure to alcohol during pregnancy. Most use is in early gestation prior to pregnancy detection and then tapers rapidly. However, past studies about the association between alcohol use and miscarriage treat alcohol as a constant exposure across pregnancy. We sought to incorporate information about timing of change in alcohol use during the first trimester in measures of miscarriage risk.

Methods: Participants in the Right From the Start prospective pregnancy cohort (2000–2012) completed a first trimester interview assessing current alcohol use, change in consumption in the past four months, and alcohol use prior to change. We used logistic regression to estimate the association between alcohol exposure in each week of gestation and miscarriage. We also measured the association between duration of alcohol exposure and miscarriage using Cox proportional hazards models. Models were adjusted for maternal age, race/ethnicity, parity, smoking status, education, and pregnancy intention.

Results: Among 5,424 participants, 50% reported alcohol use in early pregnancy and 12% experienced miscarriage. Median gestational age at change in alcohol use was 30 days (inter-quartile range 21–36). Exposure in gestational week five and onward was associated with a significant increase in miscarriage risk (range of adjusted odds ratios for week five through twelve: 1.28–4.40). Each additional week of alcohol exposure in pregnancy associated with a 6% increase in risk (hazard ratio 1.06, 95% confidence interval 1.04–1.08).

Conclusions: While it would be optimal if alcohol use in pregnancy was completely avoided, a high proportion of women are exposed until pregnancy detection. Alcohol use in week five of gestation, around the time pregnancy is detectable through a home test, and beyond is associated with increasing risk of miscarriage. Early pregnancy recognition could curtail the risk of miscarriage attributable to alcohol use in pregnancy.

Leprosy: Stigma Continuity in Brazil

Alexandra Llovet

Halle Institute for Global Research and Learning, Emory University, Atlanta, GA

Purpose: (1) spread awareness that Leprosy is still a problem to be addressed, (2) develop an understanding of stigma and stereotypes in relation to disease.

Introduction: Leprosy exemplifies the social, physical, and mental repercussions that disease can have for patients. Brazil and India have the most newly diagnosed cases of leprosy per year. From the 1920s to the 1970s, Brazil's health policies required that patients be placed in leprosaria distant from their loved ones and isolated. These policies traumatized and dehumanized patients. I use an interdisciplinary approach to analyze the continuity of discrimination and stereotyping of leprosy in Brazil.

Methods: Over two summers in Brazil, I compiled four types of sources from 1924 to 2018: (1) literary sources, (2) visual sources, (3) medical records, and (4) ten oral narratives. Using literary analysis of themes and representations, I analyze visual and literary sources of Leprosy in Brazil. I gathered ten oral narratives of healthcare workers and patients through snowball sampling in two leprosaria, a state reference hospital, and local clinics. I used medical records (1920s-1980s) from the Emilio Ribas Public Health Museum's archives in São Paulo that show a narrative of the patient's lives in the leprosaria, detailing runaways, removed children, etc. and clinical treatment.

Results: The pool of sources help to understand life when labelled a "leper" in Brazil. Through the diversity of sources across time and intended fields (literature, art, public health), I show the continuity of stigma and fear despite leprosy's curability with multidrug therapy since the 1970s.

Discussion/Conclusions: Leprosy in Brazil shows the failure of purely biomedical medicine in treating patients. Lack of empathy and consideration for patients' quality of life has longstanding effects that persist even after a biological cure is discovered. These findings are now being drafted into my Spanish and Portuguese Honors Thesis.

Improvements in Behavioral Skills in Teens with ASD Through Social Intervention

Schyler C. Said, Melissa A. Allison, Jordan A. Ko MA., Ty W. Vernon Ph.D.

Purpose:
Individuals with Autism Spectrum Disorder (ASD) often exhibit patterns of eye contact and speaking/listening ratios that differ from the typically developing individual. As these behaviors serve as measurable indicators of social competence, they are targeted through the Social Tools and Rules for Transitions (START) program.

Methods:
34 adolescents, aged 12-17 with a pre-existing diagnosis of ASD (verbal IQ > 70) enroll in the Social Tools and Rules for Teens (START) group, a 20 week social-skills intervention consisting of weekly 2-hour treatment groups facilitated by undergraduate research facilitators. Each participant participated in two 5-minute video recorded conversations with typically developing (TD) peers. Conversations were recorded at week 0 and 20 time points, and coded for behaviors of eye contact, speaking, and listening. Measures of eye contact ratios are assessed for positive gains.

Results:
Participants in START maintained eye contact 49.3% and 66.1% pre- and post-intervention, respectively. Percentages of eye contact during speaking (SEC) and eye contact during listening (LEC) for ASD individuals at week 0 are 42% and 55%, respectively. Percentages of SEC and LEC for ASD individuals at week 20 are 58% and 74%, respectively.

Discussion:
Participants in START exhibit significant increase ($p < 0.0001$) in overall eye contact following intervention. As eye contact is a social marker for individuals with ASD, these results indicate positive gains in social competency. Similar studies report TD individuals maintain eye contact 62% of the time while listening and 43% of the time while speaking (Turkstra, 2005). Percentages of SEC and LEC in individuals with ASD post-intervention show statistically significant gains in eye contact (SEC p value 0.0053, LEC p value <0.0001). These values establish that individuals with ASD follow the social norm in sustaining increased eye contact during periods of listening than during speaking.

Novel Prognostic and Therapeutic Gene Signature Upregulated in Uterine Serous Carcinoma Patients with Poor Survival

Lynn K. H. Tran*, Emily Myers*, David P. Mysona, MD+, Paul Tran*, Wonsok Lee, MD**, John J. Wallbillich, MD*++, Daniel Kleven, MD**, Sharad Ghamande, MD++, and Jin-Xiong She, PhD*+; *Center for Biotechnology and Genomic Medicine, Medical College of Georgia at Augusta University, Augusta, GA; +Department of Obstetrics and Gynecology, University of North Carolina, Chapel Hill, NC; ++ Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Medical College of Georgia at Augusta University, Augusta, GA; ** Department of Pathology, Medical College of Georgia at Augusta University, Augusta, GA

Purpose: Uterine serous carcinoma (USC) is a rare but particularly invasive histological subtype of uterine cancer, the most common gynecologic malignancy in developed countries. Though previous studies with small sample sizes have been conducted to identify transcriptomic and proteomic biomarkers for USC, none have resulted in a clinical assay for patient risk stratification. I have discovered a panel of 78 genes highly upregulated in poor prognosis patients from The Cancer Genome Atlas (TCGA) USC dataset.

Methods: To create a composite score for our biomarker panel, I applied a machine learning algorithm (elastic net regression) to the TCGA USC gene expression data set to generate a model which outputs a linear predictor score (USC78) based on individual patients' expression of the 78 genes from our signature. I then quantified gene expression from formalin-fixed paraffin embedded (FFPE) tumor tissue in our cohort of Augusta University (AU) patients and calculated their USC78 score to demonstrate the ability of this model to separate good and poor survival prognosis in a single-center, retrospective validation study.

Results: In both TCGA and AU, higher USC78 scores are associated with worse overall survival. This score is also able to risk stratify serous ovarian carcinoma patients in the TCGA data set, suggesting that USC78 may be an important prognosis prediction tool in serous gynecological cancers. Additionally, network analysis of these genes reveals increased TGF-B signaling correlates with the poor-prognosis USC78-high tumor expression profile. TGF-B inhibition has demonstrated chemosensitization effects in primary USC tumor cell lines.

Discussion/conclusion: I aim to use USC78 to better understand the biological mechanisms behind poor survival prognosis in USC patients and identify potential therapeutic targets for USC. One such target is TGF-B inhibition, and further knockdown, overexpression, and small molecule inhibitor studies will be conducted to test whether TGF-B inhibition can improve USC patient prognosis.

Discovery Pipeline of Cancer Transcriptomes Using Machine Learning

Eileen Kim[1], Lynn Tran[1,2], Paul Tran[1,2], Bruno Dos Santos[1], Sharad Purohit PhD[1], Jin-Xiong She PhD[1,3]

[1]Center for Biotechnology and Genomic Medicine

[2]University System of Georgia MD-PhD Program

[3]Department of Obstetrics and Gynecology, Medical College of Georgia at Augusta University, Augusta, GA

Introduction: Cancer affects millions of people and causes nearly 1 in 6 deaths worldwide. Precision oncology holds potential in guiding cancer treatment on a molecular basis and elucidating targetable cancer pathways. This study aims to create a discovery pipeline that uses cancer patients' transcriptomes and machine learning algorithms to predict cancer patient survival.

Methods: Transcriptomic and phenotypic data from cancer patients were downloaded from The Cancer Genome Atlas (TCGA). Initial genes were selected using univariate cox proportional hazard models to predict overall survival across all cancer types. Co-expressed selected genes were clustered, creating gene sets per cancer subtype. Then, an amalgam of LASSO and elastic-net regularized generalized linear models (GLMNET), gene bootstrapping, random forest without/with shadow features (Boruta), and recursive feature elimination were used. Monte Carlo iterations were performed for GLMNET to assess model stability and over-/underfitting.

Results: The highest hazard ratios (HRs) and lowest p-values resulted from the univariate cox proportional hazards model (e.g. for the RASGEF1A gene in a uterine serous carcinoma model, HR=2.8e8, p=0.005) and subsequent GLMNET models (e.g. 392 genes for uterine endometrioid carcinoma, HR=2.89e8, p=0.00873). Boruta before GLMNET yielded lower HRs and higher p-values (e.g. 11 genes for uterine serous carcinoma, HR=2, p=0.0517). Bootstrapping genes before GLMNET generally yielded much lower HRs and higher p-values.

Conclusions: We were able to model survival risk in cancer patients with a variety of methods. The best method so far is univariate gene selection and GLMNET. Next steps include investigating the biology of prognostic models, using disease-specific survival, analyzing possible confounders, using Breslow-Wilcoxon or accelerated failure time models, replacing histological with molecular-signature-based cancer subtypes, and exploring alternative methods for prognosis prediction.

A novel light-inducible CRISPR/dCas9 system for controlling gene expression.

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

Neurobiology, University of Alabama at Birmingham, Birmingham, Alabama.

Introduction: The tightly regulated control of gene expression is critical to life, and when these processes are disrupted disease can occur. As gene expression both directly influences and is altered by cellular activity, being able to study rapid gene expression fluctuations is necessary to understanding normal cellular physiology and many diseased states. Investigations of gene expression have relied on overexpressing or knocking down genes of interest, but most approaches lack the temporal precision to directly study how cells are modified by and respond to changes in their environment on the timescale at which these alterations occur. Development of technology capable of mimicking these fine-tuned manipulations has proven difficult.

Methods: Through the fusion of the light regulated Flavin Kelch-repeat F-box1 (FkF1) and GIGANTEA (GI) elements with deactivated Cas9 (dCas9) and a transcription activator, we created a new system to target specific genes for upregulation in the presence of blue waveform light (470nm). When this FkF1 Light Induced CRISPR Construct system (FLICC) is compared to an existing light-activated CRISPR based transcriptional control system (LACE), GFUCC confers several specific advantages. For example, previous reports suggest that GFUCC will require shorter exposure times to harsh blue light than LACE to activate.

Results: Under the control of blue waveform light, FLICC successfully upregulated both endogenous gene targets at the mRNA level and a luciferase reporter at the protein level, allowing tight temporal manipulations of gene expression. GFUCC also demonstrates less leaky baseline gene upregulation relative to LACE at targeted loci.

Conclusion: FLICC provides the tight temporal control of gene expression required to investigate fluctuations in gene expression at the time scale on which they occur, while offering specific benefits over currently available technologies.

Interactions between the HIV-1 Glycoprotein 41 Cytoplasmic Tail and the Gag Matrix Protein

Eliot Forster-Benson[1,3], Geoffrey Li[1,3], Jing Zhou[2], Arina Hadziselimovic[1,3], Chris Aiken[2], Charles Sanders[1,3]

[1]Department of Biochemistry, Vanderbilt University

[2]Department of Pathology, Microbiology, and Immunology, Vanderbilt University

[3]Center for Structural Biology, Vanderbilt University

The HIV-1 envelope spike is a protein complex that is responsible for binding to and entry into host cells by fusing the membranes of an HIV virion and a healthy immune cell. Glycoprotein 41 (gp41) is a subunit of the complex, comprising of an extracellular domain, a transmembrane domain, and a cytoplasmic tail (CT). The function of the 150-amino acid long gp41CT in viral assembly is thought to be mediated by interactions with the matrix protein subunit of the Gag polyprotein (Gag MA). The overall goal of our study is to biophysically characterize the structures and interactions of gp41CT and Gag MA in a native-like environment via NMR. We found evidence for a direct interaction between the two proteins via chemical shifts in NMR spectra and a sedimentation assay, therefore more structural studies are to follow. A more complete understanding of gp41CT's and Gag MA's structures and interactions, with both model membrane systems and each other, will shed light on mechanisms of action in HIV-1 replication and open the door to potential antiviral targets and immunotherapy.

Mechanistic Insights on the Broad Spectrum Antiviral Potential of Disrupting Host-Cell Arginine Pathways

Nazary Nebeluk[1], Diana Battaglia[1], Maria Dulfary Sanchez Pino[3,4], Augusto Ochoa[3,5], and Timothy P. Foster[1,2,3]

[1]Department of Microbiology, Immunology and Parasitology, [2]Department of Ophthalmology, [3]Department of Medicine, [4]Department of Genetics and [5]Stanley S. Scott Cancer Center, Louisiana State University Health Sciences Center, New Orleans, Louisiana, 70112.

Most antivirals have been engineered to disrupt a single viral process essential for viral replication. This approach has limited therapeutic effectiveness due to restricted viral specificity, development of drug resistance, and an inability to control disease-promoting inflammation. As obligate intracellular pathogens, viruses are reliant on host metabolism and macromolecular synthesis pathways to facilitate replication. In particular, the bioavailability of arginine and its metabolites is required for replication of most viral pathogens and initiation of disease-promoting pathophysiology. Arginine metabolites are shunted to pathways critical for viral genomic replication, protein synthesis, and intracellular energy reserves. Evaluation of two representative human pathogens, Herpes Simplex Virus (HSV) and Adenovirus (AdV), indicated that arginine was depleted during lytic viral replication. In addition, our data shows that increasing levels of arginine, but not lysine or glutamine, in the extracellular environment significantly increase the amounts of infectious progeny virion produced in a dose dependent manner. Therefore, we hypothesized that therapeutic disruption of host arginine-associated metabolic pathways would have broad antiviral activity across multiple stages of the viral replicative process. Utilizing a biological therapeutic, pegylated-Arginase-1 (peg-Arg1), which modulates arginine pathways by depleting extracellular arginine we observed that peg-Arg1: i) exhibited no cytotoxicity; ii) significantly decreased infectious viral yields; iii) reduced viral transmission and spread; iv) ameliorated virus-mediated cytopathic effects and cell lysis; v) reduced expression of select viral proteins, including viral DNA polymerase; vi) inhibited viral genomic replication and vii) modulated the transcription patterns of key viral genes. Our findings illustrate that targeting host arginine-associated metabolic pathways is a potential means of controlling both viral replication and disease. The combined antiviral and disease resolving activities of peg-Arg1 represent a novel approach with vast therapeutic potential. Future studies will explore the breadth of viruses inhibited by this approach, as well as the ability to suppress arginine-associated virus-mediated clinical disease.

DAMPs-coated Prussian blue nanoparticles as photothermal-nanoimmunotherapy agents for cancer

Armando J. Ruiz-Justiz[1,2], Juliana Cano-Mejia[1], Rohan Fernades[1], Ph.D.

[1]Department of Pediatrics, The George Washington University, Washington DC

[2]Deparment of Biology, The University of Puerto Rico at Ponce, Ponce, PR

Nanoparticle-based photothermal therapy (PTT) is a rapid and minimally invasive tumor ablation technique where synthesized nanoparticles allow the conversion of incident light into heat, which kills cancer cells due to cell temperature increases. We used Prussian blue nanoparticles (PBNPs) as photothermal agents because they exhibit strong absorbance at near-infrared (NIR) wavelengths, are stable, non-toxic, and have 20.5% photothermal conversion efficiencies. Previous studies, using PBNPs to administer photothermal therapy (PBNP-PTT) to localized tumors in a neuroblastoma model, have shown that under certain conditions PBNP-PTT generates immunogenic cell death (ICD). ICD is defined by the release or exposure of danger-associated molecular patterns (DAMPs) from dying cancer cells and these serve as powerful immunological adjuvants to activate a cytotoxic T lymphocyte response. The PBNP-PTT-stimulated release of DAMPs from dying cancer cells depends on the cell type and conditions of PBNP-PTT, DAMPs may or may not be released sufficiently to generate downstream responses. To ensure that PBNP-PTT induces the release and expression of the correct DAMPs to induce ICD, we synthesized DAMPs-coated PBNPs for use in PBNP-PTT. Our hypothesis is that PBNP-PTT allows to heat cancer cells, causing exposure of some DAMPs, while simultaneously releasing other DAMPs from the PBNPs, augmenting the immunogenic effect. Here, we show the synthesis strategy for coating PBNPs with DAMPs, the amount of DAMPs bound to PBNPs using the Bio-Red Protein Assay, and the stability of the DAMPs-PBNPs over several days. We present consistent data showing the cytotoxic capability of DAMPs-PBNP-based PTT by a luminescence-based viability assay. Additionally, we compare the effects of applying PBNP-based PTT on the surface versus interstitially, to further optimize our photothermal nanoimmunotherapy.

Adoptive cell transfer in combination with peptide vaccination enhances the anti-tumor response of genetically engineered T cells

Aaron Fan PhD*^, Takumi Kumai MD, PhD+, Hussein Sultan PhD*‡, Esteban Celis MD, PhD*

*Georgia Cancer Center, Augusta University, Augusta, GA, USA.
^University System of Georgia MD/PhD Program, Medical College of Georgia, Augusta, GA, USA.
+Department of Otolaryngology-Head and Neck Surgery, Asahikawa Medical University, Midorigaoka-Higashi, Asahikawa, Japan.
‡Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA.

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, as demonstrated by total 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.

TIP60 Depletion as a Mechanism of PARP Inhibitor Resistance in BRCA2 deficient cells

Anastasia Hale[1]; Kristen Clements[2]; George-Lucian Moldovan PhD[3]

[1] Schreyer Honors College, Penn State University

[2] Dept of Biochemistry and Molecular Biology, Penn State College of Medicine

[3] Dept of Biochemistry and Molecular Biology, Penn State College of Medicine

Purpose: Essential for double strand break repair, BRCA2, a vital tumor suppressor gene, is often mutated in human tumors, particularly breast and ovarian cancer. Poly-ADP-ribose polymerase inhibitors (PARPi) are used to treat tumors with these mutations, as loss of BRCA2 and inhibition of PARP1, an enzyme essential for DNA repair, results in synthetic lethality. However, resistance to these drugs is a major clinical problem. In order to understand this phenomenon, we performed a CRISPR knockout screen to identify proteins that, when depleted, cause PARPi resistance in BRCA2-deficient cells. From the screen results, we chose to investigate TIP60, a histone acetyltransferase with known roles in DNA repair.

Methods: To test if loss of TIP60 confers PARPi resistance, we depleted TIP60 using siRNA and used CellTiter-Glo and Annexin staining with flow cytometry to investigate how TIP60 depletion affects cell viability and apoptosis, respectively, after PARPi treatment. Furthermore, to identify the mechanisms underlying this resistance, we investigated two processes known to promote PARPi resistance: increased double strand break repair and stabilization of replication forks.

Results: We observed that TIP60 depletion rescues sensitivity to PARPi treatment in HeLa cells depleted of BRCA2. TIP60 depletion causes an increase in cellular viability in cells deficient of BRCA2 after PARPi treatment, and also prevents PARPi-induced apoptosis. Interestingly, we found that TIP60 depletion does not affect the double strand break repair mechanisms homologous recombination or non-homologous end joining, suggesting that TIP60 loss may lead to PARPi resistance through stabilization of the replication fork or perhaps an entirely novel mechanism.

Conclusion: We conclude that TIP60 depletion decreases the sensitivity of BRCA2-deficient cells to PARPi. Experiments are underway to investigate the way in which TIP60 loss causes this resistance. Ultimately, we propose TIP60 as a putative biomarker to predict patient sensitivity to PARPi, which should be validated with clinical studies.

An Osteopontin immune checkpoint controls CD8+ T cell activation and tumor immune evasion

John D. Klement,[1,2,3] Amy V. Paschall PhD,[1,2,3] Priscilla S. Redd PhD,[1,2,3] Mohammed L. Ibrahim,[1,2] Chunwan Lu PhD,[1,2,3] Dafeng Yang,[1,2,3] Esteban Celis MD PhD,[2] Scott I. Abrams PhD,4 Keiko Ozato PhD,5 and Kebin Liu PhD,[1,2,3]

[1]Department of Biochemistry and Molecular Biology, [2]Georgia Cancer Center, Medical College of Georgia, Augusta, GA 30912. [3]Charlie Norwood VA Medical Center, Augusta, GA 30904, USA. [4]Department of Immunology, Roswell Park Comprehensive Cancer Center, Buffalo, NY 14263, USA. [5]Division of Developmental Biology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA.

Purpose: Tumors evade immune-mediated clearance in part by the expression of co-inhibitory checkpoints that attenuate CD8+ cytotoxic lymphocyte (CTL) function. Despite breakthroughs in immune checkpoint inhibitor (ICI) immunotherapy such as anti-PD1 and -CTLA4 monoclonal antibodies (mAbs), many patients and cancer types remain refractory to ICI therapy, suggesting the existence of additional immune checkpoints. Here, we observed that colorectal cancer (CRC) and myeloid cells silence expression of the transcription factor interferon regulatory factor 8 (IRF8) to upregulate osteopontin (OPN) expression, which we show to be a potent inhibitor of CTL activation.

Methods: ChIP-qPCR and EMSA were used to assess IRF8 binding to the Spp1 (OPN) promoter. Human CRC gene expression levels were accessed through TCGA COADREAD database; patient CRC serum samples were obtained from the GCC Biorepository. Human and mouse CTL activation and proliferation was measured by surface marker expression and CFSE dilution, respectively, following in vitro CD3/28 mAb stimulation. OPN transcript and protein levels were assayed by qPCR, ELISA, and intracellular flow cytometry.

Results: Splenocytes from IRF8.KO mice expressed tenfold higher Spp1 transcript levels due to a massive expansion in CD11b+Gr1+ myeloid cells, which demonstrated silencing of IRF8 in wild-type controls. In a mouse inflammation-driven colorectal cancer model, IRF8 was shown to be a transcriptional repressor of OPN in colon epithelial cells, leading to increased serum OPN levels in CRC-bearing mice. Accordingly, IRF8 was found to repress OPN in human CRC patients, who display elevated OPN levels; OPNhi patients have decreased disease-specific survival. These increased levels of OPN were found to inhibit human and murine CTL activation, proliferation and effector functions in vitro.

Conclusion: Loss of IRF8 in myeloid cells and colonic enterocytes leads to increased expression of OPN, which represses CTL effector function. Future experimentation centers on the contribution of OPN to immune suppression in the tumor microenvironment.

ABSTRACTS – POSTER SESSION 1

Regulation of Central Nervous System Endogenous Opioid mRNA in Chronic Binge Alcohol (CBA) Treated Male Rhesus Macaques Infected with Simian Immunodeficiency Virus (SIV)

Brittany L. Foret, MS, Scott Edwards, PhD, Patricia E. Molina, MD, PhD

LSUHSC New Orleans, Dept. of Physiology, New Orleans, LA. Comprehensive Alcohol Research Center, New Orleans, LA.

Purpose: Over 36.7 million people are living with HIV/AIDS (PLWHA) around the world, with 1.1 million of those people living in the US. With the advent of antiretroviral therapies (ART), the lives of PLWHA have been extended, allowing for the manifestation of previously unseen comorbidities. Neuropathic pain is the most common neurological complication affecting almost 30% of PLWHA. Additionally, many PLWHA have alcohol use disorders (AUD), which are known to independently cause neuropathic pain.

Methods: Previous studies of neuropathic pain in mice have shown decreases in opioid receptor availability and remodeling in the frontal cortex. No studies, however, investigate the brain in HIV-associated neuropathic pain, or explore the comorbid effects of alcohol and HIV infection on neuropathic pain. Our study aimed to fill in these gaps by exploring how HIV infection changed the expression of opioid genes in the frontal cortex and how alcohol exacerbated these changes. We hypothesized seeing decreased levels of both opioid ligand and receptor mRNA in the frontal cortex, and those decreases being exacerbated by chronic binge alcohol (CBA).

Results: To test our hypothesis, we used a non-human primate model of CBA, simian immunodeficiency virus (SIV) infection and ART. Male rhesus macaques were either administered CBA or isocaloric sucrose through an intragastric catheter at the start of the experiment, inoculated with SIV Mac251 3 months later, and half of the animals received ART (emtricitabine/tenofovir). At 11 months post-infection, animals were euthanized, brains excised, and frontal cortex tissue used for mRNA extraction. Our results show CBA and ART interact to decrease kappa opioid receptor (KOR) mRNA levels. While not statistically significant, the effects of CBA and ART independently appear to decrease mRNA levels of KOR.

Conclusion: Our results indicate a need for future studies to better understand the molecular changes associated with neuropathic pain, and how CBA effects those changes.

Rac1-Induced Mitochondrial ROS mediates Mitochondrial Biogenesis and Pro-fibrotic Polarization of Macrophages in Pulmonary Fibrosis

Shelby Harris1,2, Jennifer L. Larson-Casey3, A. Brent Carter3,4,

Summer in Biomedical Sciences1, Jacksonville State University2, Department of Medicine, Division of Pulmonary, Allergy, & Critical Care Medicine, The University of Alabama at Birmingham, Birmingham, AL3, Birmingham VAMC, Birmingham, AL4

Purpose: Idiopathic pulmonary fibrosis (IPF) is characterized by the formation and build-up of scar tissue in the lungs. Causing the lungs to become stiff and reduce lung function. There is no known cause of IPF, and current treatments for the disease are not effective in stopping the progression of IPF. This project investigates a pathway by which Rac1, a small GTPase, induces mitochondrial reactive oxygen species (ROS) that results in mitochondrial biogenesis and alternative activation of lung macrophages to a pro-fibrotic state in pulmonary fibrosis.
Methods: For these studies, THP-1 and MH-S cells were transfected with four different plasmids: Empty (control), Rac1WT (wild-type), Rac1CA (constitutively active), or Rac1DN (dominant negative). Isolated mitochondrial samples were used for immunoblot analysis to confirm overexpression and localization of Rac1 to the mitochondria. The generation of mitochondrial hydrogen peroxide was determined by pHPA assays. RNA was also used for real-time PCR to measure the effect of Rac1 on gene expression. MitoTracker green and siRNA was used to stain mitochondria for biogenesis.

Results: Immunoblot analysis showed that Rac1 was overexpressed and localized to the mitochondria in the Rac1WT. RT-PCR revealed that fibrotic genes, such as Ym-1 and TGF-?1, were increased in cells expressing Rac1WT compared to the empty control. The pHPA assay demonstrated that the Rac1WT cells generated significantly more H2O2 than Rac1DN or empty. Silencing Rac1 with siRNA decreased fluorescence of MitoTracker green, while overexpression of Rac1CA increased TFAM in mitochondria. In contrast, cells expressing Rac1DN has a marked decrease in TFAM.

Conclusion: Macrophage mitochondrial ROS has a critical role in the pathogenesis of IPF. We found that the mitochondrial localization of Rac1 increases mitochondrial ROS, which mediated mitochondria biogenesis and increased expression of pro-fibrotic factors in macrophages. These data suggest that Rac1-mediated mtROS plays a vital role development of pulmonary fibrosis.

A functional module for a response to low nutrients in Arabidopsis

Muntathar J Alshimary[1], DJ Speed, Allosin Scott, Jean Greenberg[1]

[1]Department of Molecular Genetics and Cell Biology, University of Chicago, IL

Plant survival and performance depend on the plant's ability to efficiently explore their environment to find water and nutrients. Zinc (Zn) and Iron (Fe) are essential micronutrients for plants. A recent report suggests that the protein AZI1 promotes root growth in low Zn media. AZI1 also has a role in salt stress tolerance and systemic immunity to pathogens. In these processes, AZI1 functions in a module with its paralog EARLI1 and the proteins MPK3/MPK6. MPK3/MPK6 are kinases that regulate AZI1's localization to multiple membranes, including plastid envelopes, an important site of defense metabolite production.

I hypothesized that AZI1 acts together with EARLI1, MPK3 and MPK6 to modulate root growth in nutrient-limited conditions. To test this, I grew mutants lacking these proteins on –Zn, –Fe, and complete media and compared their root lengths over time. Unlike wild type, the mutants failed to show increased growth on Zn-limited media. Thus, AZI1, EARLI1, MPK3 and MPK6 may constitute a functional module in root growth in low Zn conditions. However, there is no evidence that they promote root growth on limited iron.

AZI1's protein features suggest that it uses a non-typical signal anchor mechanism for plastid localization. I produced a fusion protein to test whether AZI1's plastid envelope location might involve AKR2, a chaperone in the signal-anchor pathway. This construct will enable future research concerning targeting mechanism of AZI1. Investigating AZI1's function and localization is important for understanding how plants respond to environmental stressors, which is a necessary step in ensuring global food security.

Development of biomarker assays to measure urinary proteins.

Katherine Pinkerton, Roshni Patel, Lynn Tran, Paul Tran, Wenbo Zhi, Sharad Purohit, Jin-Xiong She

Glomerular damage from uncontrolled hyperglycemia and hypertension often lead to proteinuria, a common indicator of renal damage in diabetes patients. Early stages of diabetic nephropathy show inconsistent signs of proteinuria; however, as the disease progresses the concentration of proteinuria can be 10 times above normal levels. Current screening methods measure urine albumin levels as a test for diabetic nephropathy. Previous research in current lab identified several <30kDa molecular weight proteins (A1AGP, RBP4, A1ZGP) to be present in urine from diabetic nephropathy patients using mass-spectrometric analysis. The results show that low molecular weight proteome present in micro albuminuria, suggests that nephropathy can be detected earlier than appearance of albumin, which is a high molecular weight protein. These potential markers need to be validated using another method which can be more acceptable as a clinical assay. Immunoturbidimetry assays are widely acceptable clinical assays that are routinely used in clinics for routine urine analysis of diabetic nephropathy. In order to develop an immunoturbidimetry assay antibodies specific to A1AGP, RBP4, A1ZGP were needed. These proteins were produced by recombinant protein technology and peptide synthesis. The anti-protein antibodies were produced in rabbits, tested using antigen ELISA. After which, the immunoturbidimetry assays were developed in lab.

Recombinant Expression of Collagen IV from *Ministeria vibrans*: The Ancestral Collagen of the Animal Kingdom

Favour Akabogu[1,2]; Ly Thien Hoang[1]; Sergei V. Chetyrkin, Ph.D.[1,3,4]; Aaron L. Fidler, Ph.D.[1,3,4]; Julie K. Hudson, M.D., M.A.[1,5]; Billy G. Hudson, Ph.D.[1,3,4]

[1]Aspirnaut™, Vanderbilt University Medical Center, Nashville, TN, USA

[2]Berea College, Berea, KY, USA

[3]Department of Medicine, Division of Nephrology

[4]Vanderbilt Center for Matrix Biology

[5]Department of Medical Education and Administration, Vanderbilt University Medical Center, Nashville, TN, USA

The transition from unicellular protists to multicellular animals coincided with the appearance of a specialized extracellular matrix (ECM), the basement membrane (BM). BMs are dynamic structures that modulate cell differentiation and behavior during development, and help shape tissue architecture. Collagen IV, a major component of BMs, forms large networks that provide tensile strength to tissues and function as smart scaffolds organizing diverse macromolecules in the BM. Importantly, collagen IV is conserved across all animals and likely played a role in the transition to animals. However, the mechanism in which collagen IV enabled this transition is unknown. The protist, Ministeria vibrans, has recently emerged as the first unicellular organism to contain collagen IV based on genomic and transcriptomic evidence. Here, we sought to build a construct with Ministeria collagen IV for recombinant expression in Chinese hamster ovary (CHO) cells. We have successfully cloned the Ministeria collagen IV gene, however, generation of a construct for transfection into CHO cells has not been successfully completed. Future work will include biochemical characterization of Ministeria collagen IV expressed by CHO cells utilizing fast protein liquid chromatography (FPLC) and Western blotting to determine how Ministeria collagen IV behaves in a unicellular organism. Together, these will provide insight into the ancestral function of collagen IV, and how collagen IV played a role in the evolutionary transition to multicellular animals.

Elucidating the Relationship Between Skeletal Muscle Injury-Induced Inflammation and Mitochondria

Anita E. Qualls[1,2], W. Michael Southern[1,2], Jarrod A Call[1,2]

[1] Department of Kinesiology, University of Georgia, Athens, GA, USA

[2] Regenerative Bioscience Center, University of Georgia, Athens, GA, USA

Previous studies on dermal burns and sepsis have demonstrated a systemic inflammatory response related to skeletal muscle and mitochondrial dysfunction. However, the effect of inflammation from localized stress, such as skeletal muscle injury, on mitochondrial function remains largely unknown. In this review, we have identified four significantly upregulated cytokines (chemokine (C-C) motif ligand 2, chemokine (C-X-C motif) ligand 1, Interleukin-6, and Interleukin-1) from inflammatory gene array data across three skeletal muscle injury models (freeze, volumetric muscle loss, and eccentric contraction). We describe signaling pathways related to each cytokine's function in the inflammatory response (e.g., functioning as chemoattractants to tissue damage), and highlight evidence of these pathways directly impacting mitochondrial function and structure. Ultimately, we postulate that the inflammatory response from a skeletal muscle injury acts as a "stressor" that the mitochondria are sensitive to and react by initiating downstream effects that result in dysfunction of overall physiological relevance, such as negatively impacting skeletal muscle recovery and regeneration.

SP Molecular Surveyor- a tool to track resistance markers for sulphadoxine-pyrimethamine

Brenda Montanez1 , Zaira Jacome1, Taryn Strickland1 , Berenice Vazquez 1, Tamesha Oden1 , Ignacio Suay2 , Suttipat Srisutham3 ,Carol H. Sibley, PhD 2,4,5, Georgina Humphreys, PhD 2,4, Sabina D. Otienoburu, PhD 1,2

1STEM college, Johnson C. Smith University, Charlotte, NC, 2WorldWide Antimalarial Resistance Network, UK, 3 Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, 4 Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, UK, 5 Department of Genome Sciences, University of Washington, Seattle, WA.

Purpose: To build a database for the SP Molecular Surveyor, a bioinformatics tool tracking the spread of Plasmodium falciparum markers associated with resistance to sulphadoxine-pyrimethamine (SP). SP is an antimalarial drug used as intermittent preventive treatment in pregnant women.

Method: A literature search was conducted in PubMed, where publications were selected based on specific inclusion criteria. Prevalences of mutations in the dihydrofolate reductase (dhfr) and dihydropteroate synthase (dhps) genes, associated with resistance to SP, and metadata were extracted and entered into an online form. In the online Molecular Surveyor, a pin displayed each study, based on location of sampling and coloured according to marker prevalence.

Results: The data displayed on SP Molecular Surveyor can be used to visualize the prevalence of resistance markers by marker, country, year, or sample size. In the literature search, we identified 201 studies published 2013-2017, of which 92 were included in the database. Studies were excluded due to lack of genotypic data and misrepresentation of baseline samples, amongst other reasons. Most publications were done in Africa (64%, n=59) , whereas North America had fewest publications (1% , n=1). According to a draft recommendation by World Health Organization (WHO), if a country's prevalence levels reach >95% and >10% for markers 540E and 581G, respectively, a new treatment could be introduced. The data shows there are no countries that fulfill these criteria, therefore, SP can still be used.

Discussion/Conclusion: The SP molecular surveyor provides researchers and policy decision-makers with relevant, real time data on drug resistance trends. This geo-visualization tool is consistently updated, and allows users to analyze the prevalence of resistance markers to sulphadoxine-pyrimethamine. This data could be utilized to change the course of treatment in areas with decreased efficacy, and allows the user to easily identify areas in which treatment is still effective.

Enrichment of SNP-heritability of psychiatric disorders in gene and isoform modules

ANGELA WEI1,2, Michael Gandal MD/PhD3, Kathryn Burch4*, Claudia Giambartolomei PhD5*, Bogdan Pasaniuc PhD5,6,7*

1. BIG Summer Program, Institute for Quantitative and Computational Biosciences; 2. Department of Mathematics, University of Kentucky; 3. Department of Psychiatry and Biobehavioral Sciences, UCLA; 4. Bioinformatics Interdepartmental Program, UCLA; 5. Department of Pathology and Laboratory Medicine, UCLA; 6. Department of Human Genetics, UCLA; 7. Department of Biomathematics, UCLA

Purpose: Gene co-expression networks, or modules, are groups of genes that share similar expression patterns. Integrating gene modules with large-scale genetic data can help uncover mechanisms underlying complex diseases, such as psychiatric disorders. Here, we integrate 34 gene and 56 isoform modules obtained from cerebral cortex samples (n=1,322) of patients with autism (ASD), schizophrenia (SCZ), and bipolar disorder (BIP) with genome-wide association studies (GWAS) of BIP (n=46,918), SCZ (n=105,318), and ASD (n=10,610).

Methods: To quantify the enrichment of single nucleotide polymorphism (SNP)-heritability within each module, we defined genes from their transcription start site to their gene end site with either a 0 kb or 10 kb window and annotated SNPs within these gene bodies as 1 and 0 otherwise. We then used stratified linkage disequilibrium (LD) score regression to examine whether SNPs inside each module have larger effects than expected by chance.

Results: No gene or isoform modules were significant in the ASD 2017 GWAS for both gene window sizes. Altering the gene window size both increased or decreased the number of significant (FDR-corrected) gene or isoform modules for the BIP 2018 and SCZ 2018 GWAS.

Discussion/Conclusion: Lack of significant modules in the ASD 2017 GWAS may be due to its small sample size. Several gene and isoform modules were significantly enriched in the SCZ 2018 & BIP 2018 GWAS, such as isoform module 13 – associated with protein serine/threonine kinase activity. Altering the window size of the annotation affects the proportion of SNPs within a module, which may affect which modules are significant and how large enrichment standard error is. This work could be extended to analyzing other psychiatric GWAS data or recalculating LD scores using different annotations based on differing gene definitions.

Health Management in the Home: A Qualitative Study of Pregnant Women and their Caregivers

Hannah B. Huth (1,2), Ryan Skeens, M.D. (3), Simone Herzberg, B.S. (4), Shilo Anders, Ph.D (2,5) , Christopher Simpson (2), Laurie Novak, Ph.D. (2), and Gretchen P. Jackson, M.D., Ph.D. (2, 6)

1- Indiana University; 2- Vanderbilt University Medical Center, Department of Biomedical Informatics; 3- Vanderbilt University School of Medicine, Fellow of Neonatology; 4- Vanderbilt University School of Medicine; 5- Vanderbilt University Medical Center, Department of Anesthesiology; 6- Monroe Carell Jr. Children's Hospital at Vanderbilt, Department of Surgery and Pediatrics

With growth in available consumer health technologies, patients and caregivers have become increasingly involved in their health and care, with many health-related activities taking place in the home. Pregnancy is a common health condition, and for many women, their first exposure to health-management practices. This study examined the management of pregnancy in the home, not only for pregnant women, but also for a wide variety of caregivers. Pregnant women and caregivers were enrolled from the Fetal Center at Vanderbilt, Expect-With-Me group prenatal care, and genetic counseling referrals. Participants completed sociodemographic surveys and semi-structured interviews in their homes. Interviews were audio recorded, transcribed, and de-identified prior to analysis. Using an interactive, inductive coding approach, we examined home interviews for themes related to the physical home, roles and help in the home, the community, the virtual home, and biggest concerns. Fifty-nine individuals (48 pregnant women, 11 caregivers) participated. Most families encountered everyday problems with mobility, access to information, and household-management activities. Pregnant women desired more assistance from caregivers, and some caregivers did not know how to help. Caregivers who did help took on new roles often involving household chores. Pregnant women reported a desire for dedicated parking in the community. Many expectant families did not trust pregnancy advice found in online forums or social media. Over half of expectant families had biggest concerns that involved the home. These common needs and preferences of pregnant women and caregivers can inform the design of health information technologies and educational resources for expectant families.

Sulfated chondroitin sulfate hydrogel cell carriers improve angiogenesis and arteriogenesis after ischemic stroke in mice.

Myles R. McCrary, Kaleena Jessen, Stephen Tan, Xiaohuan Gu, Lohitash Karumbaiah, Shan Ping Yu, Ling Wei.

Emory University, Atlanta, GA. University of Georgia, Athens, GA.

Ischemic stroke is one of the leading causes of death and long-term disability worldwide. Transplantation of neural progenitor cells (NPCs) can help to repair damaged neural networks and enhance endogenous regeneration after stroke. However, several critical issues remain to be improved for the development of more effective and efficient stem cell treatments. Many transplanted cells die in the harsh ischemic environment and fail to augment regeneration or differentiate into neurons. Hydrogels are biocompatible carrier materials that may overcome some of these challenges. For this study, we used a sulfated chondroitin sulfate (sCS) hydrogel, which can enrich neuro-regenerative factors such as FGF2, BDNF, and the immunomodulatory protein IL-10, as a carrier for NPCs for transplantation into the stroke core. We hypothesized that encapsulation with the sCS hydrogel may improve transplanted cell survival and advance their therapeutic value. Adult transgenic mice expressing α-SMA-GFP were subjected to ischemic stroke targeting the sensorimotor cortex. Intracranial transplants were carried out 1 week after stroke. BrdU was administered daily after treatment to monitor for newly formed cells, and laser Doppler was used to measure cortical blood flow. Mice were sacrificed 1-2 weeks after treatment, and tissue was collected for Western blotting and immunohistochemistry. Western blot analyses indicate that angiogenic markers such as VEGF and VEGF-R2 are significantly increased in stroke animals treated with sCS encapsulated cells. Furthermore, the number of newly formed brain endothelial cells (Glut1+/BrdU+) and the number of microvessels within the stroke core were significantly increased. Arteriogenesis and collateral artery recruitment was also improved with sCS encapsulated NPC treatment. Taken together, these results suggest encapsulating NPCs in sCS hydrogel may augment their abilities to promote vascular regeneration after ischemic stroke.

Targeting ultrasonic neuromodulation in non-human primates with optical tracking-guided MR-ARFI

Sumeeth V Jonathan*, M Anthony Phipps**, Vandiver L Chaplin**, Aparna Singh*, Pai-Feng Yang***, Allen T Newton***, John C Gore***, Li Min Chen***, Charles F Caskey***, William A Grissom*

*Biomedical Engineering, Vanderbilt University, Nashville, TN
**Chemical and Physical Biology, Vanderbilt University, Nashville, TN
***Radiology and Radiological Sciences, Vanderbilt University, Nashville, TN

Purpose: Transcranial focused ultrasound (FUS) has the potential to become a highly specific, non-invasive technology for neuromodulation. Due to its micron-scale sensitivity, magnetic resonance imaging (MRI) guidance via MR-acoustic radiation force imaging (MR-ARFI) could target transcranial FUS procedures performed at low acoustic pressures, like neuromodulation. In this study, we used an optical tracking system to inform the geometry of MR-ARFI acquisitions for image-guided ultrasonic neuromodulation in non-human primates.

Methods: MR-ARFI images were acquired using a diffusion-weighted spin echo 2D MR-ARFI pulse sequence implemented on a high-field 7 Tesla scanner (Philips Achieva). Sonications were performed at 802 kHz with a low duty cycle (2.8 MPa peak negative pressure/3.1 mechanical index) using an MRI-compatible FUS transducer (Sonic Concepts). An optical tracking system (Polaris Vicra) was used to determine the location of the ultrasound beam in real-time, and this information was used to prescribe MR-ARFI scans parallel to the acoustic propagation axis to maximally encode MR-ARFI-derived signal. Our current protocol for in vivo ultrasonic neuromodulation uses prone/head first positioning, targeting the somatosensory network (right S1 areas 3a/3b) of sedated adult macaque monkeys (n = 2). Transcranial MR-ARFI images were acquired in situ after determining the location of the ultrasound beam with optical tracking. Touch circuit-specific neuromodulation effects were further characterized using functional MRI (fMRI), with manual tactile stimulation as a reference.

Results: Our results show that a priori knowledge of the ultrasound beam's position in space is critical for MR-ARFI, and can be determined using optical tracking. In some cases, the observed signal was completely missed if the MR-ARFI scan was prescribed in the wrong orientation. Our results represent the first demonstration of transcranial MR-ARFI feasibility in non-human primates for targeting ultrasonic neuromodulation in vivo.

Conclusion: Our ongoing studies will demonstrate the effectiveness of focused ultrasound in modulating the function of relevant neural circuits in non-human primates.

DECELLULARIZED HUMAN SKIN-DERIVED ECM AS A SUPPLEMENT TO FIBRIN HYDROGEL FOR SKIN BIOPRINTING APPLICATIONS

Zishuai Chou [1 2 3], Adam Jorgensen [2], Anthony Atala, M.D. [2]

[1]Summer Scholar, [2]Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC
[3]Department of Bioengineering, University of California, Berkeley, Berkeley, CA

Purpose: Skin bioprinting has been proposed as a new treatment for full-thickness burn injuries. However, fibrin hydrogel, a potential bioink, has limited post-printing mechanical strength. The aim of this project is to evaluate the efficiency of decellularized human skin-derived ECM supplement in improving fibrin hydrogel's mechanical strength and biological performance.

Methods: Human skin was decellularized and solubilized into an ECM solution. Decellularization efficiency was evaluated through scanning electron microscopy [SEM] and histology. Surface structures of fibrin hydrogel and Fibrin-ECM gel [FEG] were visualized by SEM. Rheological properties were evaluated at varying temperatures and ECM concentrations to optimize printability. Fibroblasts isolated from human skin were cultured in hydrogels. Cell proliferation, viability, and cell-laden construct mechanical strength and structure were evaluated.

Results: SEM and histology confirmed successful skin decellularization. All gel compositions displayed shear thinning properties and their strength decreased at higher temperatures. FEG's strength decreased with increasing ECM concentration before crosslinking, but FEG with 1% ECM demonstrated the highest post-crosslinking strength. Higher ECM concentration caused a decreased rate of cell proliferation in 2D culture, but an increased rate in 3D culture. Cell viability was better maintained in FEG over 15 days. All cell-laden constructs exhibited a decline in mechanical strength over 15 days, but FEG displayed superior storage modulus than fibrin hydrogel at all time points. Histological assessment of the constructs confirmed improved maintenance of structural integrity in the FEG construct.

Conclusion: Decellularization and solubilization of human skin yielded an ECM solution, a potential supplement to fibrin hydrogel for skin bioprinting. 1% ECM was selected as an optimal concentration for FEG based on its enhanced mechanical strength and structural stability, maintenance of cell viability, and improved cell proliferation compared with fibrin hydrogel. Further work must be done to characterize ECM solution protein composition and assess cell activities within the constructs.

Stabilization of siRNA Polyplexes for Improved Systemic Circulation and Enhanced Shelf-Life

Rachel E. Miles, Meredith A. Jackson, Craig L. Duvall

While siNPs hold great promise for in vivo treatment of solid tumors, they must be prepared fresh and have an extremely short shelf life, on the order of four hours. This research aims to test formulations of siNPs with excipients in order to find a formulation that can be stably lyophilized, but retain activity upon reconstitution. These stable siNP formulations were used to target the mTOR pathway in triple negative breast cancer (TNBC) animal models, in support of Meredith Jackson's dissertation project, on which I have collaborated since August 2017. We have used siNPs to inhibit Rictor/mTORC2, a target for which no specific drugs exist. The siNPs are an enabling technology, which will allow us to study the specific role of Rictor in TNBC and the potential therapeutic effect of its inhibition. In addition, triblock polymers have already shown promise in further increasing snNP stability and will be further investigated; the addition of PPS to the core further stabilizes siRNA in conjunction with the DMAEMA-BMA copolymer.

A Computational and Experimental Investigation of Relationships between Magnetic Resonance Diffusion Tensor Imaging Fractional Anisotropy and Applied Mechanical Strain

Ouniol T. Aklilu, Reuben H. Kraft, PhD

Department of Biomedical Engineering, The Pennsylvania State University, PA

Traumatic brain injury (TBI) and related disability affects more than 2% of the U.S. population. Diffuse axonal injury (DAI) is a common pathology associated with TBI in which deformation of axonal cells leads to rupture and axonal degeneration. DAI can be observed in white matter tissue in the brain, which consists largely of bundles of aligned, myelin-sheathed axons. State-of-the-art imaging techniques (magnetic resonance diffusion tensor imaging, or MR-DTI) can be used to visualize these fibrous structures. However, sensitive measures of structural changes due to injury that can be detected with MR-DTI, notably fractional anisotropy (FA), show conflicting trends in how FA changes in response to injury. FA values can be obtained through analyzing the MR-DTI using applicable software such as FMRIB Software Library (FSL).

In this work, we provide a detailed examination of the correlation between MR-DTI changes and mechanical strain using an experimental and computational investigation approach. The research is classified into a three step process which consists of 1) pre-imaging the sample of interest to obtain undamaged MR-DTI data of the sample, including FA measures 2) impose mechanical strain on the sample to cause deformation and rupture of the fibers in the sample and 3) obtain a post MR-DTI image of the sample to observe changes in FA. As high-quality MR-DTI "phantoms" are being manufactured from our collaborator in Germany, the approach is tested using asparagus (asparagus officinalis), which also has a fibrous internal architecture.

Tissue specificity of the DAF-7/ TGFbeta pathway in affecting sperm guidance

Muhan Hu*, BS; Michael Miller*, PhD

*Department of Cell, Developmental, and Integrative Biology, University of Alabama at Birmingham, Birmingham, AL

Purpose: Fertilization is fundamental to the development of sexually reproducing animals. In C. elegans, we have identified a group of structurally similar F-series prostaglandins (PGFs) that guide the sperm towards the spermatheca. Previous studies showed that the DAF-7/ TGF pathway, including the DAF-1 type I receptor and DAF-3 co-SMAD, is essential for regulating sperm guidance and PGF levels. The purpose of this project is to uncover the tissue in which DAF-3 co-SMAD functions to regulate sperm guidance.

Methods: Mosaic analysis was performed in C. elegans to identify the tissue in which the DAF-3 co-SMAD is expressed to modulate sperm guidance. daf-1 (m40); daf-3 (mgDf90) hermaphrodites were injected with a DNA mixture containing a wildtype copy of daf-3 and a sur-5::GFP marker. Cells containing this extrachromosomal DNA have green nuclei. As the transgenic hermaphrodites develop, the extrachromosomal DNA is randomly lost from different cell lineages. These hermaphrodites are then mated with wild-type males to assess the ability for sperm to reach the spermatheca, or the site of sperm storage in the hermaphrodite.

Results: daf-1 (m40); daf-3 (mgDf90) hermaphrodites exhibit sperm guidance levels similar to that of wildtype animals (~90% of sperm reach the spermatheca). Transgenic daf-1 (m40); daf-3 (mgDf90) hermaphrodites expressing wildtype daf-3 ubiquitously are similar to daf-1 (m40) and have poor sperm guidance (~50% of sperm reach the spermatheca). We found that loss of wildtype daf-3 from the intestine and germline of the transgenic daf-1 (m40); daf-3 (mgDf90) hermaphrodites suppresses the sperm guidance defect, restoring the ability of sperm to target the spermatheca.

Conclusion: Proper DAF-3 function in the germline and intestine are necessary for normal sperm guidance towards the spermatheca, suggesting the TGF pathway modulates genes specifically in these tissues to alter prostaglandin synthesis. Further studies are needed to identify the downstream genes responsible for PGF metabolism and sperm guidance.

Role of the Na+/H+ Exchanger in Modulating Resistance to Apoptosis in Pulmonary Arterial Smooth Muscle Cells from Rats with Pulmonary Hypertension

Micheal C. Munson, Xin Yun, Ph.D., Haiyang Jiang, M.D., John C. Huetsch, M.D., Larrisa A. Shimoda, Ph.D.

Division of Pulmonary and Critical Care Medicine, Johns Hopkins School of Medicine, Baltimore, MD.

Pulmonary arterial hypertension (PAH) is a disease characterized by the thickening of the pulmonary vessel walls and formation of vaso-occlusive lesions. Previous studies showed increased Na+/H+ exchanger 1 (NHE1) activity in pulmonary arterial smooth muscle cells (PASMCs) isolated from a rat model of severe pulmonary arterial hypertension (PAH). Inhibition of NHE1 increased apoptosis in PAH PASMCs, suggesting that increased NHE1 activity is necessary for resistance to apoptosis. Therefore, we tested the hypothesis that increasing NHE1 would be sufficient to induce apoptosis resistance in control PASMCs. PASMCs isolated from distal pulmonary arteries from male Wistar rats were infected with a viral construct to overexpress NHE1 (AdNHE1) or green fluorescent protein (AdGFP) as a control. PASMCs were treated with pharmacologic inhibitors of NHE1 (EIPA) and the apoptosis-inducing agents hydrogen-peroxide and staurosporine. PASMC apoptosis was measured via Hoechst staining and protein was isolated. Immunoblot analysis was performed to measure the levels of the apoptotic mediator, CHOP. AdNHE1 attenuated CHOP expression at baseline and in response to apoptotic stimuli. Surprisingly, AdNHE1 did not prevent caspase 3 activity or apoptosis. Overall, these findings suggest that increasing NHE1 is not sufficient to induce resistance to apoptosis.

Expressed miR-17~92 cluster in endothelial cells confers protection against kidney ischemia-reperfusion injury

1Brandon Sanders *; 1Kai Shaikh; 1Takuto Chiba; 1Andrew J. Bodnar; 1Elina Mukherjee; 1Jacqueline Ho; 1Sunder Sims-Lucas

Acute kidney injury (AKI), often caused by hypoxic stress such as ischemia-reperfusion (IR) is an independent predictor of morbidity and mortality. The kidney microvasculature is sensitive to IR-AKI and is thought to have limited or no capacity for repair. We have shown that expression of a microRNA cluster miR-17~92 in nephron progenitor cells is required for normal development of the kidneys. More recently, it has been shown that miR-17~92 expression in the endothelial cells mediates angiogenesis. We hypothesized that the expression of miR-17~92 in kidney endothelial cells promotes its angiogenesis and confers repair of kidney microvasculature after IR-AKI. miR-17~92 is Tie2+ endothelial cells-specifically inactivated in mice (miR-17~92endo-/-). Age- and sex-matched mice of miR-17~92endo-/- and of its Cre- littermate control were challenged with an IR-AKI model. RT-qPCR and immunostaining were performed with the kidney samples of control and miR-17~92endo-/- 7 days post-IR. After IR-AKI, miR-17~92endo-/- mutation increased (1) the serum level of known AKI markers, blood urea nitrogen (BUN) and creatinine and (2) mRNA level of an AKI marker, neutrophil gelatinase-associated lipocalin (NGAL). Immunostaining with an endothelial marker, Endomucin, suggested a trend to be decreased microvasculature in kidneys of miR-17~92endo-/-. In addition, mRNA levels of the known Endothelial-mesenchymal transition (EndoMT) markers including Vimentin and Zeb2 and Fatty acid oxidation (FAO) genes including Cpt1 and Cpt2 were regulated in miR-17~92endo-/- kidneys after IR-AKI. miR-17~92 expression in endothelial cells confers protection against IR-AKI. Endo-MT, and FAO are suggested to be down-stream mechanism to mediate the effect from miR-17~92.

The Influence of Nato3 on Genes Involved in Dopamine Neurogenesis and Maturation

Melina Frantzeskakis2, Dayne Martinez2, Jordan Straight2, Nick Huisingh2 , Daniel Doyle2, Merritt Taylor1,2

1.Biomedical Science, 2. Cell and Molecular Biology, Grand Valley State University, Allendale, MI

Dopamine neurons neurons arise from the floor plate of the midbrain, these midbrain dopamine (mDA) neurons are responsible for the development of Parkinson's disease when they cease to function. Genes that promote the formation of mDA have potential to be used for clinical therapy development; this is due to the influence they have on gene regulation. Transcription factors effect expression of genes that encourage mDA development. Neurogenesis and maturation of mDA are influenced by multiple genes such as: FOXA1/2, LMX1, WNT1, and several others. One gene involved in dopamine neuron maturation is the basic helix-loop-helix transcription factor Nato3 (N3), however, its mechanism of action is unknown. We hypothesized that Nato3 had the ability to upregulate genes involved in mDA neurogenesis and maturation in vivo and in the SN4741 cell line and is therefore able to drive dopamine neurogenesis. Previous data produced by our lab using qPCR and immunostaining showed that overexpression of N3 upregulates LMX1 genes in vivo. The upregulation of the genes involved in mDA neurogenesis and maturation by Nato3 overexpression was mimicked in the SN4741 cell line, shown through qPCR data. This upregulation of these genes (such as Nurr1, En1, and FOXA1/2) indicates that Nato3 influences dopamine neurogenesis.

Perfect muscle regeneration in the African spiny mouse (Acomys) after chronic injury

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

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

Regeneration is the perfect regrowth and repair of damaged tissue. The African spiny mouse (Acomys) is the only known mammal in the world that is capable of scar-free skin regeneration as an adult. In the laboratory, we are using the normal lab mouse (Mus) as a non-regenerating control to analyze the true extent of Acomys's regenerative abilities. After an ear punch wound, Acomys fully regenerated hair follicles, adipocytes, cartilage, sebaceous glands, and, most notably, skeletal muscle. To further study Acomys' skeletal muscle regeneration, we focused on the tibialis anterior leg muscle. The muscles of both Acomys and Mus were injected with cardiotoxin, a snake venom-derivative, to induce a wounding response. Subsequent immunohistochemistry with a collagen I antibody showed that Acomys recovered in 8 days, 3 days faster than Mus. Significant scarring in the Mus connective tissue was observed, whereas no scarring took place in Acomys. Regeneration in response to repeated injury was also studied. After the initial injection, the mice were given 3 weeks to heal then injected again. This was repeated for 5 injection-healing cycles. Amazingly, Acomys was still able to regenerate its muscle perfectly; however, Mus showed an intriguing result: adipocytes within the muscle. Although initially surprising, the abundance of fat cells in the Mus muscle is reminiscent of Duchenne muscular dystrophy. Since Acomys averted this dystrophy-like phenotype, continued study of the African spiny mouse will help us to better understand ways to treat and prevent this debilitating disease.

Polymeric Silicone Material Design and Utility in Medical Devices

Lauren G. Scott, T. Brian Cavitt, Ph.D

Department of Chemistry and Biochemistry, Lipscomb University, Nashville, TN

Purpose: Silicone materials are ubiquitous in medical devices; therefore, the ability to predict silicone material properties would enable diverse functionality thereof. Silicone acrylates aggregate at the substrate-air interface and are thus an excellent model system for interfacial structure-property relationship analyses. The purpose of this research is to compare the traditional algebraic approach with a novel linear algebraic approach when determining surface free energy (SFE) profile of a silicone substrate.

Methods: Interfacial analyses is expedited by using contact angle measurements to obtain the SFE profile using the van Oss-Chaudhury-Good (OCG) thermodynamic model to determine contributions of nonpolar and polar interactions. Several silicone acrylates were polymerized whereupon the OCG thermodynamic model was used to determine the SFE profile of homopolymer via contact angle measurements of fully characterized solvents. Traditional SFE profiles were obtained via an algebraic data treatment using a nonpolar solvent, a monopolar solvent, and a bipolar solvent. Our lab has pioneered a novel linear algebraic approach that can determine SFE profiles using any three, fully characterized solvents. After determining the complete SFE profile for a series of homopolymeric silicone acrylates, we were able to apply Cassie's equation to predict the SFE profile of related silicone copolymers.

Results: Our novel linear algebraic approach is more accurate compared to the traditional algebraic approach. The nonpolar component of the SFE profile is identical across both approaches with very little deviation from experimentally determined values. When using the traditional algebraic approach, the predicted polar components of the SFE profile differed significantly from experimentally determinations; however, the novel linear algebraic approach more closely resembled experimental results.

Discussion/Conclusion: For many of the copolymers, the correlation between the predicted and experimentally determined SFE profile was remarkable–especially when using the novel linear algebraic approach–thereby illustrating the utility of silicone acrylate coatings as a model system for predictive SFE analyses. Using the linear algebraic approach for each silicone homopolymer, we then can calculate the SFE profile for any cured, multicomponent formulation incorporating related silicone acrylates. The ability to accurately predict the structure-property relationships of a polymeric silicone enables the manufacture and improvement of medical devices.

Regiospecific Surface Encoding of Nanoparticles for Programmable Self-Assembly

Guillermo Pereda, Kyle J. Gibson, Margaret Lee, Yossi Weizmann

The assembly of colloidal nanoparticles (NPs) into ordered structures is of great interest in the fields of detection and nano-optics. Through the formation of homo- and hetero- assemblies comprised of various isotropic and anisotropic shapes we can create unique optical and plasmonic properties, based on the individual interactions between NPs. A common approach utilizes solution-based self-assembly, a method that spontaneously positions large numbers of NPs into more organized structures. However, assembly into pre-programmed configurations remains challenging, as NPs will randomly interact with each other due to their identical surface chemistries. To resolve this challenge, we make use of a diblock polymer (polystyrene-b-polyacrylic acid) to selectively block NPs' surfaces by tuning the interfacial free energies of the system. We then we modify the remaining polymer free surface regions with single stranded DNA (ssDNA). Through this programmable surface encoding, we realize an interparticle binding that is both specific and directional. By asserting greater control over the assemblies of NP complexes, we can produce optical-properties of greater complexity capable of responding to external stimuli. The development of nanoparticle self-assemblies offers great promise in the field of nanotechnology, and can offer additional insights into nanoparticle interactions and their influence on plasmonics, magnetics, and excitonics

Determination of a Ochratoxin A in Kentucky Black Walnuts

Bryce M. Forry, Bruce M. Branan, Ph.D.

Department of Science and Allied Health, Asbury University, Wilmore, KY

Previous research has shown that edible tree nuts are potential substrates for mycotoxins. Black walnuts (Juglans nigra) are frequently collected, dried, and consumed with minimal processing, especially in rural communities. This presents health risks if black walnuts contain high levels of mycotoxin contamination. This study aimed to determine the levels of one mycotoxin, ochratoxin A (OTA), in black walnuts from Kentucky. Black walnut samples were collected from three geographical locations near Lexington, Kentucky plus one commercially available sample (nut meat only) from another state. Analysis was performed using immunoaffinity column concentration and separation of the OTA followed by high-performance liquid chromatography with fluorescence detection (HPLC-FLD). Results from analysis of the commercial nuts and samples of the wild husks and nuts showed no quantifiable ochratoxin A present, even though analysis of OTA standards and of oat-containing cereals did test positive for OTA in expected levels.

Supporting Rural Grandparent Caregivers through Building on Appalachian Cultural Traditions

Madeline N. Dunfee*, MPH; Nancy E. Schoenberg*, PhD; Robyn L. Brown+, PhD

*Department of Behavioral Science, University of Kentucky, Lexington, KY; +Department of Sociology, University of Kentucky, Lexington, KY

Purpose: Poverty and poor health disproportionately affect older adults serving as primary caregivers to their grandchildren. Grandparent caregivers living in rural and underserved regions, including Appalachia, are especially vulnerable. However, Appalachian cultural traditions, including religious practices and spirituality, offer grandparents support when facing these challenges.

Methods: To improve understanding of the role religion and spirituality play in coping, twenty-six grandparent caregivers, recruited through community organizations and snowball sampling, engaged in a series of four interviews. A coding team applied conventional content analysis to the transcripts, employing multiple approaches to ensure rigor and transferability.

Results: Findings suggest that religion and spirituality help grandparent caregivers cope by (1) providing a sense of purpose and perspective; (2) fostering peace and perseverance; (3) encouraging forgiveness; and (4) strengthening social cohesion.

Discussion/Conclusion: An improved understanding of the strategies employed by these grandparent caregivers and the potential assets in their communities can inform interventions to improve the lives of grandfamilies. Our findings suggest numerous opportunities for practitioners, policymakers, faith leaders, and social service administrators to leverage cultural traditions in Appalachian communities. For example, acknowledging the great solace grandparents find in attending church, religious and spiritual organizations may consider expanding programming for grandparents through developing programs that facilitate grandparents' development of a sense of purpose through good works, or programs that honor grandparents such as intergenerational scripture studies. Community leaders, local service providers and spiritual leaders should also consider seeking opportunities to locate programming for grandparents in religious and spiritual locations.

The Health Impact of Housing Factors among People Living With HIV

Safiya George PhD[1], Billy Kirkpatrick[2] PhD, George Mugoya[3] PhD

[1]Capstone College of Nursing, University of Alabama, Tuscaloosa, Alabama

[2]Executive Director of Five Horizons Health Services, Inc.

[3]Department of Educational Studies in Psychology, Research Methodology and Counseling in the College of Education, University of Alabama, Tuscaloosa, Alabama

Purpose: The purpose of this study is to examine the health impact of various housing factors among people living with HIV/AIDS (PLWH) in the Deep South. Housing remains the greatest unmet need for people living with HIV/AIDS (PLWH).

Methods: A mixed-method study was conducted using a community-based participatory research (CBPR) approach to address the following main research question (RQ) is: (1) What is the association between housing, case management and HIV health outcomes, specifically HIV treatment adherence, mental health, immune function, and health-related quality of life among PLWH? The study was approved by the University Institutional Review Board and each participant gave their informed consent. Computerized surveys were conducted with 100 PLWH and individual, qualitative interviews were conducted with 28 of the participants.

Results: 100 PLWH participated, 55.4% were male and 44.6% were female. Participants' ages ranged from 25-76 years, and the average age was 51.5±8.9 years. Mean years of living with HIV was 17.1±8.6 years. Mean CD4 cell count was 699.9±298.0. On average, participants lived in their current housing for 4.6±5.7 years. Majority of participants were African American (88%), unemployed or on disability (12.0% and 73.5%, respectively), and single (59% never married, 35% separated, divorced or widowed). Majority of participants identified housing as very important (97.6%). Almost half (43.4%) of the sample reported having challenges related to housing and 37.3% received housing assistance. Satisfaction with housing was significantly associated with engagement in HIV care ($X2=12.9$, $p=.01$) such that those who reported being dissatisfied with their housing also reported not being currently in HIV care.

Conclusions: Housing factors are important to PLWH and some housing factors are associated with poor HIV-related health outcomes. Therefore, it is important to consider the impact of a PLWH's housing situation on their HIV care, HIV medication adherence and HIV outcomes.

Uncovering Genetic Interactions in Glia-Mediated Formation of The C. Elegans Brain Neuropil

Jira White 1, Georgia Rapti, PhD 2, Shai Shaham, PhD 2

1 Claflin University, Orangeburg, South Carolina; 2 Labratory of Developmental Genetics, The Rockefeller University, New York, NY

Studying how neural circuits develop is challenging because many key steps of these processes occur in utero. C. elegans, a small non-parasitic nematode, is ideal to observe these processes in vivo and dissect its key molecular events. It features a fully sequenced genome, conserved genes, cells and processes with functional counterparts in humans. The C. elegans nervous system comprises of 302 neurons and 56 glial cells. Its brain-like neural circuit is called the Nerve Ring (NR) which forms a circle of ~180 axons surrounding the pharynx and associates with glia similar to mammalian astroglia. In different organisms, brain assembly is thought to begin with “pioneer axons” extending over non-neural cells, yet its underlying mechanisms are not clearly understood. Studies in the Shaham lab have demonstrated that in C. elegans, glia are essential in the initiation of brain circuit formation. A Chimaerin – Furin double mutant identified in that study presented a massive disruption of NR axon guidance. Studying this mutant and other conserved genes suggest that identifying pathways of circuit formation is difficult in part due to gene redundancies, where single mutants cannot reveal the roles of specific genes in circuit formation. As a continuation of the previously mentioned study, a series of double mutant strains were created with selected genes affecting certain signaling pathways or the extracellular matrix. At the end of this study, more than 30 new C. elegans strains were created and found to serve as appropriate tools to uncover the redundant roles of new genes in NR formation. They also allow the opportunity to observe the morphological structures of other nerve ring components for newly identified genes found in recent genetic screens. This investigation outlines how these strains can help further understand the role that glial cells play in the development of neural circuits.

Self-Renewing Kidney Resident Macrophages Downregulate MHC class II and Promote Resolution After Acute Kidney Injury (AKI)

Jeremie M. Lever, BS* Travis D. Hull, MD/PhD**** Ravindra Boddu, PhD* Laurence M. Black, BS* Oreoluwa O. Adedoyin, PhD* Zhengqin Yang, PhD* Amie M. Traylor, BS* Anupam Agarwal, MD*,*** and James F. George, PhD**

PURPOSE: Kidney resident macrophages (F4/80HiCD11bLow, KRM) arise during embryonic development and are not replenished from the peripheral blood. In adults, all KRM express MHCII protein, required for antigen presentation. We hypothesized that KRM, in response to injury, promote recovery by generating signaling proteins and growth factors that regulate nephrogenesis during kidney development.

METHODS: We compared isolated KRM obtained from developing mice to those isolated 6d after AKI using flow cytometry and RNAseq to determine changes in protein expression and transcriptional programming. Parabiosis was used to identify bloodborne cells. AKI was induced by bilateral ischemia-reperfusion (IR).

RESULTS: In embryonic mice, KRM lacked expression of MHCII until well after birth (94.7±0.5% MHCII- at E14.5, 69.1±1.1% at P14, and 3.7±0.5% at P28). Notably, the MHCII- KRM population reappeared within 2d after IR-AKI and increased in proportion up to 6d post-injury (36.8±7.2%, $p<0.05$). Using parabiosis between congenic mice, we established that the MHCII- KRM did not result from differentiation of monocytes infiltrating from the blood. Three days after IR-AKI in parabiotic mice, percent chimerism among KRM remained low in both MHCII+ and MHCII- cells (chimerism of MHCII+ = 1.1±0.5%, MHCII- = 1.36±0.4%). RNAseq analysis of MHCII- KRM obtained at P7 in uninjured mice compared with MHCII- and MHCII+ KRM obtained 6d post-injury showed transcriptional programming of MHCII- and MHCII+ KRM were more similar to each other than P7 KRM. However, expression of Pdgfbr and Wnt4 transcripts, genes associated with nephrogenesis, were increased relative to MHCII+ KRM in both P7 and 6d post-injury MHCII- KRM.

CONCLUSIONS: A previously undescribed subpopulation of MHCII- KRM exist after AKI that phenotypically resemble developmental KRM. They are not replenished from the blood but exist as an isolated population in the kidney. After injury, KRM express transcripts that are associated with nephrogenesis, in common with KRM that exist during kidney development.

Involvement of the Croquemort Receptor in Bacterial Phagocytosis by Glial Cells in the Brain of *Drosophila melanogaster*

Allison R. Cummins, BS; Stanislava Chtarbanova, PhD

Department of Biological Sciences, The University of Alabama, Tuscaloosa, Alabama

The molecular mechanisms of innate immunity are conserved from flies to humans. However, still little is known with regard to how pathogens are recognized and eliminated following brain infection. It has been previously reported that Drosophila Croquemort (Crq), which is related to the human CD36 family of scavenger proteins, acts as a phagocytic receptor on macrophages in clearance of apoptotic cell material during Drosophila development. Additionally, Crq has been shown to be involved in the elimination of bacteria via phagocytosis in adult flies, with crq deficient flies exhibiting a decreased lifespan and higher susceptibility to various microbial infections. Using genetic and confocal microscopy techniques, we present preliminary data supporting that Crq also plays a role in bacterial clearance within the fly brain when the tissue is exposed to the bacterium Escherichia coli. We were also were able to identify the cell type within this tissue that is responsible for engulfing bacteria and also show that this process is mediated by Crq. Being able to better understand how the innate immune system works within the fly brain would be a key step in further understanding the mechanisms in the human brain that, when dysregulated, can lead to age related diseases such as Parkinson's and Alzheimer's that are becoming ever more prevalent as human populations continue to live longer.

The NLRP3 inflammasome is a critical mediator of cell-free hemoglobin-induced acute lung inflammation

Sangami Pugazenthi*, Stuart R. Landstreet*, Ciara M. Shaver*, MD, PhD

*Division of Allergy, Pulmonary, and Critical Care Medicine, Vanderbilt University Medical Center, Nashville, TN

Purpose: Cell-free hemoglobin (CFH) increases inflammation during acute lung injury through its proinflammatory effects on alveolar macrophages. In the setting of inflammation, NLRP3 is activated and forms a complex with its adaptor protein ASC and the proteases caspase-1 and caspase-11. Activation of this complex results in increased production of pro-inflammatory cytokines, thereby influencing ongoing inflammation. This study tests the hypothesis that cell-free hemoglobin induces airspace inflammation through the NLRP3 inflammasome.

Methods: For in vivo assessment of the role of NLRP3 in CFH-mediated lung injury, wild-type (WT) and NLRP3 KO mice were each treated with a 100 ug intratracheal injection of CFH and bronchoalveolar lavage (BAL) was performed after 24 hours. Inflammatory cell counts were quantified by diffquik staining. For in vitro measurement of NLRP3 upregulation, alveolar macrophages were collected from WT or NLRP3KO mice were treated with 1 mg/mL CFH or PBS for 24 hours. RNA was then isolated and qRT-PCR was performed for NLRP3 inflammasome components. For protein measurement of NLRP3 components, MHS cells, an alveolar-like macrophage cell line, were treated with 1 mg/mL CFH or PBS for 24 hours and caspase-1 protein expression was quantified by Western blot.

Results: NLRP3 KO mice had less airspace inflammation compared to WT mice after being exposed to CFH, shown through lower inflammatory cell counts, specifically neutrophils ($P=0.006$). When treated with CFH compared with PBS in vitro, alveolar macrophages from WT mice showed increased RNA expression of NLRP3 ($P<0.001$), Caspase-1 ($P=0.007$), and Caspase-11 ($P=0.0017$); and no upregulation of ASC ($P=0.421$). CFH treated MHS cells presented a significant increase of the Caspase-1 protein compared to PBS treated cells in a western blot ($P=0.003$).

Conclusion: The NLRP3 inflammasome may play a role in CFH-mediated lung inflammation, and the presence of CFH upregulates the expression of the NLRP3 inflammasome components.

Screening of harmful toxins in Cosmetics and personal care products

Akenda T Walker[1], Jazmyn Wilson[1], Zhabiz Golkar[2], PhD

[2]Department of Science, Technology, and Human services Voorhees College, SC, USA

Objectives: Our objective was to screen harmful toxins in cosmetics and personal care products that might be contaminants and cause cancer, hormone interruption, birth and pregnancy complications, contact dermatitis, and other skin disorders. Introduction: Phthalates, as key components in plastics, appear in many consumer products. The main phthalates in cosmetics and personal care products are dibutyl phthalate in nail polish, perfumes, lotions and in hair spray. Often, their presence is not noted on labels. In 2014, the FDA started to investigate WEN by Chaz Dean Cleansing Conditioners when the product received 127 complaints from users that it was causing their hair to fall out, among other problems. In one study in Brazil, 45 percent of women who used makeup religiously had skin disease related to the makeup they were wearing. At the same time, 14 percent suffered from makeup-induced acne lesions. In young teens whose hormones are just developing, these chemicals can offset the body's balance and could lead to detrimental effects like infertility and even skin cancer.

Methodology: 50 samples were collected in sterile eppendorf from students on campus or stores in Bamberg, Orangeburg, and Beaufort. Each sample was registered following lab code. To screen the toxins ingredients and contaminants for example: Coal tar, Diethanolamine(DEA), 1,4-dioxane, Formaldehyde, Butane and Isobutane, Petroleum distillates, Polyethylene glycol/ceteareth, Talcum, Nitrosamines. Ingredients on the box was recorded and mass spectrometry was performed for samples with no ingredients indicated on the box.

Results & Conclusion: Polyethylene glycol (PEG) was found in Tone Mango Body wash, outlast 3-1 foundation, Fit Me foundation, True Match foundation, NARS, LORAC Cosmetics Light Source Illuminating 3 in 1, Born this Way, Clinique Beyond Perfecting, Clinique Beyond Perfecting, Be Joyful lotion, Double Duty Beauty Shape Tape Contour, Double Duty Beauty Shape Tape Contour, Born This Way Naturally Radiant Concealer, Studio Fix Fluid SPF 15 Foundation, Idealist Pore Minimizing Skin Refinisher, No. 50 Serum Collagen Veil Anti-Aging Primer, MAYBELLINE Dream Liquid Mousse Foundation, Confidence in a Cleanser, Bare Minerals Bare Pro, Bali Blue Surf, and Pro Conceal concealer PEG is a petroleum-derivative compound that is made from ethylene glycol (ethane-1,2-diol), the main ingredient in antifreeze. PEG can strip the natural oils out the skin leaving the immune system vulnerable. The by- product can be toxic to the organ, respiratory system, and skin irritant. In addition Tone Mango Body wash contain Sodium laureth sulfate. Sodium laureth sulfate is a foaming agent used to break down water in grease. It's so powerful that it's also used in concrete floor cleaners, engine degreasers, and car wash detergents. A well-known skin irritant, it is rapidly absorbed and retained in the eyes, brain, heart and liver, which may result in harmful long-term effects. It can slow healing, cause cataracts in adults, and prevent children's eyes from developing properly, corrode hair follicles and impair ability to grow hair. These surfactants that can cause skin irritation or trigger allergies, which could be highly toxic for consumers. However in our collection we were unable to find; Coal tar, Diethanolamine(DEA), 1-4-Dioxane, Formaldehyde, Methylpropane, and Nitrosamines. Further studies require to analyze the effects and side effects of this toxic chemical on the human body by screening animal model literatures, and to screen for harmful chemical toxin in the cosmetics and personal care products.

Evaluation of hNRF2 as a Neuroprotective Gene Therapy Approach in a Transgenic Pig Model Expressing Human P23H Mutant Rhodopsin

Dana K. Oakes*, Maha H. Jabbar*, Wei Wang, MD/PhD*, Henry J. Kaplan, MD*, Constance L. Cepko, PhD+, & Maureen A. McCall, PhD*

*Department of Ophthalmology and Visual Sciences, University of Louisville School of Medicine, Louisville, KY; +Departments of Genetics and Ophthalmology, Howard Hughes Medical Institute, Harvard Medical School, Boston, MA

Purpose: Retinitis Pigmentosa (RP) is the leading cause of inherited vision loss, affecting 1.5 million people worldwide. RP is most commonly caused by mutations in rhodopsin (RHO) leading to rod photoreceptor (rod) death, disabling night and peripheral vision. Loss of rods triggers loss of cone photoreceptors (cones), leading to progressive blindness. The abundance of different RHO mutations leading to rod cell death, in addition to the high extent of rod degeneration seen in patients upon diagnosis, elucidate the need for a more general therapeutic approach to protect cones. The nuclear factor E2-related factor 2 (NRF2) works as an antioxidant, and shows therapeutic effects in mouse models of RP. The P23H rhodopsin transgenic pig has been shown to recapitulate the human disease. Therefore, we used NRF2 gene therapy in an effort to protect the RP swine model from cone degeneration.

Methods: P23H rhodopsin TG and WT pigs were subretinal injections were administered containing one of

three concentrations of adeno-associated virus (AAV) gene therapy vector containing human NRF2, vehicle, control virus at comparable titer, or an injection was not administered. Ocular coherence tomography (OCT) from baseline (postnatal day 3 – 7) up to 18 weeks post injection (wpi) were taken to assess ongoing changes. Retinas were recovered for analysis and processed for immunohistochemical analysis of outer retinal morphology.

Results: There is good correlation between our OCT imaging and our immunohistochemical analyses. BSS injection revealed no significant damage, and outer laminar pattern disruption associated with hNRF2 injection was greater in WT than TG pigs. Immunohistochemical analyses in retinal sections shows that the changes in the OCT images are related to a loss of photoreceptor somata, and changes in the overall cone morphology.

Conclusion: At the viral concentrations used, all structural and functional assessments show no evidence of a significant neuroprotective effect of hNRF2 in this model of RP.

Subclinical Compromise in Cardiac Strain Relates to Lower Cognitive Performances in Older Adults

Hailey A. Kresge, 1; Omair A. Khan, MAS, 2; Madison A. Wagener, MA, MEd, 1,3; Dandan Liu, PhD, 2; James G. Terry, MS, 4; Sangeeta Nair, DVM, MS, 4; Francis E. Cambronero, AB, 1; Katherine A. Gifford, PsyD, 1; Katie E. Osborn, PsyD, 1; Timothy J. Hohman, PhD, 1; Kimberly R. Pechman, PhD, 1; Susan P. Bell, MBBS, MSCI, 1,5,6; Thomas J. Wang, MD, 5; J. Jeffrey Carr, MD, MSc, 4,5; Angela L. Jefferson, PhD, 1

1 Vanderbilt Memory & Alzheimer's Center, Department of Neurology, Vanderbilt University Medical Center, Nashville, TN; 2 Department of Biostatistics, Vanderbilt University Medical Center, Nashville, TN; 3 Department of Psychology, Vanderbilt University, Nashville, TN; 4 Radiology & Radiological Sciences, Vanderbilt University Medical Center, Nashville, TN; 5 Division of Cardiovascular Medicine, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN; 6 Center for Quality Aging, Division of General Internal Medicine, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN

Purpose:
Global longitudinal strain (GLS), reflecting total shortening of the myocardium during the cardiac cycle, has emerged as a more precise myocardial function measure than left ventricular ejection fraction (LVEF). Longitudinal strain may be selectively affected in subclinical heart disease, even in the presence of normal LVEF. This study examines subclinical cardiac dysfunction, assessed by GLS and LVEF, and cognition among older adults.

Methods:
Vanderbilt Memory and Aging Project participants who were free of clinical dementia, stroke, and heart failure (n=318, 73±7 years, 58% male) completed neuropsychological assessment and cardiac magnetic resonance to quantify GLS and LVEF. Linear regression models related GLS and LVEF to neuropsychological performances, adjusting for age, sex, race/ethnicity, education, Framingham Stroke Risk Profile, cognitive diagnosis, and APOE*?4 status. Models were repeated with a cardiac×cognitive diagnosis interaction term.

Results:
Compromised GLS (reflected by higher values) related to worse naming (?=-0.07, p=0.04), visuospatial immediate recall (?=-0.83, p=0.03), visuospatial delayed recall (?=-0.22, p=0.03), and verbal delayed recall (?=-0.11, p=0.007). LVEF did not relate to worse performance on any measure (p>0.18). No diagnostic interactions were observed.

Discussion/Conclusion:
Our study results are among the first to suggest that compromised GLS relates to worse episodic memory and language performance among older adults who are free of clinical dementia, stroke, and heart failure. Subclinical cardiac dysfunction may correlate with cognitive health in late life, even when LVEF remains normal. The results add to growing evidence that GLS may be a more sensitive and preferred method for quantifying subclinical changes in cardiac function.

Funding: IIRG-08-88733, R01-AG034962, K24-AG046373, K23-AG045966, K23-AG048347, K12-HD043483, K01-AG049164, R24-HL085343, UL1-TR000445

Dopamine Regulation via Allosteric Modulation of the M1 Receptor: Implications for the Negative Symptoms of Schizophrenia

Jordan A Galbraith*, Samantha E Yohn PhD*, Ellen Rieth*, P Jeffrey Conn PhD*, Craig W Lindsley PhD+

*Vanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University, Nashville, TN, +Department of Pharmacology, Vanderbilt University, Nashville, TN

Purpose: Schizophrenia is a devastating neuropsychiatric disorder that is characterized by three symptom domains. Currently approved therapeutics are effective at treating positive symptoms, which are dependent on elevated dopamine (DA) release. However, they offer little to no benefit for negative and cognitive symptoms; thus, there is a critical need to develop novel therapeutics. Our lab has previously reported that positive allosteric modulators (PAMs) of the muscarinic M1 receptor are beneficial for cognitive deficits frequently observed in schizophrenia. However, the role of M1 on negative symptoms has not yet been explored. The manifestation of negative symptoms, such a motivational dysfunction, has been correlated to reduced DA release in the nucleus accumbens (NAc). M1 is highly expressed in the NAc and global M1 knockout mice have deficits in motivation. Therefore, I hypothesize that M1 will regulate NAc DA release and attenuate impairments in motivation.

Methods: To address my research question, I am (i) conducting ex vivo fast scan cyclic voltammetry (FSCV) to monitor changes in NAc DA following electrical stimulation and (ii) employing two validated preclinical models of motivational dysfunctions, a progressive ratio task and a concurrent choice experiment.

Results: Excitingly, I now report that activation of M1 can increase NAc DA release and restore motivation. Furthermore, I report that M1 activation can significantly attenuate motivational impairments induced by the first-generation antipsychotic haloperidol, a DA D2 antagonist used clinically in patients who display severe positive symptoms but can exacerbate negative and cognitive symptoms.

Discussion/Conclusion: These findings suggest that activation of M1 may not only hold efficacy by itself but be additive to current therapeutics. We are currently investigating the mechanism of action to learn more specifically how M1 is regulating DA and plan to run further experiments to answer these questions.

Identifying and Localizing Novel Genes in Zebrafish Spinal Cord Controlling Locomotion

Marlisa Shaw, Kristen D'Elia, Basak Rosti, Paula Cruz, David Schoppik, Jeremy Dasen

Animals and requires many subtypes of motor neurons. However, it is still unknown what genes regulate the specification of motor neuron subtypes, specifically those that regulate speed. The goal of this project is to discover candidate genes that could be specifiers for motor neurons that regulate speed. I analyzed a single-cell RNA sequencing dataset, obtained from motor neurons of zebrafish embryos, to identify genes that are localized to specific motor neuron subpopulations. By analyzing gene expression, in combination with a brief literature review, I identified pax2a, dmrt3a, nkx1.2lb, rgs4, chga, ache, and otpa as genes of interest. Then, I designed RNA probes using the Digoxigenin (DIG) labeling protocol. The RNA probes were used in in situ hybridization to confirm, in vivo, if the identified genes are expressed in a subpopulation of motor neurons and if that subpopulation is likely a speed-specific subtype. The in-situ results of Dmrt3a and Pax2a confirmed that the genes of interest are localized in the desired ventral region of the zebrafish spinal cord, which is where motor neurons are typically positioned. Now that possible novel genes are found, merging antibody staining with in-situ hybridization can be done to verify that the DIG labels are only labeling motor neurons. In addition, knock-outs of each gene may be done to further understand the genes' function in motor neuron specification.

Effect of D-Limonene on Developing Human Neurons

SaDazia Driffin and Omar Bagasra, M.D., Ph.D.

Department of Biotechnology, +N.I.H. R.I.S.E., Claflin University, Orangeburg, SC

D-limonene is a chemical that is predominantly used in fragrances and is used as an additive for a lemon-like flavor. Common consumer goods that have synthetic D-Limonene an additive include fragrances, soaps, automobile tire cleaner, cleaning products, shampoo, beverages, and chewing gum. Currently, widespread use of synthetic limonene is due to its easy biosynthetic. These are not natural chemicals, but are synthesized chemically on a mass scale. Since, most of the women are exposed to limonene during pregnancy we explored the effects of limonene on human developing neurons. Methods: To determine the potential adverse effects of D-limonene, we utilized four neuroblastoma cell lines (NBC's): two of male origin (CRL-2267& 2142) and two from female origin (CRL-2266 & 2149). The NBCs were exposed to low amounts of D-Limonene (i.e. from 20 nM to 0.2 nM). After exposure for 48-96 hours in these chemicals the cells were fixed and stained with hematoxylin and eosin (H&E). We carried out a detailed histological analysis of the NBCs that were exposed to D-limonene and compared them to unexposed controls for cell growth, growth pattern changes, central chromatolysis, axonal length, axon degeneration, and syncytia formation. The morphologic analyses showed significant changes in the NBCs for both male and female cell lines that were exposed to limonene. The exposures to the chemical at all concentration imparted profound morphologic adverse effects including significant decline in cell growth, changes in axonal lengths, increased degeneration of neurons and increased central chromatolysis, and syncytia formation. We concluded that exposures to D-limonene even at very low concentrations induce significant neuro-modifications in male and female NBCs. Our observations are first of show a potential link between exposures to D-limonene and neurodevelopmental disorder in human developing neurons that represent fetal brain development in utero. Molecular and immunological analyses are in progress.

The Role of Prelimbic Cortex Neuronal Ensembles in Fear vs. Reward

Alexander J Yue, Maribel Vazquez-Silva, Jessica Frazier, and Jamie Peters, PhD

Department of Neuroscience, Medical University of South Carolina, Charleston, South Carolina

Fear serves a critical role in human and animal evolution, motivating organisms to avoid threats and survive. In humans, when these neuronal fear pathways dysfunction, phenotypic abnormalities such as phobias or post-traumatic stress disorder emerge. Animal model studies have shown that the medial prefrontal cortex (mPFC) plays a critical role in fear expression, in particular the prelimbic subregion (PL). Neuronal ensembles are thought to be the memory-encoding units by which the brain stores and retrieves memories. In this study, PL neuronal ensembles were hypothesized to be critical for the encoding and retrieval of a fear memory trace. We "trapped" PL neuronal ensembles in adult Wistar rats after Pavlovian fear conditioning using a Targeted Recombination in Active Populations (TRAP) approach. Fear was measured as conditioned suppression of sucrose seeking. Rats were trained on a random interval (RI) schedule of sucrose training and allowed to seek sucrose throughout all fear procedures. Inhibitory designer receptors (Gi-DREADDs) were TRAPped in PL ensembles after fear conditioning, wherein rats learned to associate a tone with an electric footshock. After allowing time for the Gi-DREADD to express, clozapine-N-oxide (CNO) was used to activate the DREADD and inhibit the TRAPped PL ensembles. Thirty minutes later, an extinction test was conducted consisting of tones but no shocks. Three days later, another extinction test was conducted to measure any persistent effects of CNO on fear expression. PL cortex tissue was subjected to immunohistochemistry to quantify expression of the TRAPped Gi-DREADD. Surprisingly, there were no statistically significant effects on conditioned suppression of sucrose seeking; however, a significant effect on baseline sucrose seeking during the extinction test was observed. These data do not support a critical role of PL ensembles in fear encoding and retrieval but do suggest the potential existence of a PL reward ensemble.

The effects of peripheral amyloid beta on spermatogenesis in APPswe/PS1dE9 mouse model for Alzheimer's disease

*Emily R. Stephens, *Breanna N. Harris, *Christine M. Prater, +Paul Soto, *James A. Carr;

*Department of Biological Sciences, Texas Tech University, Lubbock, TX, +Department of Psychology, Louisiana State University, Baton Rouge, LA

Purpose: Amyloid-beta (AB) is formed by the cleavage of the amyloid precursor protein (APP) and forms the hallmark plaques of Alzheimer's disease (AD). The formation of AB can occur within the brain and other organs. Peripheral AB has been shown to circulate through the blood and enter the brain through the blood-brain barrier (BBB). In this experiment, we examine the testis which has a blood-tissue barrier (BTB) similar to the BBB and may be susceptible to similar AB accumulation. Cell degeneration is observed in areas affected by AD; therefore, we expected that the processes of spermatocytogenesis and spermiogenesis in male APPswe/PS1dE9 transgenic (Tg) mouse models for AD will be altered relative to their wild type (WT) littermates.

Methods: Testes from adult transgenic (N=10) and wild-type (N=5) mice were prepared for routine paraffin embedding, sectioned at 10 microns, and stained with hematoxylin and eosin. Cells at each stage of spermatogenesis were counted from representative regions and comparisons were determined statistically. Currently, we are verifying the presence/absence of AB immunohistochemically and will move on to quantify AB levels using ELISA. These additional methods will provide the first rigorous assessment of AB transport into the mammalian testes.

Results: Contrary to our predictions, Tg and WT mice did not differ significantly in any stage of spermatogenesis.

Conclusion: Our preliminary data suggests that the testis is excluded from the physiological symptoms of AD. The presence or absence of AB in these tissues has yet to be determined, yet for either outcome, we speculate that differences in gene expression between the BTB and BBB in normal and disease states serves a protective function. Through future quantitation and genetic analysis, we hope to uncover more about the differences between the body's blood tissue barriers that account for variation in AB plaque load throughout the body.

Calcification and Risk Stratification in Femoral Artery Stenosis

Shyam P. Desai*, Elizabeth K. McCourt+, Homayoun Valafar+, PhD, Susan M. Lessner*, PhD

*Department of Cell Biology and Anatomy, University of South Carolina School of Medicine, Columbia, SC;
+Department of Computer Science and Engineering, University of South Carolina, Columbia, SC

Purpose: Peripheral arterial disease (PAD) is a growing concern in older populations. About one in every 20 Americans over age 50 has PAD, which is heavily linked with an increased chance of having a myocardial infarction or stroke. Vascular calcification, which is commonly observed in PAD patients with co-morbid diabetes or chronic kidney disease, complicates interventional treatment and correlates with increased morbidity and mortality. The specific aim of this project was to compare calcium levels in atherosclerotic plaques measured using a biochemical approach to calcification volume calculated using image analysis of computed tomographic angiogram (CTA) scans. By developing a validated calcium score for the lower extremities, we aim to better stratify PAD patients undergoing femoral endarterectomy according to risk of future disease progression.

Methods: Preliminary studies were performed on samples from patients undergoing carotid endarterectomy (CEA). Plaque samples were lyophilized and weighed to calculate calcium content as a fraction of dry weight. A colorimetric assay for calcium was performed on a plate reader to measure the total calcium content. To estimate extent of calcification in lower extremity CTA scans, calcified areas in non-contrast enhanced images were segmented and measured using Fiji image analysis software, while total vasculature volume was obtained using ITK-SNAP in contrast-enhanced CTA scans.

Results: Two samples from the same plaque had calcium content of 0.77 mg/mg dry wt and 0.31 mg/mg dry wt, demonstrating considerable intraplaque variability. Estimates for fractional volume of calcification throughout the lower extremities were 0.74% and 3.62% in two CTAs.

Conclusion: We successfully demonstrated a biochemical method to measure total calcium content in human plaque specimens. We also developed an approach to quantify calcification volume from CTA images. Future work will focus on automating the analysis of CTA scans, and on comparing biochemical results to image analysis results in the same specimens.

Optimizing Imaging of Headaches in the Pediatric Emergency Department

Kristen D. Williams[1,4,5]; Angelica M. Garcia, MD[3]; Brian J Dillon, MD[2]; Karen Santucci, MD[3]; Michele H Johnson, MD[2]

[1]Frank H. Netter MD School of Medicine at Quinnipiac University, North Haven, CT

[2]Department of Radiology and Biomedical Imaging, Yale University School of Medicine, New Haven, CT

[3]Pediatric Emergency Medicine, Yale University School of Medicine, New Haven, CT

[4]Nth Dimensions, Chicago, IL

[5]American College of Radiology, Reston, VA

Purpose:

Evaluation of children with headaches is a significant health concern in any practice. While most cases are due to primary headaches, such as migraines or tension headaches, circumstances exist that indicate serious illnesses. Within the past year, Yale New Haven Hospital (YNHH) treated 1,095 headaches of the 38,000 encounters within the Pediatric Emergency Department (PED). Because the incidence of serious, life-threatening headaches is small, there is a possibility of under-diagnosis. The infrequency of such cases may offer less cohesive protocols and allow errors; primarily, due to lack of training and experience. A review of significant, yet rare headache cases may offer an approach to enhance diagnostic protocols of pediatric headaches. We wanted to determine if an educational module designed to focus on clinical diagnosis and imaging selection for pediatric patients with headache would favorably impact care in the PED.

Methods:

We performed literature review regarding pediatric headaches and diagnostic imaging protocols in the PED. We performed clinical shadowing in the PED to observe clinical workflow.

We identified important examples of pediatric headache cases through teaching-file material from the PED and Radiology Department at YNHH. The data collected was used to develop a teaching module.

Results:

A teaching module of case vignettes was developed offering pertinent questions and references in approaching pediatric headaches.

Conclusion:

A teaching module focused on diagnostic imaging of pediatric headaches can be used to train Resident and/or Attending Physicians regarding headache care in the PED. Plans exist to develop tests to evaluate the validity of the teaching module as a tool to improve patient care.

Preoperative administration of L-Arginine, Omega-3 Fatty Acids and Ascorbic Acid effects on postoperative complications in diabetic orthopedic patients

Kimberly Gillens, University of South Carolina School of Medicine, Columbia, SC

J. Benjamin Jackson III, M.D., Palmetto Health USC Orthopedic Center, Columbia, SC

Purpose: The primary objective of this study is to determine whether preoperative supplementation of L-Arginine, Omega-3 Fatty Acids and Ascorbic Acid will affect postoperative complications in diabetic orthopedic surgical patients. Diabetes mellitus is already one of the most prevalent chronic diseases in the world and its incidence is expected to increase. Although there are different etiologies, the pathophysiology of the disease is the same: poor peripheral blood flow, decreased cellular response at injury sites, hyperglycemia, and poor nutrient transport. This altered physiology does not allow for the proper nutrients and factors to mobilize to the site of injury, leaving wounds at high risk for dehiscence or infection, even with proper care. Nutrition therapy has proven useful in modulating inflammation and the immune response, optimizing glucose control and attenuating the hypermetabolic response to surgery. Multiple studies have produced strong evidence that perioperative nutrition therapy reduces the incidence of post-surgical complications, optimizing healing and recovery. Preoperative immunonutrition may be a viable, low cost way to the greatest enhancing of the body's response to surgery by taking a proactive approach.

Methods: Participants of this study will start a daily regimen of 77.2g of immunonutrition containing 20g of L-Arginine, 17g of Omega-3 Fatty Acids and 500mg of Ascorbic Acid six days prior to surgery and continue until the day before surgery. Subjects with successful completion of the regimen will be monitored postoperatively for rates of wound healing, infection and revision surgery due to dehiscence. These parameters will be recorded and analyzed to determine the effectivity of immunonutrition in this subset of patients.

Discussion: This project is still in pre-submission. Favorable results could be pertinent to remodeling the postoperative management of diabetic patients and the overall population.

The risk for anterior cruciate ligament injury is higher when serum relaxin concentrations peak

Gabrielle G. Gilmer, Gretchen D. Oliver

Sports Medicine and Movement Laboratory, School of Kinesiology, Auburn University, Auburn. AL

Purpose: Biomechanical and neuromuscular anterior cruciate ligament (ACL) injury risk factors have been identified via the use of clinical tests. Studies revealed athletes are at a greater risk for injury during the pre-ovulatory phase of the menstrual cycle. Recently, relaxin, a peptide hormone similar in structure to insulin, was identified as interfering with the structural integrity of the ACL. We aimed to determine how biomechanical (knee flexion and valgus, and tibial rotation) and neuromuscular (gluteus medius, rectus femoris, and biceps femoris) risk factors differed between the maximum and minimum SRC during three clinical tests. It was hypothesized that athletes would be at a greater risk for injury when SRC peaked, when compared to when SRC was at a minimum.

Methods: Kinematic data were collected at 100 Hz using an electromagnetic tracking system, and electromyography data were collected at 1000 Hz. Participants performed a single leg squat (SLS), drop vertical jump (DVJ), and single leg crossover dropdown at two visits during their menstrual cycle: 1-2 days after the menstrual cycle begins (SRC minimum) and 22-24 days after the menstrual cycle begins (SRC maximum).

Results: Independent samples t-tests revealed significant differences in tibia rotation during the SLS and DVJ, gluteus medius activation and quadricep to hamstring ratio during the DVJ and single leg crossover dropdown, and knee valgus during the single leg crossover dropdown. Results revealed that when SRC peaked, the tibia was more internally rotated, gluteus medius activation was decreased, quadricep to hamstring ratio was increased, and knee valgus was increased.

Conclusion: These movement patterns suggest athletes are at a greater risk for injury when SRC peaks. These findings disagree with previous studies, since SRC peaks during the post-ovulatory phase, and suggest there is a need for a more detailed classification of the menstrual cycle for evaluating risk for injury.

Mucus Matters: Ferrets Demonstrate Sustained Fibrosis, Mucociliary Decrement, and Aberrant Lung Repair Following Bleomycin-Induced Pulmonary Fibrosis

Jacelyn E. Peabody!,@,#, Scott E. Phillips PhD#, Balu Chako PhD$, Vivian Y. Lin!, A. Timothy Adewale!, Sandeep Bodduluri PhD#, Jeremie M. Lever@,#, Ren-Jay Shei PhD!,#, John F. Engelhardt PhD%, Guillermo J Tearney MD PhD&, Victor Darley-Usmar PhD$, David A. Schwartz MD^, Victor J. Thannickal MD#, Steven M. Rowe MD, MSPH!,#

!Cystic Fibrosis Research Center; @Medical Scientist Training Program; #Department of Medicine; and $Department of Pathology, University of Alabama at Birmingham, Birmingham, Alabama
%Department of Medicine, University of Iowa, Iowa City, Iowa
^Department of Medicine, University of Colorado Anschutz Medical Campus, Denver, Colorado
&Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, Massachusetts

Introduction: A gain-of-function promoter variant is the strongest risk-factor for the development of idiopathic pulmonary fibrosis (IPF); yet, the role of Mucin 5B (MUC5B) in IPF pathogenesis is unknown. Ferrets, unlike mice, have submucosal glands and human-like distribution of MUC5B in the lung.

Purpose: Determine if bleomycin (BL)-exposed ferrets model human IPF and elucidate the role of Muc5b in fibrosis pathogenesis.

Methods: BL (5U/kg) or saline control (SCt) was administered via intratracheal microspray to wild-type ferrets. Fibrosis was assessed with micro-computed tomography (µCT), hydroxyproline (Hyp), and histology. Muc5B, alpha-Smooth Muscle Actin (a-SMA), acetylated tubulin and Club Cell Secretory Protein (CCSP) expression were assessed by immunohistochemistry (IHC) and immunofluorescence (IF). Mucociliary transport (MCT) and ciliary beat frequency (CBF) were assessed ex-vivo using micro-optical coherence tomography (µOCT). Bioenergetic profiles were measured in peripheral blood mononuclear cells (PBMCs) via Seahorse XFe96 Extracellular Flux Analyzer.

Results: Apparent at 2wks, ground-glass opacities persisted through 6wks on µCT. Volumetric µCT analysis revealed increased fibrosis in BL lungs (increased 18.1±2.2% vs -0.8±0.8% in SCt, $P<0.001$). At 6 wks, Hyp was elevated (8.1±1.4 µg/mg BL vs. 4.3±0.7µg/mg SCt, $P<0.04$). BL ferrets demonstrated significant increase in maximal oxygen consumption rate in PBMCs at 3wks (37.7±1.8 pMol/min BL vs 53.8±6.13 pMol/min SCt, N=4/group, $P=0.05$), suggestive of increased metabolic flux. µOCT demonstrated reduced CBF in the bronchi at 3wks and 6wks post-BL exposure (mean decrement -2.4 and -1.8 Hz vs. SCt, $P<0.01$). IF revealed collagen-rich matrices, scattered myofibroblasts, and a-SMA+ fibroblastic foci (FF). Abnormal expression of mucin-rich proximal AW markers was noted in cystic distal airspaces, akin to MUC5B positive honeycomb change.

Discussion/Conclusion: BL-exposed ferrets exhibit features of IPF not found in rodent models and may be related to Muc5b expression: FF, mucin-rich honeycomb cysts, bronchiolized distal airspaces, and sustained fibrosis associated with aberrant MCT. Our ongoing studies of contemporaneous fibrosis development and mucociliary physiology in Muc5b knockout and pharmacologically-induced Muc5b over-expression ferrets will help elucidate the role of Muc5b in pathogenesis of fibrosis.

Injection of Adipose-derived Stromal Vascular Fraction Restores Adrenergic Function and Alters Reactive Oxygen Species Signaling in Coronary Arterioles from Aged Females

Evan P. Tracy, B.S., Jason E. Beare, M.S., Pallavi Katragadda, Fangpin Yuan, M.D., Amanda J. LeBlanc, Ph.D.

Department of Physiology, Cardiovascular Innovation Institute, University of Louisville, Louisville, KY, USA

Purpose:

Small coronary vessel disease is the predominant heart disease presentation post-menopause. Adipose-derived Stromal Vascular Fraction (SVF) incorporates into vascular walls and improves coronary flow reserve following intravenous injection. Understanding mechanisms of SVF-mediated small coronary vessel recovery is necessary before establishing therapeutic applicability. Potential mechanisms include improvements in adrenergic function and reactive oxygen species (ROS) signaling. We hypothesize that there are differences in adrenergic function and ROS signaling in advanced age, reversible by SVF injection.

Methods:

Female rat coronary arterioles isolated from young control (YC), old control (OC), or old injected with SVF (OSVF) were mounted in a pressure myography system. Concentration-response curves to the B1 agonist dobutamine, B1 and B2 agonist norepinephrine, and norepinephrine + B1 antagonist atenolol were performed. Arterioles were stained with B1 or B2 antibodies and confocal imaged. Separate arterioles were infused with the H2O2 dye Mytopy1 and the NO dye 2,3-diaminonaphthalene (DAN). Changes in mean fluorescence intensity (MFI) were monitored with varied flow. Two-Way ANOVA was performed with significance as $p < .05$.

Results:

Age-related decreases in adrenergic vasodilation were fully restored by SVF injection. Atenolol attenuated adrenergic response in YC and OSVF, but not OC. OC H2O2 MFI was significantly higher than YC and OSVF for all flow rates. With flow, YC NO MFI was significantly higher than in OC, but there was no statistical difference between OSVF treatment and YC or OC. MFI for B1 receptors were increased in arterioles from YC and OSVF compared to OC.

Conclusions:

Advanced age significantly decreases B1 and B2 adrenergic response and B1 expression, which is rescued by SVF. OC and YC had significantly higher H2O2 and NO MFI, respectively, both increasing with flow. SVF treatment had H2O2 and NO response comparable to YC and OC, respectively.

Investigation of 1,2,3-triazoles as amide bioisosteres in modulators of the cystic fibrosis transmembrane conductance regulator.

Jake Doiron*, Christina Le^, Britton Ody*, Jon Brace*, Savannah Post*, Nathan Thacker*, Liping Tang DVM+, Wei Wang PhD+, Steven Rowe MD MSPH+$, Steve Aller PhD^, Mark Turlington PhD*

* Chemistry, Berry College, Mount Berry, GA, United States; + Gregory Fleming James Cystic Fibrosis Research Center, University of Alabama-Birmingham, AL, United States; $ Pulmonary, Allergy & Critical Care Medicine, University of Alabama-Birmingham, Birmingham, AL, United States; ^ Pharmacology & Toxicology, University of Alabama-Birmingham, Birmingham, AL, United States

Cystic Fibrosis (CF) results from mutation of the CF transmembrane conductance regulator (CFTR) gene that codes for the CFTR anion channel necessary for proper pulmonary and pancreatic function. In 2015, Orkambi® was approved to treat the most common CF mutation, ?F508, and consists of two drug molecules, VX-809 and VX-770. While revolutionary, combined administration of VX-770 and VX-809 only increases chloride ion passage from 3% to ~25% in CF-?F508 HBE cells, which corresponds to a 2-4% increase in forced expiratory volume. Recently, the 1,2,3-triazole moiety has been successfully used as an amide surrogate in medicinal chemistry, and in many instances, improves drug potency and/or physiochemical properties. Here we describe the synthesis, pharmacological analysis, and crystallographic data of VX-770 and VX-809 1,2,3-triazole containing analogs. Ussing chamber analysis across CFBE-?F508 monolayers displayed that the VX-809 1,2,3-triazole analogs have similar efficacy and slightly reduced potency to the parent amides, which displays that the 1,2,3-triazole can be tolerated in this chemical series. However, the VX-770 1,2,3-triazole analogs were inactive in four different CF cell lines (CFBE-?F508, CFBE-G551D, FRT-G551D, CFBE-WT). Interestingly, a VX-770 1,2,3-triazole analog had similar potency (5nM) in patch-clamp studies when compared to its amide counterpart (1nM). This finding suggests a membrane permeability issue for the 1,2,3-triazole VX-770 analogs as the VX-770 binding site is hypothesized to exist in an intracellular region of CFTR. In addition, we report X-ray crystallographic structural comparison of the respective amide and 1,2,3-triazole containing analogs. Compared to its parent amide, the 1,2,3-triazole analog increases the spacing of attached groups by 1Å and widens the angle between the groups by 17°, providing structural information that may partially explain differences in biological activity. The implementation of the amide-triazole substitution utilizing CF modulators provides insight into the resulting physical and pharmacological changes induced by inclusion of the 1,2,3-triazole.

Two open source designs for a low-cost operant chamber using Raspberry Pi(TM)

Katelyn M. Gurley, MS

Department of Cell Biology and Anatomy, Louisiana State University Health Sciences Center, New Orleans, LA

Operant chambers remain a significant financial investment for experimenters despite a near-century of use and development. Small powerful single-board computers such as the Raspberry PiTM offer researchers a low-cost alternative to expensive operant chambers. In this paper, we demonstrate designs for two operant chambers, one using nose-poke ports as operanda and another using a touchscreen. To validate the design, rats acquired both visual discrimination and delayed alternation tasks in each chamber. Designs and codes are open source and serve as a starting point for researchers to develop behavioral experiments or educational demonstrations.

Effects of a Nature Walk with Canopy on Cortisol and Cognition in Children with Autism Spectrum Disorder

Emily Vernet and Melissa Birkett, Ph.D.

Department of Psychological Sciences, Northern Arizona University, Flagstaff, Arizona

Purpose: Autism Spectrum Disorder is a neurodevelopmental disorder that affects social interactions, communication, and behavior. Cortisol is a glucocorticoid secreted from the adrenal cortex that plays a major part in the hypothalamic-pituitary-adrenocortical axis in maintaining adaptive stress response. Effects in social, communicative, and behavioral functioning in Autism Spectrum Disorder causes susceptibility to stress and anxiety. The purpose of this study is to examine the impact of nature environments have on the stress levels and executive functioning of children with Autism Spectrum Disorder. More specifically, this study aims to measure the cortisol levels and the attention, memory, and impulse control among children with Autism Spectrum Disorder following a tree-canopy nature walk and a non-canopy nature walk.

Methods: Participants included children between the ages of 5 and 17 that have been diagnosed with Autism Spectrum Disorder by a medical professional. Acute stress was induced using the Wechsler Intelligence Scale for Children—Fourth Edition prior to the nature walk. Participants' attention, inhibition, and spatial working memory were measured before and after the nature walk using the Psychology Experiment Building Language software. Children were randomly assigned to either an outdoor tree-canopy or an outdoor non-canopy walk and performed the next walk on the following day. Cortisol samples were taken at three time points on each day: after the pre-walk measures, halfway through the walk, and after the post-walk measures and then analyzed.

Results: Current data does not provide any conclusive results to the study.

Discussion/Conclusion: Currently, no conclusion can be drawn from the present study. However, previous research has shown that environmental events can cause an impairment in stress levels due to social challenges or disruption in routines. Research has also shown a positive correlation with cortisol and stress levels.

ABSTRACTS – POSTER SESSION 2

Uncovering novel inhibitors of Type III CRISPR-Cas systems in Streptococcus thermophilus

Clare A. Edwards*┼, Michael P. Terns, PhD*;

*Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA; ┼Medical College of Georgia, Augusta University, Augusta, GA

Prokaryotic CRISPR (clustered, regularly interspaced, short palindromic repeat) systems provide adaptive immunity against bacteriophages and mobile genetic elements through historically-encoded, sequence-specific defense. During the first encounter with an invader, adaptation proteins incorporate nucleic acid fragments, known as spacers, between the conserved repeats of the CRISPR locus. Upon future invasion, transcribed spacers are processed into crRNAs that recognize the target nucleic acid through base-paired interaction and recruit nucleases for target degradation. Despite this sophisticated defense mechanism, certain lytic and lysogenic phages have evolved a way to evade CRISPR-mediated defense. This mechanism of phage survival was recently attributed to anti-CRISPR (Acr) proteins that are encoded in phage genomes and function as selective inhibitors of CRISPR-Cas pathways. The recently identified Acr proteins add to the understanding of phage and bacterial dynamics and are beginning to be exploited in gene editing approaches to increase nuclease specificity. Though Acr proteins share few similarities in sequence or structure, previous findings demonstrate that Acr coding regions have a characteristic genomic architecture with little intergenic space and an encoded HTH regulatory protein. Acrs have been identified against Type I, II, V, and VI CRISPR-Cas systems, but not against the unique functionality of bacterial Type III systems. Type III systems are novel in their requirement for transcriptional coupling during defense and the ability to degrade both viral genes and their mRNA transcripts. To identify Type III candidates, we searched for homologues of previously identified Streptococcal Acr proteins, identifying regions of phage genomes with characteristic Acr architecture. Genes surrounding homologues in these regions were expressed in S. thermophilus strains and tested for their ability to inhibit the endogenous Type III (as well as co-existing Type I and II) CRISPR systems. This quick in-vivo approach yields the potential to find novel Acr proteins with a range of type specificity and inhibitory mechanisms, further uncovering the intricacies of bacterial and phage dynamics.

Biochemical studies of HIV-1 MA mutants affecting Envelope glycoprotein incorporation

Gunnar N. Eastep*, Jamil, S. Saad*, PhD

Department of Microbiology, University of Alabama at Birmingham (UAB)

The incorporation of the envelope glycoprotein (Env) is an essential step in the assembly of retroviruses. Though it is not the case for all retroviruses, the Env cytoplasmic domain of some viruses such as HIV-1 bears an especially long cytoplasmic-tail (CT) domain. It is presently unclear what role the long CT domain plays in assembly. It is established that assembly of HIV-1 particles is initiated by the targeting of the structural Gag protein to the plasma membrane (PM). Biochemical and genetic evidence suggest that HIV-1 Env incorporation is mediated by interactions with the matrix (MA) domain of Gag. Recent biochemical studies of an HIV-1 MA mutant that enhances Env incorporation, Q63R MA, have attempted to link Q63R's enhanced trimerization properties to increased Env incorporation. The model proposed by the authors asserts that the Q63R mutation changes the arrangement of MA hexamers on the PM resulting in a larger "gap" in the hexamer center which better accommodates the CT of Env. Furthermore, it has been shown that Q63R MA, but not WT MA, can bind CT directly. In order to further explore this model, we characterized the Q63R mutant, as well as MA mutants (L75G, L75E) which negatively affect Env incorporation. Using 1H-15N HSQC Nuclear Magnetic Resonance (NMR) we have identified that minimal structural changes occur as a result of these mutations. Analytical ultracentrifugation (AUC) data indicate that these mutations do not significantly affect trimerization, shedding doubt on the prior hypothesis of how Q63R affects Env CT accommodation and incorporation. Furthermore, using direct NMR titration approaches we probed for a direct interaction between MA (Q63R and WT) and CT. This research provides a basis for further studies in our lab investigating oligomerization of MA mutants and its potential effect on CT binding, and challenges recent data on the Q63R MA mutant.

CTRP3 Overexpression Attenuates Ethanol-Induced Changes to the Liver Fatty Acid Profile

Greta H. Trogen[1], W. Andrew Clark Ph.D. R.D.[2], Jonathan M. Peterson Ph.D[3]

College of Arts and Sciences[1], College of Clinical and Rehabilitative Health Sciences[2], College of Public Health[3],
Department of Health Sciences,
East Tennessee State University, Johnson City, Tennessee

Alcoholic fatty liver disease (AFLD) is a leading cause of death due to hepatic complications, with no approved pharmaceutical treatments. CTRP3 is a protein that prevents diet-induced fatty liver, however, the effect of CTRP3 on preventing alcohol-induced fatty liver has not been previously studied. We hypothesized that CTRP3 overexpression would attenuate ethanol-induced changes to the liver lipid accumulation and profile. Ethanol feeding significantly increased total hepatic lipid accumulation and decreased the relative palmitic acid while increasing linoleic acid, α-linolenic acid concentration. This lead to an overall decrease in the C18:C16 fatty acid ratio, indicating an overall ethanol-induced change to lipid metabolism. CTRP3 not only prevented the ethanol-induced increase to total hepatic lipid accumulation, but CTRP3 also attenuated the ethanol-induced changes to the proportional concentration of the specific fatty acids. These results indicate that CTRP3 overexpression prevents alcohol-induced fatty liver and maintains normal lipid metabolism. These data support further studies for the use of CTRP3 as a potential treatment for AFLD.

Exploring the Role of Nuclear Lamin A in the Regulation of Stress Response Using a Cell Culture Model of Aging

Jasmine S. Carter*, Benedicth Ukhueduan+, Rekha C. Patel, PhD+

*Department of Biology, Claflin University, Orangeburg, SC; +Department of Biological Sciences, University of South Carolina, Columbia, SC

Hutchinson-Gilford progeria syndrome (HGPS) is a rare disorder that causes premature aging and affects young children. The affected children begin to display hair loss, growth retardation, osteoporosis, and other age-related diseases usually associated with aged individuals. HGPS is caused by a point mutation in the LMNA gene responsible for the production of Lamin A and Lamin C, which serve as scaffolding proteins for the nuclear lamina. The nuclear lamina is a structure inside the nucleus that is composed of intermediate filaments and membrane-associated proteins that provide nuclear support and chromatin organization for the cells. The nuclear lamina also plays an important role in the transcriptional regulation of several genes, including the anti-oxidant genes induced by oxidative stress. The point mutation in the LMNA gene results in the loss of 55-amino acids generated by aberrant splicing; the mutant protein is known as progerin. Progerin is responsible for the premature aging phenotype seen in individuals with HGPS. Proteotoxicity due to misfolded proteins is thought to be a central component of aging. The heat shock response (HSR) triggered by heat stress and misfolded proteins is one of the pathways that cells use to protect themselves against proteotoxicity. HSR is downregulated in old organisms in most previously established models of aging. The objective of this project is to study how HGPS cells respond to heat shock. Using an inducible Tetracycline system, Wt-LMNA and progerin expressing cells were treated to a heat shock time course experiment and a Trypan blue viability assay. We found that progerin expressing cells had a higher basal level of HSP70 compared to the Wt-LMNA expressing cells and a lower cell viability/survival rate in the absence of doxycycline in comparison to the Wt-LMNA expressing cells. Therefore, we concluded that progerin might affect HGPS cells ability to respond to heat shock.

Synthesis of a Macrocyclic Triamine for Metal Ion Binding

Kevan J. English, Christina V. Pizza, Alexander M. Condoroteanu-Orovean, Ajay R. Lajmi, PhD;

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

Macrocylic polyamines serve as ligands to coordinate with metal ions and are commonly used to mimic the active sites of enzymes. Our goal was to synthesize one such polyamine, namely, 1,5,9-Triazacyclododecane (TACD). This triamine serves as a ligand to coordinate zinc ions (Zn(II)) in solution to mimic the active sites of physiologically significant enzymes such as carboxypeptidase and thermolysin. The synthesis was performed in two steps. In the first step, a substitution nucleophilic bimolecular reaction (SN2) between hexahydropyrimidopyrimidine and 1,3-propane ditosylate at room temperature resulted in a tricyclic salt of a carbocation intermediate at the most centered carbon in the molecule. This intermediate was subsequently reduced in situ by a mild reducing agent, sodium borohydride to form an orthoamide. In the second step, the orthoamide was hydrolyzed to form TACD in an overall 25-35% yield. The product was confirmed using nuclear magnetic resonance spectroscopy (1H NMR and 13C NMR) and gas chromatography – mass spectrometry (GC-MS).

Synthesis of a Polyamine Ligand as Metalloenzyme Mimic

Alexander M Condoroteanu-Oroveanu, Markis J Harris, Ajay R. Lajmi, PhD

Cyclic amines have been used in the literature for coordination with metal ions as mimics of enzyme active sites. Our goal was to synthesize 1,5,9-triazacyclododecane (TACD) as a zinc (Zn (II)) ion to serve as a functional mimic of the active site of carboxypeptidase and thermolysin. The three amine nitrogen atoms coordinate with Zn(II) ion while the fourth site is occupied by a water molecule that is deprotonated at physiological pH to form a zinc-bound hydroxide that catalyzes amide and ester substrates. The synthesis was performed in three steps. In the first step, the three amino groups in 3,3'-diaminodipropylamine were tosylated by reacting it with p-toluenesulfonyl chloride to form a tritosylated triamine. The product of the first step was cyclized by reacting it with 1,3-dibromopropane to form a 1,5,9-tritosylated-triazacyclododecane. In the final step, the tosyl group on the three amines was hydrolyzed using hydrobromic and glacial acetic acid to form TACD.

Molecular mapping and subtyping of gliomas from The Cancer Genome Atlas

Dakota Booth[1,2], Paul Tran[1,2], Lynn Tran[1,2], Sharad Purohit[1,3,4], and Jin-Xiong She[1,3]

[1]Center for Biotechnology and Genomic Medicine, [2]University System of Georgia MD-PhD Program, [3]Department of Obstetrics and Gynecology, [4]Department of MLIRS, Medical College of Georgia at Augusta University, Augusta, GA

Gliomas affect around 20,000 patients every year. Grading is based on pathological observations, however, there is a need for molecular subtyping due to high interobserver and intraobserver variations in glioma grading. The Cancer Genome Atlas (TCGA) Research Network performed multi-omics analysis of several hundred glioma patient tumors and identified molecular subgroups with prognostic value. Known factors for this classification include IDH1 mutation, 1p19q codeletion, ATRX mutation, MGMT methylation, and other genetic aberrations. The goal of this project was to use a single platform, gene expression, to classify patients with low grade gliomas (grades II and III) and glioblastomas (grade IV) from TCGA into prognostically relevant molecular subgroups. Gene expression was chosen as a platform, because, based on the central dogma of molecular biology, many genetic aberrations (mutation, methylation, etc.) can cause the same transcriptomic effects. This study used t-Stochastic Neighbor Embedding (t-SNE), differential expression analysis, and gene set enrichment analysis (GSEA). TCGA genomic, transcriptomic, and phenotypic data was downloaded from the UCSC Xena Data Portal. We identified 3 prognostically relevant subgroups of glioma patients, including subgroups that had been previously identified using multiple platforms. IDH1 mutation status, TERT promoter status, and 1p19q codeletion status were all relevant factors for the subgroups. These findings suggest that using gene expression could be a more efficient way to evaluate prognosis of patients with gliomas. Future directions include further evaluation of the patients using GSEA to identify actionable pathways, and in vivo testing in animal models to target these pathways. Supported by the University System of Georgia MD-PhD Program and by the Center for Biotechnology and Genomic Medicine, Medical College of Georgia, Augusta, GA.

Discovery Pipeline of Cancer Transcriptomes Using Machine Learning

Eileen Kim[1], Lynn Tran[1,2], Paul Tran[1,2], Bruno Dos Santos[1], Sharad Purohit PhD[1], Jin-Xiong She PhD[1,3]

[1]Center for Biotechnology and Genomic Medicine

[2]University System of Georgia MD-PhD Program

[3]Department of Obstetrics and Gynecology, Medical College of Georgia at Augusta University, Augusta, GA

Introduction: Cancer affects millions of people and causes nearly 1 in 6 deaths worldwide. Precision oncology holds potential in guiding cancer treatment on a molecular basis and elucidating targetable cancer pathways. This study aims to create a discovery pipeline that uses cancer patients' transcriptomes and machine learning algorithms to predict cancer patient survival.

Methods: Transcriptomic and phenotypic data from cancer patients were downloaded from The Cancer Genome Atlas (TCGA). Initial genes were selected using univariate cox proportional hazard models to predict overall survival across all cancer types. Co-expressed selected genes were clustered, creating gene sets per cancer subtype. Then, an amalgam of LASSO and elastic-net regularized generalized linear models (GLMNET), gene bootstrapping, random forest without/with shadow features (Boruta), and recursive feature elimination were used. Monte Carlo iterations were performed for GLMNET to assess model stability and over-/underfitting.

Results: The highest hazard ratios (HRs) and lowest p-values resulted from the univariate cox proportional hazards model (e.g. for the RASGEF1A gene in a uterine serous carcinoma model, HR=2.8e8, p=0.005) and subsequent GLMNET models (e.g. 392 genes for uterine endometrioid carcinoma, HR=2.89e8, p=0.00873). Boruta before GLMNET yielded lower HRs and higher p-values (e.g. 11 genes for uterine serous carcinoma, HR=2, p=0.0517). Bootstrapping genes before GLMNET generally yielded much lower HRs and higher p-values.

Conclusions: We were able to model survival risk in cancer patients with a variety of methods. The best method so far is univariate gene selection and GLMNET. Next steps include investigating the biology of prognostic models, using disease-specific survival, analyzing possible confounders, using Breslow-Wilcoxon or accelerated failure time models, replacing histological with molecular-signature-based cancer subtypes, and exploring alternative methods for prognosis prediction.

Identifying genetic drivers of poor prognosis in uterine serous carcinoma using regulatory network analysis

Emily K. Myers[1,2], Lynn K. H. Tran[1,3], Paul M. H. Tran[1,3], David Mysona, MD[4], Sharad Purohit, PhD[1,5], J.J. Wallbillich, M.D.[1,5,] and Jin-Xiong She, PhD[1,5]

[1]Center for Biotechnology and Genomic Medicine, Medical College of Georgia at Augusta University, Augusta, GA, [2]Medical College of Georgia at Augusta University MD Program, Augusta, GA, [3]University System of Georgia MD-PhD Program, Augusta, GA, [4]Department of Obstetrics and Gynecology, University of North Carolina Chapel Hill, Chapel Hill, NC, [5]Department of Obstetrics and Gynecology, Medical College of Georgia at Augusta University, Augusta, GA

Uterine serous carcinoma (USC) is a subtype of endometrial cancer (EC), which is the most common gynecological malignancy in the developed world. Though USC is a Type II EC, which are only responsible for 20% of EC cases, Type II EC causes 74% of EC deaths. In our previous work, we developed and validated a 78-gene expression signature, USC78, which predicts USC patient prognosis by dividing patients into good (USC high) and poor (USC low) prognosis groups. However, little was understood about why differential expression of these particular genes predicts patient prognosis. In the current study, we investigated the key transcription factors driving the differential survival between the two groups by using the Passing Attributes between Networks for Data Assimilation (PANDA) algorithm. As described by Glass, et al., PANDA uses message passing to integrate information from multiple data types to construct robust interaction networks between transcription factors (TF) and genes. We selected potential key regulators from the PANDA-generated regulatory networks. First, we use linear regression analysis to select TFs involved in gene interactions with the greatest edge weight differential between the two groups. Second, we identify TFs involved in unique regulatory interactions to each group. Third, we analyze the TF-gene signature gene interaction networks for both patient populations to select hub transcription factors. TFs selected in all three analyses as potential key regulators are ranked using summed residual values to determine in which group the TFs were weighted more strongly. Literature review revealed that the TFs most heavily weighted in the poor survival group are implicated as tumor suppressors. The TFs most heavily weighted in the good survival group are implicated as tumor promoters. This suggests that fast-growing tumors correlate with good survival and slow-growing tumors correlate with poor survival, and we plan to test this hypothesis in future studies.

Perfluorocarbon Nanodroplets for Extravascular Contrast-Enhanced Ultrasound in Cancer

Steven K. Yarmoska*, Heechul Yoon+, Vadakkancheril S. Jisha, Ph.D.+, Yiying I. Zhu, Ph.D.+, Eleanor M. Donnelly, Ph.D.+, 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

Perfluorocarbon nanodroplets (PFCnDs) are a class of phase-change contrast agents for ultrasound and photoacoustic (US/PA) imaging. They are stable at sub-micrometer sizes prior to core vaporization via user activation. These agents have the potential to traffic into the tumor stroma via neovascular fenestrations prior to imaging, and thus have the ability to serve as image-guided diagnostic or therapeutic agents in cancer via bioconjugation and drug encapsulation, respectively. Still, this theranostic US/PA paradigm relies on PFCnD extravasation, a phenomenon that has not been conclusively demonstrated by the field. The purpose of this work is to design a particle capable of robustly assessing PFCnD extravasation in realistic animal models of breast cancer.

Methods

PFCnDs were synthesized using a perfluorohexane core, phospholipid shell, and encapsulated near-infrared photoabsorber to initiate optical droplet vaporization during US/PA imaging. PFCnDs also included encapsulated Cy3 carboxylic acid and 18:0 Cy5 PE within the shell, conferring on-particle FRET capability for intact PFCnDs on histology. The PFCnD size distribution was assessed by nanoparticle tracking analysis. Fluorescence emission was analyzed using a spectrofluorometer with 540-nm excitation. US/PA images were captured with a custom setup utilizing a programmable ultrasound system coupled to a pulsed Nd:YAG laser. For preliminary animal imaging, athymic female mice were orthotopically inoculated with 4T1 triple-negative rat breast carcinoma cells. Tumors were allowed to grow for 5-7 days prior to US/PA imaging. After preliminary imaging, mice were systemically injected with PFCnDs. Following 24 hours of PFCnD circulation, mice were imaged again both before and after perfusion.

Results / Discussion

PFCnDs exhibited on-particle FRET in vitro, with attenuation of peak fluorescent emission compared to Cy3-Cy5 nanomicelles. Imaging studies showed US/PA contrast enhancement at 24 hours compared to pre-imaging in both pre- and post-perfusion images, suggesting that PFCnDs are extravascular. Ongoing studies will examine tumor histology for FRET signal.

Assessing treatment response of GBM to an HDAC inhibitor, belinostat (PXD101)

Saumya Gurbani, Soma Sengupta, MD, PhD, Alfredo Voloschin, MD, Zhongxing Liang, MD, PhD, Younghoon Yoon, PhD, Jose Velazquez Vega, MD, Chad Holder, MD, Jeffrey Olson, MD, Hui-Kuo Shu, MD, PhD, Hyunsuk Shim, PhD;

Emory University, Atlanta, GA

The current standard of care for glioblastoma (GBM; WHO grade IV glioma) is maximal safe resection followed by radiation therapy (RT) with concurrent and adjuvant chemotherapy. Despite this aggressive treatment, the median survival remains only 15 months. Thus, there is a need for better treatment options. Histone deacetylases (HDAC) are proteins with broad impact on many cellular functions implicated in oncogenesis. Belinostat (PXD101; Spectrum Pharmaceuticals) is a pan-HDAC inhibitor (HDACi) with increased blood-brain barrier uptake compare to the previous HDACi's. in this work, we evaluate the mechanism of belinostat in vitro and its anti-tumor effect in an in vivo orthotic rat glioma model. The data shows that belinostat has an excellent dose-dependent anti-tumor effect as evaluated by behavioral changes and tumor volume, and that it restores MR-spectroscopy-detectable metabolism of N-acetylaspartate and myo-insolitol in vitro better than other HDACi's. We also present data from several representative cases of patients treated with belinostat in addition to standard chemoradiation as part of an ongoing clinical trial (NCT02137759), assessing their metabolic response on spectroscopic MRI (sMRI) using the ratio of choline to N-acetylaspartate (Cho/NAA). It is observed that a patient who weakly stained for acetyl H4 immunohistochemical analysis (IHC) showed an increase in metabolically-active tumor volume (as measured by Cho/NAA > twice contralateral white matter) at one month post-radiation, indicative of tumor proliferation, whereas two patients with strong acetylated H4 IHC showed sMRI signal decrease, indicative of tumor reduction. A patient with intermediate acetyl H4 IHC showed a small decrease in tumor volume. In summary, the preclinical data and these cases suggest that acetyl H4 can be a good biomarker for predicting HDACi treatment efficacy, and that their early response can be reliably monitered non-invasively using sMRI.

The Effect of Epigenetic Pharmacological Agents on Abnormal Nuclear Morphologies in Cancer

Aliasger Ezzi, Andrew C. Tamashunas, Vincent J. Tocco, James H. Matthews, Hendrik Luesch, Jonathan D. Licht, Richard B. Dickinson, and Tanmay P. Lele

Abnormal nuclear shapes are hallmarks of many diseases. Nuclear size and shape are prognostic and diagnostic indicators of cancer. Irregular nuclear shapes can be characterized by blebs, bulges, and concavities in the contour. To identify families of proteins which may play a role in nuclear shaping, our lab conducted a high-content RNAi screen of 615 epigenetic-related genes and screened for nuclear shape. Building on the results of the siRNA screen which revealed that chromatin regulators, particularly those that regulate histone modifications, play a substantial role in nuclear shaping, we then asked if pharmacological agents with chromatin-regulating targets could similarly dysregulate nuclear shape. To investigate this possibility, we are systematically screening 146 drugs in MCF-10A and MDA-MB-231 cells using confocal and epifluorescence microscopy and assayed for nuclear shape. MCF-10A (non-tumorigenic breast epithelial) cells have regular nuclear shapes compared to MDA-MB-231 (human breast adenocarcinoma) cells. We hope to find that drugs that inhibit similar molecules as those that produced irregular nuclear shapes when knocked down in the siRNA screen, also produce irregular nuclei in MCF-10A cells when treated with the drug. We are also interested in identifying if drugs that have the opposite effect on these molecules can make the nucleus more regular in cancer cells with irregular nuclei. This would allow us to use pathway analysis software to identify potential pathways involved in regulating nuclear shaping in breast epithelial cells. Many epigenetic drugs require several cell divisions cycles for the effects to be apparent. Thus, an incubation period of seven days is being used to allow for approximately seven cell cycles.

The Effects of Diet-Induced Obesity on Tumor Microenvironment and Immune Checkpoint Blockade Efficacy

Stephanie O. Dudzinski.*, Kathryn E. Beckerman M.D., Ph.D.+, Todd D. Giorgio, Ph.D.*,@, Jeffrey C. Rathmell, Ph.D.^ ,

*Department of Biomedical Engineering, @Department of Cancer Biology, Vanderbilt University, Nashville Tennessee; +Department of Medicine, ^Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN

Immune checkpoint blockade treatment is life-saving for some patients, but most do not respond. Obesity is a risk factor for many different types of cancer, and while it is known that systemic immune dysfunction occurs in obesity, no studies have linked how this dysfunction affects immunotherapy efficacy. Obesity promotes immune dysfunction through T cell exhaustion, which is well understood, and changes in macrophage polarization, which is not well characterized in the setting of obesity and cancer. Therefore, we hypothesize that obesity-induced immune changes would decrease immunotherapy efficacy. We used a 60 kcal high-fat diet for twelve weeks in C57BL/6 mice. Male mice a received a subcutaneous injection of MC38 colon cancer cells. Female mice received an orthotopic injection of E0771 breast cancer cells. Tumor growth was measured, collected three-weeks post-injection, and processed into single cell suspensions for flow cytometry. Samples were stained for helper T cells, cytotoxic T cells, PD1 and CD44 expression, M1 and M2 macrophages, and myeloid derived suppressor cells (MDSCs). In both models, DIO mice have larger tumor growth rate and burden than lean mice. Tumors in DIO mice have fewer CD8+ and CD4+ T cells, but the T cells express higher levels of CD44+ and PD1. MC38 tumors in DIO mice have a more M2 macrophages and fewer M1 macrophages, while exhibiting more splenic MDSCs. We will investigate how baseline DIO immune differences immunotherapy efficacy. These studies have important translational implications in understanding the efficacy of immunotherapy treatment in obese patients. When giving anti-PD-1 antibodies in immunotherapy studies to DIO and lean mice, the DIO tumors are larger, indicating a smaller response to anti-PD-1 therapy compared to the lean mice. Future studies will characterize the mechanism of these differences.

Tumor-derived exosomes drive tumor metastasis through metabolic reprogramming of macrophages in a pre-metastatic niche

Samantha M. Morrissey*, Jun Yan, M.D.,Ph.D+

*Department of Microbiology and Immunology, University of Louisville, Louisville, KY + James Graham Brown Cancer Center, University of Louisville, Louisville, KY

Purpose: Lung and bronchial cancers are the leading cause of cancer mortality. Although new immunotherapies targeting PD-1 (programmed death-1) and its ligand (PD-L1) are improving this situation, progression free survival (PFS) rates are still low. The question then remains as to what mechanisms enable tumor growth and metastasis despite active treatment? The goal of this project is to determine if tumor derived nanoparticles, namely exosomes (TDE), can drive cancer metastasis through polarization of immunosuppressive cell phenotypes capable of restricting αβ T cell effector functioning in a distant pre-metastatic niche.

Methods: TDE were isolated using serial ultracentrifugation from Lewis Lung Carcinoma cells. Metabolic function was assessed using an XFe96 extracellular flux analyzer along with 1uM 2-DG and 10uM BAY inhibitors. RT-PCR was performed following Trizol RNA extraction using the SYBR Green system.

Results: TDE stimulation differentially upregulates immunosuppressive PD-L1 on CD11b+ macrophages both in vitro and in vivo despite the absence of circulating tumor cells. This polarization is thought to occur through two separate pathways, one mediated by NF-κβ and the other via metabolic reprogramming. TDE have been shown in vitro to metabolically reprogram macrophages to display a “Warburg” phenotype characterized by increased lactate production, which has been shown to limit effector T cell function and increase tumor associated macrophage production.

Discussion/Conclusion: The average PFS rate for immune checkpoint therapies is 4 months. We hypothesize that TDE released from the primary tumor antagonize anti-PD-1 therapy by seeding distant “soils” for metastasis increasing the probability for progressive disease. Data from our lab has shown that TDE are capable of metabolically reprogramming cells and markedly increasing their PD-L1 expression. Future experiments will focus on the effect elimination of TDE has on primary tumor metastasis in addition to recapitulating the phenotype with human lung cancer patient derived exosomes and human PBMCs.

Applications of a Cellular Proliferation Gene Signature in Precision Oncology

Paul Tran+,*, Shuchun Li+, Hai-Tao Liu+, Lynn Tran+,*, Sharad Purohit+,^ , Boying Dun+,^, and Jin-Xiong She+,^
+Center for Biotechnology and Genomic Medicine,

*University System of Georgia MD/PhD Program, and ^Department of Obstetrics and Gynecology, Medical College of Georgia at Augusta University, Augusta, GA

An important focus in current cancer research is in precision oncology, which is the concept of the right treatment for the right patient. Current research include basket trials like the MATCH trial and identifying biomarkers of response to current FDA approved therapies. However, only +0-20% of patients screened have an actionable genomic aberration, and thus, 80-90% of cancer patients do not benefit from current precision oncology trials. Additionally, these approaches do not account for the molecular drivers of poor survival outcome in cancer patients. This suggests that many important cancer pathways are not yet targeted in clinics. Thus, we aim to identify potentially druggable gene signatures associated with poor prognosis in cancer.

We analyze data from The Cancer Genome Atlas (TCGA) to identify patient groups with differential survival characteristics using gene expression data. We applied a univariate Cox regression model to identify genes associated with patient survival and identified a 67 gene signature of highly correlated genes significant for cell cycle related gene ontologies on over-representation analysis.

Patients from the TCGA dataset whose tumors have a higher gene expression score are more likely to have worse survival prognosis compared to tumors with a lower gene expression score, demonstrated through Kaplan-Meier survival analysis and univariate and multivariate Cox proportional hazard models. This suggests we have identified a co-regulated gene expression network associated with cellular proliferation which predicts overall and recurrence-free survival in many of the cancers in the TCGA dataset. We further analyzed data from the NCI-60 Human Tumor Cell Lines Screen and found the cellular proliferation gene signature expression is correlated with response to cell cycle inhibitors. We further validated this finding using resistant cell lines and gene knockdown models in vitro. This gene signature may provide an important tool for selecting patients for chemotherapy administration.

Use of a phospholipid binding MARCKS mimetic for the targeted killing of glioblastoma cells

Nicholas J. Eustace[1], Jason M. Warram[2], Hayley N. Widden[3], Joshua C. Anderson[1], Rune T. Pedersen[6], Patricia H. Hicks[1], William J. Placzek[3], Yancey G. Gillespie[4], Anita B. Hjelmeland[5], Christopher D. Willey[1]

Department of [1]Radiation Oncology, [2]Otolaryngology, [3]Biochemistry and Molecular Genetics, [4]Neurosurgery, [5] Cell Molecular and Developmental Biology, The University of Alabama at Birmingham, Birmingham Alabama, [6]ChemoMetec, DK-3450 Allerod, Denmark.

Introduction: Glioblastoma (GBM), like most cancers, harbors frequent mutations in phospholipid signaling that contributes to growth and therapeutic resistance. Myristoylated alanine-rich C-kinase substrate (MARCKS) effector domain (ED) is a phosphatidylinositol 4,5-bisphosphate (PIP2) and phosphatidylserine (PS) binding domain that has been used in other cancers types for its anti-tumor effects.

Purpose: Investigate the cytotoxic effects of a cell-penetrating version of MARCKS ED peptide against GBM patient-derived xenografts (PDX) and identify the specificity and mechanism of its cytotoxicity.

Methods: Cytotoxicity of a MARCKS ED featuring a cell penetrating trans-activator of transcription (TAT) sequence (TAT-ED) against seven GBM PDX lines, neural progenitor cells and normal human astrocytes (NHA) was evaluated using an ATP luminescence assay. Quantitative immunofluorescent microscopy was utilized to compare cellular accumulation of a CY7 labeled TAT-ED and investigate the mechanism of cell death through Annexin V and Sytox staining, pan-caspase inhibitors, cell cycle analysis and calcium imaging. Blood-brain barrier penetration of TAT-ED was determined In-vivo using biodistribution studies 3 hours after intravenous delivery of the peptide.

Results: TAT-ED has dose-dependent cytotoxic effects against GBM PDX lines at low micromolar concentrations, which remained non-toxic in normal human astrocytes. Quantitative Immunofluorescent imaging revealed selective binding of TAT-ED to GBM PDX lines compared to NHA's and approximately 2 times higher rates of cellular accumulation. TAT-ED was found to rapidly induce membrane ruffling, cytoplasmic contraction, Annexin Vpos /Sytoxpos staining, and trigger formation of Annexin Vpos giant plasma membrane vesicles in a caspase-independent manner. TAT-ED also triggered a rapid and sustained increase in intracellular calcium that was greater in GBM PDX lines compared to NHA's. TAT-ED crossed the BBB and accumulated in intracranial implanted GBM tumors.

Conclusions: TAT-ED preferentially accumulates in GBM PDX compared to NHA's triggering a rapid caspase-independent death characterized by calcium influx and membrane permeabilization.

Surrounding the enemy: investigating the influence of cervical microbiome and tumor microenvironment on the prognosis of cervical cancer patients.

Dewey Brooke[1,2], Jessica Blair[1], Aishwarya Sundaresan[1], Vinodh Srinivasasainagendra[1], Jianqing Zhang[2], Akinyemi Ojesina[1, 2]

[1]Dept. of Epidemiology, [2]Cancer Control and Population Sciences Program, Comprehensive Cancer Center, University of Alabama at Birmingham

While nearly all cervical tumors are infected with HPV, infection alone is not sufficient for tumor development. Although most cervical HPV infections are cleared by cell-mediated immunity, progression to malignancy is linked to an immunosuppressive tumor microenvironment comprising of a subset of protumorigenic lymphocytes and immunosuppressive stromal fibroblasts. While the tumor microenvironment directs the biology of many cancers, recent evidence has linked dysbiosis of the vaginal microbiome with the extensive reprogramming and remodeling of the cervical stroma. Using expression-based cell-deconvolution methods on RNAseq from 372 cervical carcinomas, we performed hierarchical clustering on principle components to identify three patient clusters, which were identified as either immune-rich, stromal-rich, or an intermediate immune/stromal type. Patients with immune enriched tumors exhibited a favorable prognosis. However, both the intermediate and stromal enriched tumors had significantly worse overall and disease-free survival ($p = 0.038$ and 0.0021) , with the stromal type caring the worst prognosis. Gene Set Enrichment Analysis found that genes associated with epithelial-mesenchymal transition were more strongly associated with the stromal subtype, while the immune type was strongly associated with genes involved in p53 pathways and networks. Furthermore, we used microbial transcriptomics to identify microbes significantly associated for both patients with or without recurrence.

These results are significant in that they confirm previous studies that tumors with higher stromal invasion and marked immunosuppression exhibit the worst prognosis, while identifying for the first time to our knowledge microbes associated with prognosis. Furthermore, we identified possible genetic markers that would aid both in differentiating tumor types and likelihood of recurrence.

IL-6 trans-signaling regulates cell migration in ovarian cancer cell lines

Rebekah L. Robinson* & Shruti Sharma, PhD*+

*Center for Biotechnology & Genomic Medicine, +Department of Ophthalmology, Medical College of Georgia, Augusta University, Augusta, GA

Purpose

Ovarian cancer is a significant cause of cancer death in women. Interleukin-6 (IL-6) is an important pro-inflammatory cytokine that mediates its effects by two mechanisms: IL-6 classical signaling through the membrane-bound IL-6 receptor (mIL-6R) and IL-6 trans-signaling through a soluble form of the IL-6 receptor (sIL-6R), which can act on cells that do not express mIL-6R by associating with the IL-6R co-receptor gp130. Both IL-6 and sIL-6R are elevated in epithelial ovarian cancers, and IL-6 trans-signaling has been shown to drive the development of malignant ascites in ovarian tumors. IL-6 trans-signaling can be selectively inhibited with a soluble gp130 (sgp130) molecule produced endogenously, or by the drug sgp130Fc, a fusion protein of sgp130 and the constant region of IgG1.

Methods

mRNA expression of IL-6 trans-signaling related genes were quantified by qRT-PCR in three ovarian cancer cell lines (OVCAR3, OVCAR8, SKOV3). IL-6 trans-signaling was selectively inhibited with sgp130Fc and effects on cell proliferation and migration were measured by cell counting kit-8 (CCK8) and scratch assay.

Results

We measured the mRNA expression of IL-6 trans-signaling related genes including IL6, IL6R, sIL6R (alternative splice), gp130, ADAM10, and ADAM17. All three ovarian cancer cell lines express these genes, though expression of IL6 and IL6R was significantly higher in OVCAR8 cells. After inhibition of IL-6 trans-signaling using sgp130Fc, a significant decrease in cell migration was observed in all three cell lines. However, no change was observed on cancer cell proliferation.

Discussion/Conclusion

Preliminary results indicate that IL-6 trans-signaling occurs in an autocrine manner in ovarian cancer cell lines and this signaling regulates cell migration, but not proliferation. Further studies are needed to elucidate the specific molecular mechanism of this effect, and to determine the effects of IL-6 trans-signaling on additional parameters including cell adhesion, oxidative stress and angiogenesis.

MTG16 is a transcriptional corepressor that regulates intestinal stem cell differentiation and lineage specification

Rachel E. Brown, B.S.[1,2,] Bobak Parang, M.D., Ph.D.[1,2], Sarah P. Short, Ph.D.[1,4], Pankaj Acharya, Ph.D.[3,6], Joshua J. Thompson, B.S.[1,2], Jennifer M. Pilat, B.A.[1,2,] Niyati Vaccharajani, Ph.D.[1,4], Mukul Mittal, Ph.D.[4], Amber M. Bradley, M.S.[4], M. Kay Washington, M.D., Ph.D.[5], Stephen J. Brandt, M.D.[6,7,8], Utpal P. Davé, M.D.[1,6,7], Scott W. Hiebert, Ph.D.[3,6], and Christopher S. Williams, M.D., Ph.D.[1,2,4,7,8]

[1]Program in Cancer Biology, Vanderbilt University School of Medicine, Nashville, TN; [2]Medical Scientist Training Program, Vanderbilt University School of Medicine, Nashville, TN; [3]Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN; [4]Department of Medicine, Division of Gastroenterology, Hepatology, and Nutrition, Vanderbilt University Medical Center, Nashville, TN; [5]Department of Pathology, Vanderbilt University Medical Center, Nashville, TN; [6]Department of Medicine, Division of Hematology/Oncology, Vanderbilt University Medical Center, Nashville, TN; [7]Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN; [8]Veterans Affairs Tennessee Valley Healthcare System, Nashville, TN

Inflammatory bowel disease (IBD), which affects nearly 1.5 million people in the United States, is a known risk factor for colorectal cancer (CRC) due to chronic intestinal injury leading to mutations and epigenetic alterations in the intestinal epithelium. MTG16 is a transcriptional co-repressor first identified in translocations driving acute myeloid leukemia. We previously identified an additional role of MTG16 in the intestine: Mtg16-/- mice exhibit aberrant secretory lineage differentiation and increased proliferation at baseline, are sensitized to dextran sodium sulfate (DSS)-induced colitis, and develop higher tumor burden in the azoxymethane/DSS model of inflammatory carcinogenesis. MTG16 contains highly conserved Nervy homology regions (NHRs1-4) that orchestrate the formation of repression complexes via protein-protein interactions. Thus, we hypothesized that the NHRs of MTG16 coordinate transcriptional repression programs in intestinal stem cells and secretory lineage precursors. We identified MTG16 occupancy of an LDB1 bipartite E-box motif within an enhancer in intron 1 of LGR5, a gene important in the maintenance of the intestinal stem cell compartment. In vivo studies using the Lgr5-EGFP-IRES-creERT2 reporter mouse indicated expansion of the LGR5+ intestinal stem cell population in the absence of Mtg16, implicating MTG16 as a previously unknown regulator of Lgr5. Additionally, the Hiebert lab performed a yeast two-hybrid screen for MTG16 binding partners and identified novel interactions with the transcription elongation-associated proteins MLL1, DOT1L, and AFF4 and demonstrated that deletion of NHR3 and NHR4 disabled these interactions. Furthermore, Mtg16ΔNHR3 mice exhibited increased susceptibility to DSS-induced colitis. These data suggest that NHR1, NHR3, and/or NHR4 of MTG16 bind to transcription and elongation factors to regulate intestinal epithelial Lgr5 expression, homeostasis, injury, and tumorigenesis. This work may ultimately improve IBD and CRC screening and therapeutics.

RASSF1A forms a direct complex with DAB2IP

Desmond R. Harrell-Stewart, M. Lee Schmidt, PhD, Geoff J. Clark, PhD; Department of Pharmacology & Toxicology, University of Louisville, Louisville, KY.

Purpose: Ras is the most frequently activated oncoprotein in human cancer, with gain-of-function point mutations occurring in roughly one third of all tumors. Ras activates multiple mitogenic pathways that, when overstimulated, synergize to mediate survival, proliferation, and transformation. Ras is also able to oppose tumor formation by activating pro-apoptotic and pro-senescent pathways through interactions with a family of scaffold proteins termed RASSF. The best characterized member of this family, the tumor suppressor RASSF1A, is frequently downregulated in human cancer by promoter methylation, disconnecting Ras from its apoptotic effector pathways and thus favoring proliferation. How RASSF1A is able to mediate its tumor suppressive effects is not yet fully understood. We have identified a novel binding partner for RASSF1A that is a tumor suppressor in its own right. This investigation seeks to identify the role of this novel complex in tumor suppression, particularly as it pertains to modulation of Ras signaling.

Methods: Novel binding partners for RASSF1A were identified in a yeast two-hybrid screen. Positive hits were confirmed via overexpressed and endogenous co-immunoprecipitation studies. Effects on target pathways were determined via in vitro knockdown and overexpression studies.

Results: RASSF1A forms a stable complex with the RasGAP DAB2IP independent of Ras activation. The RASSF1A-DAB2IP interaction may influence the activity of each protein, evidenced by Hippo pathway activation and NFkB suppression.

Discussion: The ability of Ras to induce cell death is critical for tissue homeostasis; however, the mechanisms involved are not well understood. Negative effectors such as RASSF1A, and regulators such as DAB2IP, play important roles in controlling Ras activation and preventing transformation. Until recently, control of Ras activation and signaling output were thought to be independent. These data suggest that the regulators and effectors of Ras may cooperate to mediate tumor suppression.

Adrenergic signaling promotes cancer cell resistance to standard chemotherapies through a mechanism that is calcium-mediated.

Jordy F. Botello, Tengfei Bian, and Chengguo Xing.

Department of Medicinal Chemistry, College of Pharmacy. University of Florida

Resistance to standard chemotherapies remains a major obstacle for the success of cancer treatment. Adrenergic stimulation, such as that seen on patients under bio-behavioral stress, adversely affects cancer cell survival. Although previous reports have attempted to define a mechanistic perspective, these remain not well understood. Here we show that norepinephrine promotes overall cancer cell survival and inhibits apoptosis induced by standard chemotherapy drugs, which are used to treat NSCLC. This resistance to apoptosis correlated with the triggering of calcium signaling responses due to norepinephrine treatment. Chelation of calcium completely blocked norepinephrine-induced cell survival and growth. The same results were obtained by the blockade of calcium influx at the membrane channel level. Furthermore, chemical probes show that these effects are largely mediated by signaling through the β2-adrenergic receptor. Ongoing research will uncover the precise protein and small-molecule interplays which result in these phenotypes, as well as the discovery of a novel drug candidate for the treatment of these malignancies. Taken together, our study has led to the discovery of a novel mechanism through which adrenergic signaling promotes resistance to standard chemotherapies and tumor survival.

Exosomes as Therapeutic Biomarkers in Prostate Cancer

Chimsom D Agbim[1], Irene Casanova Salas[2], Lloyd Trotman[2]

[1] Vanderbilt University, Nashville, Tennessee
[2] Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Purpose: Metastasis presents an obstacle in prostate cancer (PC), yet we currently lack robust methods to monitor therapeutic response in aggressive disease. Additionally, mitochondrial function and the mitochondrial genome are known to be altered in many cancers. We examine if prostate cancer cells and the exosomes they secrete influence the mitochondrial genome and function upon treatment with docetaxel, an anticancer therapy commonly used in the treatment of PC.

Methods: PC3 metastatic prostate cancer cells and 22RV1 prostate cancer cells from primary tumor were sourced for analysis. All cells were plated and separately treated with docetaxel in RPMI media. Exosomes were isolated from cells via serial centrifugation. DNA was isolated from exosomes, and changes in exosomal mitochondrial DNA (mtDNA) levels were analyzed using Quantitative Polymerase Chain Reaction (qPCR). Mitochondrial stress was measured in cells using the Seahorse assay.

Results: First, we showed that mitochondrial genes can be detected in exosomes isolated from prostate cancer cells. Additionally, exosomes from PC3 and 22RV1 cells treated with docetaxel exhibited decreased mtDNA levels. Prostate cancer cells also appear to release fewer exosomes upon treatment with docetaxel. Docetaxel treatment also induced mitochondrial stress in cells, as measured by the decreased oxygen consumption and increased extracellular acidification rates in the seahorse assay.

Conclusions: Docetaxel has an apoptotic method of cell death, and mtDNA copy number is believed to increase in cells during apoptosis. We conclude that mtDNA copies increase in cells and this decreases the mtDNA that can be packaged into exosomes. Therefore, the same reduction of exosomal mtDNA is observed in both cell lines. These results speak to the growing interest in mtDNA in cancer and suggest the potential use of mtDNA levels as therapeutic biomarkers in prostate cancer.

Assessing the effects of oncohistone mutations in H3.3 on cell proliferation in Drosophila melanogaster

Berenice Vazquez[1,2], Christopher Abdullah[3], and Robert J. Duronio[4,5,6,7]

[1]Johnson Crane Smith University, [2]SPIRE Summer Undergraduate Research Program, [3]SPIRE Postdoctoral Fellowship Program; [4]Department of Genetics; [5]Department of Biology; [6]Integrative Program for Biological and Genome Sciences; [7]Lineberger Comprehensive Cancer Center University of North Carolina at Chapel Hill

Histone proteins provide the basis for the chromatin structure and function. Histones, therefore, play a significant role in DNA packaging and gene regulation and expression. Histones are incorporated into DNA in either replication-dependent (canonical) and -independent(non-canonical or variant) manner. One example, Histone H3, has two canonical histones (H3.1/2) and one variant (H3.3) isoform which differs from H3.1/2 by only 4 or 5 amino acids. Interestingly, mutations (K36M and G34R as well as others) specific to histone H3.3 variant have been found in tumors such as pediatric brain and bone cancers diagnosed in children as early as the age of 10. Interestingly, these cancer-associated histone, or oncohistones, mutations are not found in the germline, and therefore, are not inherited or passed down into offspring. Currently, only transgenic oncohistones animal models exist and none exist that express knock-in mutations from the endogenous gene locus. We are currently generating a knock-in Drosophila model, and in parallel are testing the effects of these mutations in an in vitro cell culture model. We engineered the Drosophila melanogaster S2 cell line to transiently express the oncohistones mutations in the replication-independent histone gene, H3.3B. We then used these cells to examine cell proliferation in cells expressing the oncohistones mutations, K36M and G34R. Additionally, we are assessing expression of the mutants in parallell to the proliferation assays using immunofluorescence and antibodies specific to the oncohistone mutations. Findings from this study will help to inform future experiments in the flies.

PD-L1 and PD-L2 Differ in their Molecular Mechanisms of Regulation Mediated via PD-1 Engagement in T cells

Edie A. Osuma[1,2], Anupallavi Srinivasamani [2,3], Michael A. Curran[3]

[1]Wesleyan College, [2]Department of Immunology, MD Anderson Cancer Center
[3]MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences

Checkpoint blockade therapies targeting the PD-1/PD-L1 signaling axis show promise in obtaining durable responses across several malignancies. Monoclonal antibodies targeting either PD-1 or PD-L1 enable T cells to bypass inhibitory signals of the tumor microenvironment and mediate potent anti-tumor activity. Despite the clinical success of PD-1/PD-L1 antibodies, especially in melanoma, not all patients are responsive to treatment. Current studies have yet to establish a complete understanding of the basic immunobiology of the PD-1/PD-L1/PD-L2 signaling circuit. Most efforts are focused on delineating PD-1/PD-L1 interactions, leaving the investigation of the highest affinity binding ligand of PD-1, PD-L2 largely unexplored. The role of PD-L2 as co-stimulatory or co-inhibitory is unclear. Hence, it is critical to elucidate the precise role of PD-L2 in regulating T cell activation and effector functions. The Promega Assay was enlisted to determine whether PD-L2 is suppressive or stimulatory. The carboxyfluorescein succinimidyl ester (CFSE) proliferation assay was utilized to investigate the effects of PD-L2 on T cell proliferation. A cell viability assay utilizing 7-aminoactinomycin D (7-AAD) and Annexin V was performed to determine the effect PD-L2 has on T cell apoptosis. A cell cycle assay utilizing the synthetic nucleoside bromodeoxyuridine (BrDu), 7-aminoactinomycin D (7-AAD), and Annexin V was performed to determine the effect PD-L2 has on T cell progression through the stages of the cell cycle. Our data suggest that human PD-L2 is an inhibitory ligand of the PD-1 co-receptor that regulates T cells in a manner distinct from that of PD-L1. We also provide evidence that though PD-L2 has a higher affinity to PD-1, PD-L1 mediates a more potent inhibition of TCR signaling in T cells.

Dysregulated DNA Double-Strand Break Repair in Stem Cells from Uterine Fibroids Promotes Mutagenesis and Propagates Uterine Tumor Development

Lauren Prusinski Fernung*, Ayman Al-Hendy^, Qiwei Yang^

*Division of Translational Research, Department of Obstetrics and Gynecology, Medical College of Georgia, Augusta University, Augusta, GA, USA; ^Department of Obstetrics and Gynecology, University of Illinois at Chicago, Chicago, IL, USA

Purpose: Uterine fibroids (UFs) negatively impact female reproductive health. Somatic mutations in MED12, detected in ~85% of sporadic human UFs, are currently thought to arise in myometrial stem cells (MSCs) converting them into UF-tumor initiating cells. Defective DNA repair increases the risk of tumorigenic somatic mutations, suggesting that impaired DNA repair in fibroid stem cells (FSCs) may ultimately propagate tumor growth. We hypothesized that FSCs isolated from human UF uteri demonstrate decreased capacity to repair DNA double-strand breaks (DSBs) than adjacent MSCs, leading to increased risk of mutation and UF tumorigenesis.

Methods: Human fibroid (F) and adjacent myometrial (Myo) tissues from reproductive age women undergoing hysterectomy to treat UF were enzymatically digested into single-cell suspensions. Stro-1 and CD44 cell-surface markers were used to isolate MSCs and FSCs. A DNA Damage PrimePCR array was utilized to select candidate genes for gene expression analyses by qRT-PCR. In addition, bleomycin was used to induce DNA DSBs (with or without recovery in normal media to evaluate DSB repair), and western blot was used to examine the phosphorylation of key DSB repair proteins.

Results: FSCs demonstrated significantly decreased expression of DSB repair-related genes, specifically homologous recombination (HR) and non-homologous end-joining (NHEJ), and FSCs showed disrupted expression of phosphorylated proteins, ATM, ATR, CHK1, CHK2, MRE11, and NBS1 following DSB induction and recovery. These are important in mammalian cells for sensing and repairing chromosomal DSBs by HR, a pathway protective against tumorigenesis suggesting their impairment may be involved in UF development.

Discussion/Conclusion: Our data suggest impaired DNA repair capacity, specifically in HR, in human FSCs contributes to UF development. Further studies are needed to reveal early changes in MSCs and/or FSCs leading to UF development and their possible contributions to the ethnic disparity of this disease. Support: Augusta University Start-up Package, R01 ES028615-01 (AA), and F30 HD089585-01A1 (LPF).

Genetic modeling and pathophysiological analysis of a putative human disease gene, FAM109A

Kristin M. Ates [1,2,3], Tong Wang, Ph.D. [3], Graydon B. Gonsalvez, Ph.D. [3], Hyung-Goo Kim, Ph.D. [3], Wolfgang Wenzel, Ph.D. [4], Priya Anand, Ph.D. [4], Lynne Wolfe, MS, CRNP [5,6], David Adams, Ph.D. [5,6], Thomas Markello, M.D., Ph.D. [5,6], Cynthia J. Tifft, M.D., Ph.D. [5,6], Joshi Stephen, Ph.D. [5,6], May Christine Malicdan, M.D., Ph.D. [5,6], William Gahl, M.D., Ph.D. [5,6], Y. Albert Pan, Ph.D. [1,3]

1 Developmental and Translational Neurobiology Center, Virginia Tech Carilion Research Institute; 2 University System of Georgia MD/PhD Program; 3 Medical College of Georgia at Augusta University; 4 Institute of Nanotechnology (INT), Karlsruhe Institute of Technology (KIT); 5 National Human Genome Research Institute (NHGRI); 6 National Institute of Health (NIH) Undiagnosed Diseases Program (UDP)

A critical barrier in the understanding and treating of endocytic diseases is the lack of information and understanding of the in vivo mechanisms of endocytosis. Our goal is to improve the understanding and ability to treat endocytic diseases by investigating a de novo arginine (R) to cysteine (C) mutation in FAM109A that has recently been identified in a patient within the NIH's Undiagnosed Diseases Program (UDP). This UDP patient presents with craniofacial abnormalities, developmental delays in comprehension, motor deficits, vision impairments, and renal dysfunction. FAM109A has been identified as a key regulator in endosomal trafficking, but not much is understood regarding its function in vivo. Interestingly, FAM109A interacts with OCRL1, a gene that is mutated in Lowe syndrome, and is essential in endocytic trafficking and intracellular signaling. This suggests that FAM109A and OCRL are closely linked in a common endocytic pathway, but they may also have some functions independent of each other.

Our central hypothesis is that (1) the R6C mutation in FAM109A is causal to disease manifestations in the human patient and (2) this FAM109A mutation disrupts endocytic sorting. We will test this by recapitulating this particular human disease in a zebrafish model to study the functions of FAM109A in an organismal context. Utilizing the CRISPR genome engineering and transgenic manipulations, we have generated both a FAM109A-null zebrafish model, as well as a FAM109A-R6C model that better mimics the gene variants seen in our human patient. To test for potentially redundant functions of FAM109A and its close homolog, FAM109B, in the zebrafish, we have also generated FAM109B-null models so that we can test the effect of R6C in the absence of FAM109A/B.

After generating the mutant zebrafish models, we have performed extensive physiological and behavioral analyses on the phenotypes relevant to that of our UDP patient, with an increased focus on craniofacial deficits, sensory function, and renal abnormalities/endocytosis. The outcomes of this project will provide insight into the function of FAM109A in vivo as well as a better understanding of endocytic disorders in general.

The Effect of Aripiprazole on Neutrophil Adhesion

Amara S. Ejikemeuwa, Peter J. Cavnar, Ph.D., Department of Biology, University of West Florida, Pensacola, Florida

Atypical antipsychotic drugs (AADs) administered to treat schizophrenia have been found to positively correlate with neutropenia in a small subset of patients. Neutropenia is a disease characterized by a low level of neutrophils, which are white blood cells of the innate immune system. This decreased number of neutrophils can increase the risk of infection and illness. Our lab has previously shown that the AAD aripiprazole exhibited a dose-dependent pro-apoptotic effect on neutrophils, which could explain the neutropenia observed in patients. Here, we investigate the effect of aripiprazole on neutrophil adhesion by utilizing the chemoattractant, N-formyl methionyl-leucyl-phenylalanine (fMLF) on the neutrophil model cell line PLB-985 cells. Neutrophils were treated with aripiprazole in the presence or absence of fMLF and adhesion was measured via plate reader. Our results show that aripiprazole exhibits a dose-dependent inhibition of neutrophil adhesion. This inhibition suggests that patients using aripiprazole may have neutrophil defects resulting in an increased risk of infection.

Compound library analysis identifies a combination of small molecules that increase the terminal differentiation of human induced pluripotent stem cells into myotubes and their maturation.

Holly McKee[1], Sridhar Selvaraj[1], Ricardo Mondragon-Gonzolas[1], Fabrizio Rinaldi[2], Joy Aho[2], & Rita Perlingeiro[1]

[1]Lillehei Heart Institute, Department of Medicine, University of Minnesota, Minneapolis, MN, USA
[2]R&D systems, a Biotechne brand, Minneapolis, Minnesota

Patient-specific pluripotent stem cell skeletal muscle derivatives represent an attractive potential alternative for in vitro disease modeling without the requirement of muscle biopsy. The technology of reprogramming somatic cells into induced pluripotent stem (iPS) cells offers tremendous potential for the generation of large amounts of lineage-committed cells for disease modeling, as well as for other applications, including drug screening. Nevertheless, the overall embryonic nature of iPS cell-derivatives (across lineages) stands as a barrier for reliable disease modeling studies. Several signaling pathways have been reported to affect myoblast fusion and their differentiation into myotubes. Thus, screening for the effect of different signaling pathways and epigenetic modifiers is a promising approach to further promote the differentiation of pluripotent stem cells into myotubes and their subsequent maturation. Following an initial screening using a small molecule compound library, six compounds were selected based on initial improvement of differentiation efficiency of iPS cell-derived myogenic progenitors into myotubes, as identified through the quantification of all myosin heavy chain (MHC) isoforms by immunofluorescent staining. Of note, our results revealed that the combined exposure of the selected compounds during differentiation promotes a significant increase in fusion index (2-fold). More importantly, there was a 100-fold increase in the expression of the neonatal isoform of MHC(MHC-neo) in compound-treated myotubes in comparison to untreated controls, indicating this compound combination induced maturation of IPSC-derived myotubes.

Novel Crystal Forms of the Antibiotic Cefixime

Win S. Hon*, Kortney M. Kersten*, and Adam J. Matzger*+

*Department of Chemistry and +Macromolecular Science and Engineering, The University of Michigan, Ann Arbor, MI

In the pharmaceutical field, solid form selection is often plagued by low bioavailability, a property determined by intestinal permeability and water solubility of a drug. The poor performance of these pharmaceuticals, however, can be improved through the use of novel crystal forms. Methods used to approach and adjust the solubility include the use of various crystallization techniques as well as crystal engineering of multicomponent forms, such as solvates or cocrystals. Crystals are analyzed using different techniques including optical microscopy, Raman spectroscopy, and powder X-ray diffraction. Herein, we describe the novel crystalline forms discovered for the antibiotic cefixime. Crystallization conditions used in our research include evaporation at room temperature, heated evaporation, cooling, addition of an antisolvent, vapor diffusion, grinding, and slurrying. Our results show that molecules containing carboxylic acids interact best to form crystals with cefixime. Scale up is needed in order to perform further characterization on these novel crystal forms.

Examining the Effects of Activating SNORD116 in a CRISPR/Cas9 Mouse Model

Rachel V. Levy, University of Florida, Gainesville, FL
Jim Resnick, PhD, Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

Prader-Willi Syndrome (PWS) is a genetic disorder characterized by behavioral problems, hyperphagia, and obesity. In these individuals, approximately 12 genes normally transcribed only from the paternal Chromosome 15 are not expressed. Individuals with deletions restricted to just one gene in this region- SNORD116- show many of the major traits of PWS. A potential therapeutic approach is the attempt to activate the silent, maternal copy of SNORD116.

This project aims to examine the effects of beginning development without SNORD116 and then turning on its expression. This will reveal which traits of PWS improve with SNORD116 activation, and it will aid in determining at what point in development these traits can effectively be improved. Additionally, this will show which downstream target genes will change when SNORD116 is activated.

Cre-lox technology is being used to create a conditional knockout of SNORD116 in mice. This is accomplished by inserting a floxed transcriptional terminator into SNRPN intron 3. This mouse with the conditional knockout is mated with several different Cre lines which will lead to removal of the transcriptional terminator and subsequent expression of SNORD116 at different pre and post-natal times. Preliminary results have proven successful. In future projects, the vector necessary for the insertion of the transcriptional terminator will be built.

This model will show the implications of activating SNORD116 and provide a foundation for future work on PWS. It will be essential in studying the benefits and limitations of gene therapy approaches to PWS, and in optimizing timing and location of gene activation.

Appropriately Timed Histone Deacetylase Inhibition Empowers T Cell-Mediated Immunity to Reject Established Breast Tumors in Pre-Clinical Models

Tyler R. McCaw1; Mingyong Liu[1]; Mei Li, PhD[2]; Dmytro Starenki, PhD[3]; Sara J. Cooper, PhD[3]; Rebecca C. Arend, MD[4]; Andres Forero, MD[5]; Donald J. Buchsbaum, PhD[2]; Troy D. Randall, PhD[1];

[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/Oncology, University of Alabama at Birmingham, Birmingham, AL

Purpose: Histone deacetylase (HDAC) inhibitors can impair tumor growth and render tumor cells more immunogenic; however, the effects of HDAC inhibition on tumor-infiltrating T cells have been controversial. These agents can impede T cell activation but paradoxically boost effector functions. This led us to hypothesize that appropriately timed HDAC inhibition might mitigate the negative effects on T cell activation, while increasing tumor cell immunogenicity, and promote cytotoxicity of infiltrating T cells.

Methods: We treated two murine mammary adenocarcinoma models, TS/A and 4T1, daily with the class I HDAC inhibitor entinostat starting at various times, later elaborating this schedule with anti-PD1. Tumor cells and infiltrating T cells were assessed by flow cytometry and RNA sequencing.

Results: We found that simply adjusting timing of HDAC inhibition relative to T cell activation could abolish anti-tumor effects or lead to rejection of established tumors in 40% of mice. Impairment of tumor growth was absolutely dependent on adaptive immunity, specifically CD8 T cells and IFNγ production. Indeed, CD8 T cells from entinostat treated tumors possessed superior cytotoxic and proliferative capacity, while maintaining a transcriptional program less susceptible to exhaustion. Additionally, treatment of tumor-bearing mice with entinostat turned on many components of an IFNγ signature recently reported to identify patients that will respond to anti-PD1. Although TS/A tumors do not respond to anti-PD1 monotherapy, treating tumor-bearing mice with entinostat then anti-PD1 at precisely the right times led to tumor rejection in nearly all mice.

Conclusions: Our work shows that HDAC inhibition using entinostat can lead to rejection of established breast tumors but only when given at precisely the right time—after CD8 T cell activation and expansion but before development of T cell exhaustion. Appropriate timing of HDAC inhibition can also sensitize tumors to anti-PD1 therapy, turning “non-responders” into “responders”, and lead to consistent rejections.

Glutaminase inhibition and CD8 T cell fate in cancer immunotherapy

Matthew Z Madden*, Marc O Johnson*, Gongbo Li PhD+, Marco L Davila MD PhD+, Jeffrey C Rathmell PhD*;

*Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville TN;
+Moffitt Cancer Center, Tampa FL

Purpose: Cancer immunotherapy such as immune checkpoint blockade and adoptive cell therapy has revolutionized oncology but does not cure the majority of patients. One factor limiting immunotherapy efficacy is tumor-specific T cell metabolic fitness, which may limit T cell effector and memory-like phenotypes important for cancer clearance. To optimize T cell metabolism and increase the efficacy of cancer immunotherapy, we propose transient inhibition of the enzyme glutaminase (GLS) in T cells.

Methods: Murine T cells were treated with GLS inhibitor CB839, a small molecule currently being tested in cancer clinical trials. A T cell specific genetic knockout of GLS (CD4-Cre Gls fl/fl) was also studied. CD19-specific chimeric antigen receptor (CAR) T cells were created in vitro by transducing murine T cell with retrovirus and were then adoptively transferred into mice. T cell differentiation, activation, and metabolism were examined by flow cytometry and other methods.

Results: Transient GLS inhibition in vitro increased markers of T cell effector function including Perforin, Granzyme B, and Tbet. GLS inhibition also increased markers associated with a memory-like phenotype including CD62L, BCL2, TCF1, and EOMES. Mitochondria in CB839-treated T cells had lower membrane potential and demonstrated reduced turnover. CB839-treated CAR T cells controlled target cell growth more effectively in vitro and attained higher numbers in vivo.

Conclusion: Transient GLS inhibition in T cells increases effector function, induces a memory-like phenotype, and modulates mitochondrial fitness, which overall may enhance cancer immunotherapy efficacy. Future studies will more closely examine the cellular consequences of GLS inhibition and will directly test T cell GLS inhibition with immunotherapy.

Mitochondrial Metabolism in M2 Macrophage Polarization: The Role of MIF and Lactate

Jordan T. Noe*ǂ, Samantha M. Morrissey+, Mariana Barbosa de Souza Rizzoǂ, Beatriz Rendonǂ, Eun Jung Kimǂ, Robert A. Mitchell*+ǂ, PhD

Department of Biochemistry and Molecular Genetics* and Microbiology and Immunology+; J.G. Brown Cancer Centerǂ, University of Louisville, Louisville, KY

Purpose:
M2-polarized tumor-associated macrophages (M2-TAMs) promote cancer progression through angiogenesis, metastasis, and immunosuppression. Cancer cells exhibiting the "Warburg Effect" highly produce lactate for enhanced malignancy, in part, by promoting M2-TAM polarization. As M2 macrophages require mitochondrial metabolism and lactate was recently found to support mitochondrial metabolism, this suggests that metabolic cooperation exists between cancer cells and M2-TAMs. To utilize lactate, M2 macrophages must first undergo metabolic reprogramming towards mitochondrial metabolism. We have previously found that macrophage migration inhibitory factor (MIF) promotes M2-TAM polarization in vivo and current evidence indicates a role for MIF in mitochondrial metabolism. The purpose of this investigation is to delineate the underlying mechanisms by which mitochondrial lactate metabolism supports M2-TAM polarization and determine the mechanistic contribution of MIF in promoting this process.

Methods:
Bone marrow-derived macrophages (BMDMs) from MIF+/+ or MIF-/- C57BL/6 mice were obtained by ex vivo differentiation with M-CSF. The cells were then plated in glucose-free media and supplemented with the exogenous metabolites and/or compounds before M2 polarization with IL-4. BMDMs were subsequently analyzed by gene expression assays, extracellular flux analysis, and flow cytometry for determinations of M2 polarization, mitochondrial metabolism, and immunosuppression.

Results:
Decreasing glucose levels reduces M2 polarization and this effect is rescued with exogenous lactate. Inhibition of mitochondrial lactate metabolism blocks M2 polarization. MIF-deficient macrophages have decreased M2 polarization, mitochondrial metabolism, and NRF2 expression. Activating CSN5 rescues M2 polarization in MIF-deficient macrophages.

Conclusions:
Macrophages maintain M2 polarization in tumor microenviroment-like conditions through mitochondrial lactate metabolism, which suggests that Warburg-like cancer cells enhance tumor progression by promoting immunosuppression through M2-TAM polarization. In addition, MIF functionally regulates CSN5 to stabilize NRF2 and enhance mitochondrial metabolism during M2 polarization, which suggests that MIF is a critical determinant of metabolic reprogramming in M2-TAMs and indicates the mechanisms by which MIF promotes M2-TAM polarization in vivo.

CD4 T Cell Restricted HIV Cryptic Epitopes, an Implication of Novel Antisense Proteins

Jacob Files, Jason Peng, Anju Bansal, Paul Goepfert

Background- The alternative reading frames (ARF) of HIV-1 have been shown to encode and produce peptide antigens of unknown function, termed cryptic epitopes (CE). Much of the current work on CE have mainly focused on the recognition of these epitopes by CD8 T cells, due in part to the fact that CE are often thought to be products of translational errors and are thus recycled and presented solely through the MHC-I pathway. This work investigated whether CD4 T cells could recognize CE.

Method- We generated overlapping peptides to previously described antisense open reading frames of HIV-1 and optimized it for CD4 T cell recognition (CD4-CE). Isolated PBMC from chronic stage HIV-1 infected patients were first depleted of CD8 T cells, the remaining cells were then stimulated with CD4-CE and tested for immunogenicity via IFN-γ ELISpot and flow cytometry.

Results- Two of the CD4-CE utilized were found to be immunogenic and strictly recognized by CD4 T cells within the small cohort tested (N=20). These results were further confirmed by flow cytometry, demonstrating CD4 production of effector cytokines in response to CD4-CE, but a lack of CD8 T cell response to these epitopes.

Conclusion- We show that immune responses to CE are not only limited to the CD8 branch of T cells but can also broaden immune responses to the virus by triggering CD4 subsets as well. Our observation suggests that in addition to ASP, HIV-1 can generate other potential novel and yet unidentified proteins from its antisense transcripts.

Human neutrophils depend on accessory cells for their survival response to LPS

Cassandra R. Woolley*, Shuvasree SenGupta*, Madhavi J. Rane+, Silvia M. Uriarte+, and Thomas C. Mitchell*

Department of Microbiology and Immunology* and Department of Medicine+, School of Medicine, University of Louisville, Louisville, KY.

Purpose: Lipopolysaccharide (LPS) prolongs neutrophil survival via activation of Toll-like receptor 4 (TLR4), but the mechanism(s) are not fully known. We aimed to identify cells, soluble factors, or pathways regulating neutrophil survival downstream of TLR4 activation.

Methods: We tested the hypothesis that neutrophils require accessory cells for survival by measuring viability of enriched (90-95% pure, "N90") or purified (>99% pure, "N99") neutrophils after culture in the presence or absence of TLR4 agonist, lipid A. N90 were prepared by percoll/plasma gradient; N99 were prepared with StemCell Technologies "EasySep Human Neutrophil Isolation" kit. Cells were cultured with medium alone or supplemented with lipid A. After 24hrs, viability was assessed with APC-conjugated Annexin V and 7-AAD stains. To identify soluble factors, cell-free supernatants (SUPs) of N90 after culture with lipid A were collected and added to N99 with or without neutralizing antibodies for candidate factors. Select cell-types were depleted from N90 using StemCell Technologies "EasySep DIY Selection" kit. Flow cytometric data were analyzed with FlowJo; T-tests of mean values after normalization were performed with GraphPad Prism7.

Results: Lipid A increased survival of N90 but not N99, suggesting accessory cells mediate survival. Culture SUPs from N90+lipid A enhanced survival of N99 cell populations, suggesting presence of soluble survival factors. Neutralization of IL12, IL24, IL27, and IL36 gamma did not affect survival. Neutralization of GM-CSF in multiple SUPs significantly decreased neutrophil survival, but not to the untreated level, indicating GM-CSF is not the only factor responsible. Depletion of CD36+ cells abolished survival activity of lipid A in N90, but depletion of CD3+ (T lymphocytes), CD41+ (platelets) or Siglec-8+ (eosinophils) had no effect.

Conclusions: These observations indicate CD36+ cells, likely monocytes, are activated by TLR4 in response to LPS and secrete factors like GM-CSF to regulate neutrophil survival. The dependence of neutrophils on monocytic cells for TLR4 survival highlights the need for consideration of accessory cells in therapeutics targeting neutrophil persistence in inflammatory pathologies.

Influence of Immune System on Osteochondromas

Spencer Richardson+#, Marie Wehenkel*, PhD, Jenny Johnson*, PhD, Cliff Guy*, PhD, Mareen McGargill*, PhD;

+College of Medicine, University of Tennessee Health Science Center , Memphis, TN; #Howard Hughes Medical Research Fellowship; Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN

PURPOSE: Osteochondromas are benign cartilage-capped bone tumors that are normally solitary. Individuals with the condition multiple hereditary exostoses (MHE) present with multiple osteochondromas due to heterozygous loss of function of exostosin glycosyltransferase 1 (EXT1) or 2 (EXT2). Mice with a heterozygous deletion of Ext1 (Ext1+/-) model the mutations observed in MHE patients; however, these mice show very limited penetrance of osteochondromas. Therefore, it is widely assumed that loss of EXT heterozygosity is required for disease to occur. However, evidence of loss of EXT heterozygosity in human or mouse osteochondromas is lacking. Thus, it is not understood how EXT mutations manifest in multiple osteochondromas nor why there is a discrepancy in the severity of disease among MHE patients. We and others have demonstrated that disruption of immune cell signaling cascades unexpectedly caused formation of multiple osteochondromas in mice and that absence of B and T cells can enhance the severity and incidence of these tumors. Since no immune regulator of osteochondroma growth has been described, we hypothesize that the immune system influences the growth of tumors in Ext1+/- mice.

METHODS: We activated the immune system of Ext1+/- mice with serial adjuvant treatments, an asthma model to induce airway hyperresponsiveness, and infectious pathogens.

RESULTS: Induction of an allergic asthma model and exposure to multiple adjuvants in Ext1+/- mice influenced the incidence and size of osteochondromas. Pathogen exposure was not pro-tumorigenic.

DISCUSSION: Our results support our hypothesis that stimulation of the immune system can impact osteochondroma growth in mouse models of multiple osteochondromas. Further work will determine whether specific cells types or global activation of the immune system is responsible for the enhanced incidence and severity of osteochondromas. Importantly, these data reveal a novel role for the immune system in development of osteochondromas.

Localization of Immunological Niches Associated in Human Renal Cell Carcinoma

Adriana Reyes(1), Caroline S Jansen(1), Nataliya Prokhnevska(1), Jennifer W Carlisle MD(2), Viraj A Master MD PhD(1), Haydn T Kissick PhD(1,3)

1. Department of Urology, Emory University, Atlanta, GA
2. Department of Hematology/Oncology, Emory University, Atlanta, GA
3. Department of Microbiology and Immunology, Emory University, Atlanta, GA

Recent medical and scientific advances established new anti-tumor therapies using the field of immunology—including but not limited to checkpoint inhibitors, adoptive cell transfers, and cytokines. One way to explore the immune system's role in anti-tumor immunity is through the localization of immune cells in tumors and describing its general structure. By extrapolating the different interactions occurring within such systems, more in-depth studies may lead to better targeted therapies for patients with specific types of cancers. The primary purpose of this study is to localize different immune cell types in Renal Cell Carcinoma (RCC) specific tumors.

The primary mode of immune cell localization utilizes immunofluorescence (IF) and confocal microscopy to identify cells expressing molecules of interest. The images are further quantified using cell profiler—analyzing the shape, size, and intensity of cells. Statistical analysis of the quantitative data is performed using R.

Our analysis found evidence of immune cell infiltrated and immune cell non-infiltrated phenotypes in patients with RCC using flow cytometry.

Within RCC specific tumors that are immune cell infiltrated, we found lymphoid aggregates of CD8+ T cells and MHC-II+ cells using IF. Importantly, these aggregates contained TCF1+CD8+ T cells which represent a stem-like population contributing to the maintenance of anti-tumor immunity. Additionally, we identified TCF1+CD8- cells localized both inside and outside of CD8+ staining—indicating the presence of other stem-like T cells.

We hypothesize that these TCF1+ CD8- cells also contribute to the immunological niche of the tumor and furthermore predict these cells to be CD4+ T cells.

Through our investigation, we discovered the presence of lymphoid aggregates in RCC tumors. We plan to continue exploring the structure and composition of these immunological niches using IF to investigate the role of these niches in the anti-tumor response.

Peripheral Blood Markers and Intraoperative Pathology Are Helpful in Predicting Non-Cutibacterium Acnes Shoulder Prosthetic Joint Infections

Thomas Stovall^; Marion Burnier, M.D.*; Tram Nguyen*; Bassem Elhassan, M.D.*; Joaquin Sanchez-Sotelo, M.D., Ph.D*

^Meharry Medical College School of Medicine
*Mayo Clinic Rochester Department of Orthopedic Surgery

Introduction. Periprosthetic joint infection (PJI) of the shoulder is a rare and potentially devastating complication. The purpose of this study was to compare the preoperative complete blood count (with differential), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and intraoperative pathology results of patients undergoing surgical treatment for deep infections by Cutibacterium acnes versus non-Cutibacterium acnes bacteria.

Methods. Between 2004 and 2016, 150 shoulder PJIs were treated at our institution. Medical records were reviewed to extract: microorganisms isolated in preoperative aspiration, microorganisms isolated in surgical cultures, results of intraoperative pathology, and preoperative peripheral blood cell count (including differential), ESR, and CRP. Shoulders were further grouped into those that were positive only for C. acnes (37 shoulder) and those that were positive for any other microorganism (including polymicrobial with or without C. acnes or culture negative, 113 shoulders). To calculate sensitivity, specificity, and predictive values, the same data was collected for a separate group of 150 shoulder arthroplasties that underwent revision surgery for non-infectious reasons during the same time period.

Results. For 150 infected shoulder arthroplasties, values were considered abnormal for ESR in 42.6%, CRP in 57.8% and pathology in 73.6%. Abnormal values were more common in the non-C. acnes group. The sensitivity, specificity and positive and negative predictive values of these preoperative tests also varied between the C. acnes and non-C. acnes groups. The following positive predictive values were obtained: ESR 71% (24% C. acnes, 68% other), CRP 81% (43% C. acnes, 78% other), and pathology 80% (42% C. acnes, 76% other).

Conclusions. Although the value of preoperative peripheral blood markers and intraoperative pathology is low for C. acnes PJIs, obtaining these tests should not be discontinued, since their positive predictive value for non-C. acnes infections seems reasonable.

Characterizing human target cells infection by three geographically distinct isolates of Mayaro virus

Aum Patel*, Jordan Dailey*, Melissa Dulcey*, Ruiyu Pu^, Amy Vittor*

*Department of Medicine, University of Florida, Gainesville, Florida; ^Department of Comparative, Diagnostic, and Population Medicine, University of Florida, Gainesville, Florida

Background: Mayaro virus (genus Alphavirus, family Togaviridae) is an emerging arthropod-borne virus transmitted by Haemagogus mosquitoes in sylvatic regions of Central and South America. Mayaro virus (MAYV) infection leads to fever, maculopapular rash, and arthralgia. Limited data exist pertaining to regional differences in MAYV in vitro infectivity in human cells. Here we describe viral kinetics, cytopathic effects, and human target cell susceptibility to three geographically distinct MAYV isolates represented genotypes D and L (Uruma, Peru and Brazil).

Methods: MAYV susceptibility of key human target cells (human dermal fibroblasts, human embryo kidney cells (HEK293), monocytes and skeletal muscle satellite cells), including Vero E6 cells was visualized using immunofluorescence microscopy at 0, 24, 48 and 72h post infection (p.i.). Viral kinetics and titers were determined for each cell line from 0h to 72h p.i. at MOI=1, through viral plaque assays in Vero E6 cells, while visualizing cytopathic effect by staining with crystal violet.

Results: Immunofluorescence and flow cytometry revealed that human dermal fibroblasts, skeletal muscle satellite cells and Vero E6 cells were all susceptible, but to differing degrees. HEK293 became infected at significantly lower rates, and monocytes were nearly refractory to infection. Viral replication kinetics assays revealed that peak viral titers occurred for all three viral isolates at different times, reaching 1x108 pfu/ml. MAYV-Uruma reached this peak the fastest, followed by MAYV-Brazil and then MAYV-Peru. Crystal violet staining also demonstrated differences in viral pathogenesis.

Conclusions: These results indicate that MAYV can infect human dermal fibroblasts and skeletal muscle cells, in keeping with clinical symptoms associated with this virus. Some differences in infectivity are apparent across different MAYV isolates, and may contribute to variable virulence and pathogenicity. These findings advance our understanding of MAYV infection of human target cells, and provide some initial data with regards to MAYV phenotypic variation according to geography.

Stochastic Microbiome Development Associates With Acute Mammalian Colitis Severity

Mahesh Krishna[1,2], Karen Queliza[1], Rajesh Shah[3], Richard Kellermayer[1,4]

1. Section of Pediatric Gastroenterology, Baylor College of Medicine, Houston, TX.
2. Wiess School of Natural Sciences, Rice University, Houston, TX.
3. Department of Internal Medicine, Section of Gastroenterology, Baylor College of Medicine, Houston, TX.
4. USDA/ARS Children's Nutrition Research Center, Texas Children's Hospital, Houston, TX.

Introduction/Purpose: Inflammatory Bowel Diseases (IBDs) are characterized by chronic intestinal inflammation in genetically susceptible individuals. The etiology and pathogenesis of IBD are not clearly understood, including the fact that genetically identical twins frequently do not share the disorders. This high monozygotic twin discordance may originate from randomly established IBD susceptibility. The gut microbiome is thought to play an important role in the development of IBD and has been observed to establish in a stochastic fashion in mammals. We hypothesized that stochastic microbiome variation plays a role in IBD development and severity.

Methods: We studied how “spontaneous” microbiome variation in genetically identical C57BL/6J mice influenced acute colitis severity in the dextran sulfate sodium (DSS) model. In a discovery and validation cohort of animals, we compared fecal microbiomes between DSS sensitive and DSS resistant mice prior to DSS.

Results: Richness and diversity of the microbiomes did not differ, but distinct separation between these two groups was found with Principal Coordinate Analysis. The genus Faecalibaculum was more abundant in mice with worse colitis ($p<0.01$), while Blautia and Lactobacillus were less common ($p<0.05$) in these animals. In the validation cohort, the separation between DSS sensitive and DSS resistant mice was not as distinct as the discovery cohort. Additionally, there was not the same separation with Principal Coordinate Analysis that was found in the discovery cohort. The difference in breeding location (commercial for discovery and local for validation) was determined to account for these differences. However, the genus Lactobacillus was again found to have a similar trend found in the discovery cohort of less common abundance in mice with worse colitis, although less significant ($p=0.20$).

Conclusions: Therefore, we concluded small vivarium based breeding decreased stochastic phenotype variation and microbiome separation in respect to colitis susceptibility in mice. Additionally, modest bacterial taxonomy variation based DSS susceptibility was detected and Lactobacillus was consistently increased in the DSS resistant mice, suggesting potential as a probiotic.

Autologous virus neutralizing monoclonal antibodies in HIV-infected, vertically transmitting and non-transmitting U.S. and Malawian women

Jesse F. Mangold, David R. Martinez, Amit Kumar, PhD, Riley J. Mangan, Elena Georgi, Juilin Chen, Michael Mengual, Holly Heimsath, Joshua A. Eudailey, Giovanna Hernandez, Papa Kwadwo Morgan-Asiedu, Kevin Wiehe, PhD, Feng Gao, MD, Sallie R. Permar, MD, PhD

Duke Human Vaccine Institute, Duke University, Durham, NC

Each year >150,000 infants become infected with HIV via mother-to-child transmission (MTCT). While some studies have shown a protective role of maternal Env-specific IgG responses against MTCT, their autologous virus neutralizing functions are not fully elucidated.

We sorted gp120-specific memory B cells from near the time of delivery in HIV-infected, non-transmitting (NT, n=3) and transmitting (T, n=4) women and sequenced their VH and VL pairs. Next, we screened functional monoclonal antibodies (mAbs) against an HIV Env antigen panel and defined their VH and VL affinity maturation characteristics. We isolated 20-30 plasma Env single genome amplicons (SGA) from NT and T women and paired infant viruses. Then, we evaluated the neutralization sensitivity of maternal and infant viruses to broadly neutralizing antibodies (BnAbs) and maternal HIV Env-specific IgG mAbs.
We found no bias in VH gene usage between NT and T women. Env-specific IgG mAbs isolated from HIV-infected T women had significantly higher rates of VH somatic mutation levels compared to NT women ($p< 0.001$, Mann-Whitney test). Moreover, gp120-specific IgG mAbs had significantly higher rates of VH somatic mutations in T women compared to NT women (9.5 vs 7.5%, $p< 0.009$, Mann-Whitney test). Neighbor-joining trees and highlighter plots revealed low genetic diversity in infant and high genetic diversity in maternal Env populations. Most Env-specific IgG mAbs isolated from T women neutralized some tier-1 viruses, yet did not neutralize maternal autologous viruses or infant viruses. However, V3-specific IgG mAbs mediated autologous virus neutralization for 6 out of 9 (67%) non-transmitting maternal viruses in a NT woman.

Env-specific mAbs in HIV-infected NT and T women target similar epitopes yet may have affinity maturation differences. NT women may have higher V3-specific IgG mAb autologous virus-neutralizing activity than T women, and these responses may select for neutralization resistant viruses in the maternal plasma virus pool.

Expression and Purification of Full-length Recombinant Plasmodium falciparum PfMC-2TM Maurer's cleft Protein

Alberto Williams-Medina, Kush Addepalli, Tobili Sam-Yellowe, Ph.D., MPH

Malaria caused by Plasmodium falciparum remains the most virulent form of malaria, resulting in 216 million cases and 445,000 deaths globally. Invasion of red blood cells by P. falciparum leads to the formation of membranous structures known as Maurer's clefts (MC). Virulence markers of P. falciparum such as PfEMP1 are transported across the MC to the surface of the infected red blood cell. Insight into the formation and function of the MC will be important for the discovery of new vaccine and drug candidates. The PfMC-2TM is encoded by a multi-gene family of 13 members. PfMC-2TM is a protein localized to the MC. We induced expression of PfMC-2TM encoded by 1 family member [PF3D7_0114100 (PFA0680c)] in BL21 DE3 strain of Escherichia coli following transformation with recombinant pET-28a plasmid containing a chemically synthesized gene. The purpose of this study was to determine immunogenic properties of the resulting recombinant protein using western blot analysis. The recombinant plasmid was isolated and analyzed in 1% agarose gel and an approximately 5kb band was identified. Pilot expression of transformants showed expression of recombinant PfMC-2TM by western blot. Recombinant PfMC-2TM protein will be expressed and purified for antibody production to allow subsequent domain analysis and characterization.

Metabolism of Sunitinib in Genotyped Primary Human Hepatocytes

Arsany A. Abouda, Elizabeth Burnham, Jennifer E. Bissada, Kj Li, Vivian Truong, and Klarissa D. Jackson, PhD;
Department of Pharmaceutical Sciences, Lipscomb University College of Pharmacy, Nashville, TN.

Sunitinib is a multitargeted tyrosine kinase inhibitor associated with idiosyncratic hepatotoxicity. The mechanisms of this toxicity are unknown. Previous studies from our lab have shown that sunitinib undergoes metabolic activation by cytochromes P450 (CYP) 1A2 and CYP3A4 to form a chemically reactive, potentially toxic quinoneimine metabolite. We hypothesize that variation in P450 enzyme activity contributes to individual differences in the generation of reactive metabolites of sunitinib, which may play a role in development of sunitinib-induced hepatotoxicity. The purpose of this study was to characterize the metabolism of sunitinib in primary human hepatocytes. Commercially available human hepatocytes were purchased from XenoTech. CYP genotype and enzyme activities for each donor were characterized by the company. Hepatocyte samples were pooled from three individual donors per lot based on their CYP3A5 genotype and predicted CYP3A5 activity level, which were classified as high activity (CYP3A5*1/*1), moderate activity (CYP3A5*1/*3), and no activity (CYP3A5*3/*3). Sunitinib was incubated with the genotyped hepatocytes in suspension for 2 hours, and sunitinib metabolites were analyzed by liquid chromatography-tandem mass spectrometry. Our preliminary data showed that hepatocytes with moderate to high CYP3A5 activity generated higher levels of the major metabolite N-desethylsunitinib, compared to donors with no CYP3A5 activity. Formation of defluorinated sunitinib and the sunitinib glucuronide conjugate was inversely related to CYP3A5 activity. These data suggest that variation in P450 activity may influence the level of sunitinib metabolites generated through competing metabolic pathways. Additional metabolism experiments will be performed to confirm these results. Future studies will focus on characterizing the cytotoxicity of sunitinib in primary human hepatocytes to identify the factors that may contribute to the development of sunitinib-induced liver injury in patients.

The Visual Opsin Genes of Diurnal and Nocturnal Frogs.

Clorissa Campbell, (Mentors: Einat Hauzman, Dr. Dora Ventura).

Minority Health International Research Program, Rhodes College, Memphis, TN; University of São Paulo, São Paulo, Brazil.

This project focuses on the visual pigments of the retina and various aspects of visual adaptations to the environment. The purpose of this project is to investigate the opsin genes expressed in retinas of frog species that are highly diverse and compare differences in the opsins associated with species ecology. We aim to estimate the spectral peaks (lmax) of opsins based on the amino acid composition at specific spectral tuning sites. To explore these qualities, the methods of RNA extraction, PCR, and DNA sequencing analysis were used. Retinal cDNA of five tropical frog species were analyzed: the nocturnal species Adenomera sp, Ischnocnema parva, and Aplastodiscus albofrenatus, and the diurnal species Brachycephalus nodoterga and Hylodes phyllodes. Using specific primers for amphibian opsins we were able to amplify the rhodopsin gene RH1 of the species Adenomera sp, I. parva, and A. albofrenatus. The nucleotide sequences were analyzed and compared to the RH1 sequence of the frog Xenopus tropicalis. As a result, a slight spectral shift was observed in Aplastodiscus albofrenatus and the project will be continued to compare this result to other tropical frog species. Prior to project construction, the hypothesis of a difference in opsin gene adaptations across various nocturnal or diurnal frog species being related to species ecology and behavior was created.

SEMSS ORGANIZING COMMITTEE

Thank you to the SEMSS planning committee and program representatives!

EMORY UNIVERSITY SCHOOL OF MEDICINE

SAUMYA GURBANI
AIMEE VESTER

MEDICAL COLLEGE OF GEORGIA AT AUGUSTA UNIVERSITY

JOHN KLEMENT

MEDICAL UNIVERSITY OF SOUTH CAROLINA

TONY KWON

MEHARRY MEDICAL COLLEGE

JEROME ARCENEAUX

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM SCHOOL OF MEDICINE

JACOB FILES
MUHAN HU

UNIVERSITY OF LOUISVILLE SCHOOL OF MEDICINE

SAMANTHA MORRISSEY

VANDERBILT UNIVERSITY SCHOOL OF MEDICINE

MARGARET AXELROD (CO-CHAIR)

ELIZABETH FLOOK (CO-CHAIR)

MEGAN WILLIAMS, PHD, MSTP ASSISTANT DIRECTOR

BRYN SIERRA, MS, MED, MSTP PROGRAM MANAGER

NOWRIN CHOWDHURY

JUAN COLAZO

EMILIE FISHER

GRAHAM JOHNSON

ZACHARY JONES

DONALD OKOYE

MICHAEL RADDATZ

MAXWELL ROESKE

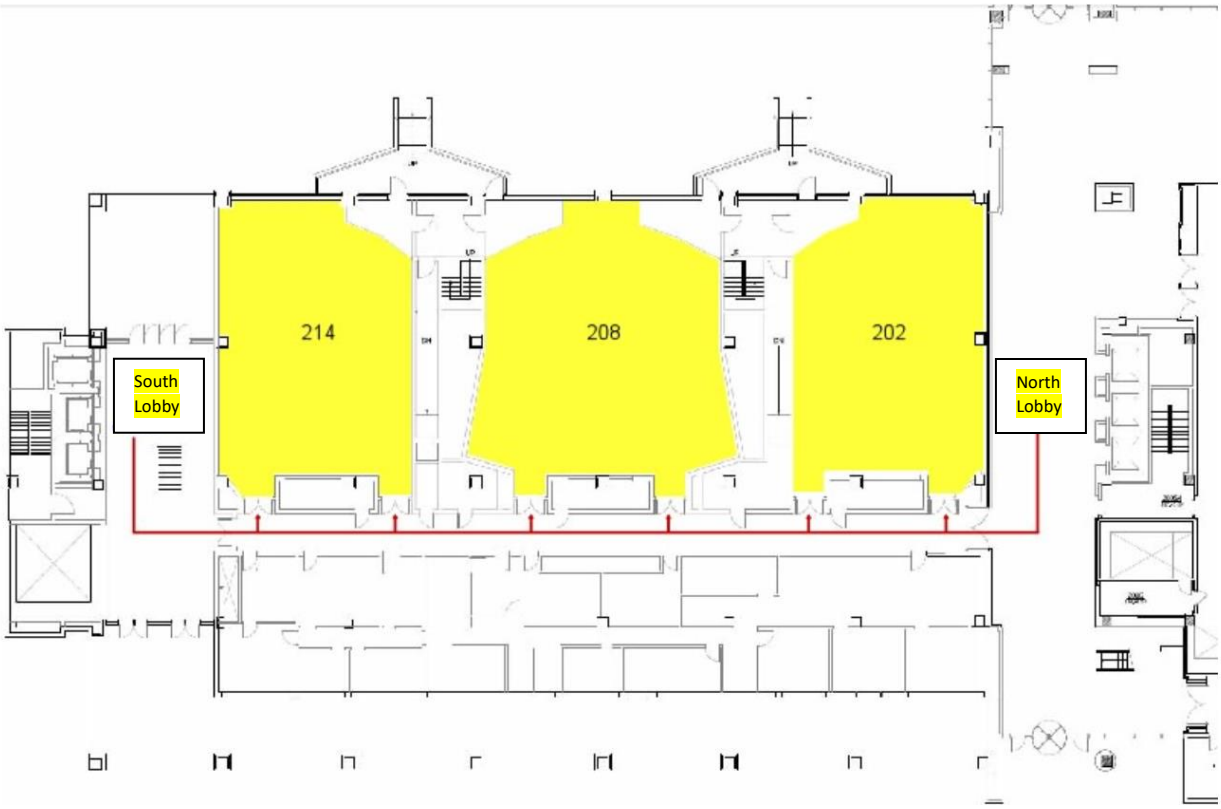
JOHN SHELLEY

ALEX SILVER

CAMILLE WANG

MAPS

2nd Floor (RLH)



4th Floor (RLH)

Please park in Zone 3 of 25th Avenue Garage (2401 Highland Ave. Nashville, TN 37212)

From 25th Avenue Garage, head north on 24th Ave. to Garland Ave.

Turn right on Garland to the round-about.

Turn right on foot-path towards Light Hall.