2015 SOUTHEAST REGIONAL IDeA MEETING

ARKANSAS, KENTUCKY, LOUISIANA, MISSISSIPPI, PUERTO RICO, SOUTH CAROLINA, WEST VIRGINIA

SE IDeA 15 Meeting

Biloxi, Miss.

NOVEMBER 11-13, 2015

Beau Rivage Resort & Casino
Biloxi, Mississippi

Mississippi INBRE
IDeA Network of Biomedical Research Excellence

Dear IDeA Program Colleagues,

I would like to welcome you to the beautiful coastal regional of our state for this exciting meeting. The Southeast Regional IDeA Meeting has become an important gathering for scientists from the seven states of the southeastern region. This critical meeting brings together the regional INBRE and COBRE programs, allowing us to exchange best practices and share ideas with each other to promote and enhance biomedical research within our individual states. I encourage each of you to network and engage with the NIGMS staff, program administrators, researchers and students from the many programs within our region. I want to thank all of you for your scientific contributions to this conference. We are pleased to have high participation numbers in this year's meeting and are encouraged by the excellent quality of the scientific presentations.

As this conference takes a significant amount of planning, I want to thank Mrs. Jamie Lott and Mrs. Mary Ann McRaney for their efforts to make this meeting possible. I also want to thank the steering committee members for their willingness to assist in the planning process and for their hard work putting together the outstanding scientific program. Finally, I want to thank the NIGMS staff for their continued support of the INBRE and COBRE programs.

Thank you all for coming. I look forward to exchanging ideas with you. Let's have a great meeting, learn a lot from each other and make some great memories!

Cheers,
Moe

Mohamed O. Elasri, Ph.D.
Associate Dean for Research and Graduate Affairs
Professor, Biological Sciences
Director, Mississippi INBRE

Biloxi
established 1699

Greetings. It is a pleasure for me to welcome you to Biloxi and the Mississippi Gulf Coast. I'm delighted that you are able to attend the 2015 Southeastern Regional IDeA Conference.

I hope that each of you have a great time while you are here, and that you have a chance to get out into the community and see what we have to offer. You'll find plenty of great seafood restaurants, family attractions, deep sea fishing, educational museums, and, of course, the world's longest man-made sand beach.

I hope that each of you share a wealth of information at this conference. I want you to have fond memories of your fun times in Biloxi and return again and again.

All the best,

Andrew "FoFo" Gilich

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Meeting Agenda

Wednesday, November 11th, 2015

2:00 p.m.-5:00 p.m.	Registration & Poster Setup ("A")	Beau Rivage Foyer/Camellia Ballroom
5:30 p.m.-8:00 p.m.	Welcome Reception	Maritime & Seafood Industry Museum

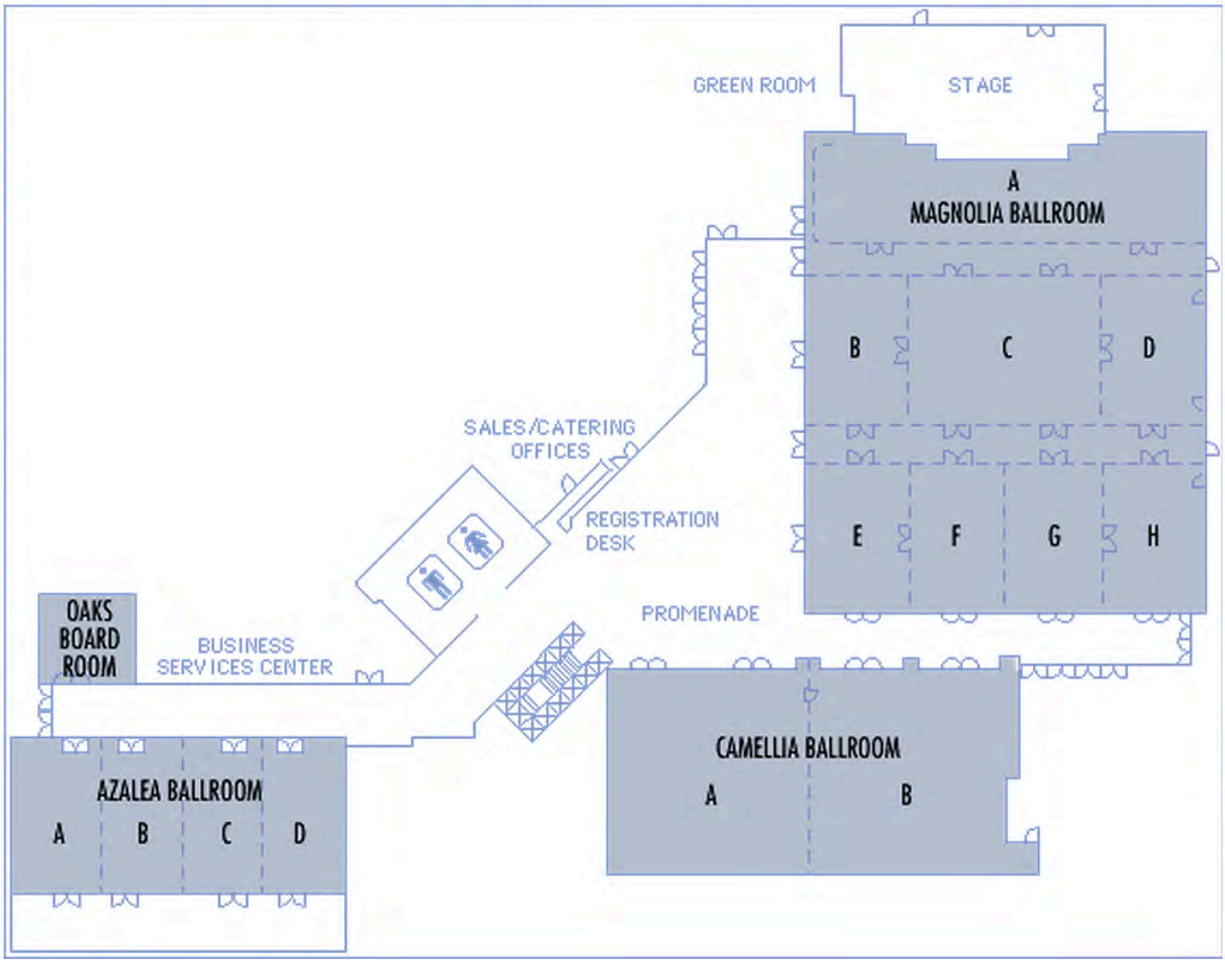
Thursday, November 12th, 2015, Beau Rivage Resort & Casino

7:15 a.m.-8:00 a.m.	Poster Setup ("A")	Camellia Ballroom
8:00 a.m.-9:00 a.m.	Breakfast Discussion Forum I - Alternative Careers & Innovation in Academia	Magnolia A,B,C,D
9:00 a.m. - 10:30 a.m.	Opening Session	Magnolia E, F, G, H
10:30 a.m.-11:00 a.m.	Break	Foyer
11:00 a.m.-12:30 p.m.	Poster Session I ("A")	Camellia Ballroom
12:30 p.m-1:30 p.m.	Lunch	Magnolia A, B, C, D
	Student Research/Career Development Lunch	Azalea A
	Mentoring Workshop/Luncheon	Azalea B
1:30 p.m.-3:00 p.m.	Bioinformatics	Azalea A
	Cancer	Azalea B
	Cardiovascular Research	Azalea C
	Cell Signaling	Azalea D
	Grants Administrators'/Program Managers' Workshop	Magnolia E, F, G, H
3:00 p.m.-3:15 p.m.	Break	Foyer
3:15 p.m.-4:35 p.m.	Science Commercialization Workshop	Magnolia E, F, G, H
4:35 p.m.-4:45 p.m.	Break	Foyer
5:00 p.m.-6:00 p.m.	Continue Science Commercialization Workshop	Magnolia E, F, G, H
6:00 p.m.-7:00 p.m.	Poster Setup ("B")	Camellia Ballroom
6:00 p.m.	Dinner on your own	
6:30 p.m.	IDeA Program PI Dinner	Azalea C, D

Friday, November 13th, 2015, Beau Rivage Resort & Casino

7:15 a.m.-8:00 a.m.	Poster Setup ("B")	Camellia Ballroom
8:00 a.m.-9:00 a.m.	Breakfast Discussion Forum II - Alternative Careers & Innovation in Academia	Magnolia A,B,C,D
9:00 a.m.-10:30 a.m.	Gen. Biomedical Sciences	Azalea A
	Infectious Disease/Immunology	Azalea B
	Neuroscience	Azalea C
	Genomics	Azalea D
10:30 a.m.-10:45 a.m.	Break	Foyer
10:45 a.m.-12:15 p.m.	Poster Session II ("B")	Camellia Ballroom
12:15 p.m.-2 p.m.	Lunch/Awards	Magnolia A, B, C, D

Meeting Floor Plan



Welcome Reception:
Maritime & Seafood Industry Museum

The Welcome Reception for the 2015 Southeast Regional IDeA meeting will be held at the Maritime & Seafood Industry Museum, 115 1st Street, Biloxi, Mississippi.

Buses will be available from 5:15 p.m. until 9:00 p.m. to transport attendees round-trip between the Beau Rivage and the Museum. Please go to the bus lobby (ground floor of the parking garage) at the Beau Rivage in order to load the buses. Buses will continually travel between the Beau Rivage and the museum to allow attendees to come and go as they please.

Attendees will enjoy a "Shrimp Boil" themed reception complete with fresh shrimp and local favorites Vegetarian and non-seafood options will be available.

Following the welcome, guided tours of the museum will begin. Attendees are welcome to browse the museum on their own as well. Food and beverages are allowed throughout the museum.

Information on the Maritime & Seafood Industry Museum:

The Maritime and Seafood Industry Museum was established in March 1986 to preserve and interpret the maritime history and heritage of Biloxi and the Mississippi Gulf Coast. It accomplished this mission through an array of exhibits and an extraordinary collection of photographs, and hundreds of one-of-a-kind artifacts that tell the story from the time of the first Indian settlement through generations of immigrants whose journey contributed to the melting-pot culture of the Gulf Coast. Featured exhibits include shrimping, oystering, recreational fishing, wetlands, managing marine resources, charter boats, marine blacksmithing, net-making, boat building, and hurricanes, all the while telling the tale of over 300 years of history, culture and heritage.

The museum was destroyed in 2005 by Hurricane Katrina, but in August 2014 it reopened as a beautiful three story museum, once again calling Point Cadet home and serving as the "Gateway to Biloxi", continuing to tell the story of how it all came to be.

Alternative Careers & Innovation in Academia Breakfast Discussion Forum – Day 1
Thursday, November 12th, 8:00 a.m.-9:00 a.m.

Location: Magnolia A, B, C, D
Chair: Dr. T. Ryan Withers, Principal Research Scientist, Progenesis Technologies, LLC

Panelist: Dr. Regine Douthard, Medical Officer in the Center for Research Capacity Building, National Institute of General Medical Sciences, National Institutes of Health
Panelist: Dr. Rafael Gorospe, Medical Officer in the Center for Research Capacity Building, National Institute of General Medical Sciences, National Institutes of Health
Panelist: Dr. Krishan Arora, Program Director, Center for Research Capacity Building, National Institute of General Medical Sciences
Panelist: Dr. Matthew Portnoy, NIH SBIR/STTR Program Manager
Panelist: Chase Kasper, Assistant Vice President for Research, Technology Transfer & Corporate Relations

Alternative Careers-Undergraduate students, graduate students and postdoctoral fellows often may not have a clear understanding of what career options exist, partly because many academic institutions and mentors themselves do not have a broad perspective. The round table offers students and postdoctoral fellows the opportunity to have either small group or one-on-one discussions with the NIGMS staff and peers to ask questions, get general advice, and learn about careers paths in academia and beyond.

Innovation in Academia-Scientists in the academic settings may have projects with viable commercial potential, but often have no experience or ability to move the research into the market place. Commercialization of academic research is a difficult undertaking, but possible with the proper insight, knowledge, and resources. The round table offers the chance to meet with NIGMS staff to explore how one goes about moving research towards the market place.

General Session,
Thursday November 12th, 9:00 a.m.-10:30 a.m.

Location: Magnolia E, F, G, H

- **Welcome**, *Dr. Mohamed Elasri, Director of Mississippi INBRE, The University of Southern Mississippi*
- **Updates On The IDeA Program And The Center For Research Capacity Building,** *Dr. Regine Douthard, Medical Officer in the Center for Research Capacity Building, National Institute of General Medical Sciences, National Institutes of Health*
- **NAIPI: National Association Of IDeA PIs**, *Dr. Carolyn Bohach, President, National Association of IDeA Principal Investigators*

Student Research/Career Development Luncheon,
Thursday November 12, 12:30-1:30 p.m.
Lunch is provided in the room

Location: Azalea A

- **"Expanding And Enhancing Undergraduate Research As A Primary Strategy To Increase Research Capacity: Lessons Learned From The Council On Undergraduate Research (CUR)"**
  Dr. Elizabeth Ambos, Executive Officer, Council on Undergraduate Research

Mentoring Workshop/Luncheon,
Thursday November 12, 12:30-1:30 p.m.
Lunch is provided in the room

Location: Azalea B

- **"Mentoring for an Evolving Academic Career"**
  Dr. Ian Paul, Professor & Director, Medical Student Education, Assistant Director, Division of Neurobiology & Behavior Research, Department of Psychiatry & Human Behavior, The University of Mississippi Medical Center

Scientific Sessions,
Thursday, November 12, 1:30 p.m.-3:00 p.m.

Bioinformatics

Location: Azalea A
Co-Chair: Dr. Shahid Karim, Associate Professor of Biological Sciences, The University of Southern Mississippi
Co-Chair: Dr. Bindu Nanduri, Associate Professor, Department of Basic Sciences, College of Veterinary Sciences, Mississippi State University

1:30 p.m.
Highlighted Speaker: Fully mining RNA-Seq data for noncoding RNA analyses
Glen Borchert, Assistant Professor, Biology Department and Pharmacology Department, University of South Alabama, Mobile, AL

2:00 p.m.
"HPIDB: A Curated Database for Host-Pathogen Interactions"
Dr. Bindu Nanduri, Associate Professor, Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Starkville, MS

2:15 p.m.
"An automated pipeline for whole genome sequencing data analysis"
Mary Yang, The University of Arkansas at Little Rock, Little Rock, AR

2:30 p.m.
"An Assessment of TMAP for Host-Pathogen Genomic Pipelines"
John Caskey, Louisiana State University, Baton Rouge, LA

2:45 p.m.
"The Implications of the Presence of Lycotoxin in the Venom of the Wolf Spider Rabidosa rabida (Areanae Lycosidae)"
Sara Wilmsen, Harding University, Searcy, Arkansas

Cancer

Location: Azalea B
Co-Chair: Dr. Paul Lockman, Inaugural Douglas Glover Chair of Pharmaceutical Sciences, Associate Center Director for Translational Research, the Mary Babb Randolph Cancer Center, West Virginia University
Co-Chair: Dr. Martin Hauer-Jensen, Professor of Pharmaceutical Sciences, Surgery, and Pathology Director, Division of Radiation Health, Associate Dean for Research, College of Pharmacy, University of Arkansas for Medical Sciences

1:30 p.m.
Highlighted Speaker: "The Mechanism of Cancer Cell Death Mediated by Knockdown of COPZ1 Gene Encoding Coatomer Protein Complex Subunit Z 1 – New Target Gene for Cancer Therapy"
Michael Shtutman, Assistant Professor, The University of South Carolina, Columbia, SC

2:00 p.m.

"Drug Designing for Prostate Cancer"
Raquema Williams, Student, Tougaloo College, Tougaloo, MS

2:15 p.m.

"**The Cancer CURE: A Course-embedded Undergraduate Research Experience"**
Lori Hensley, Chair & Professor, Biology, Ouachita Baptist University, Arkadelphia, AR

2:30 p.m.

"Development of New Patient-Derived Xenograft (Pdxs) Models for Accurate Assessment of New Therapeutics in Pre-Clinical Settings for Breast Cancer Patients"
Elena N. Pugacheva, Assistant Professor, West Virginia University, MBR Cancer Center, Morgantown, WV

2:45 p.m.

Patient Advocate Video
Dr. Martin Hauer-Jensen, Professor of Pharmaceutical Sciences, Surgery, and Pathology, Director, Division of Radiation Health, Associate Dean for Research, College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, AR

Cardiovascular Research

Location: Azalea C
Co-Chair: Dr. John Hall, Arthur C. Guyton Professor & Chair, Director of Mississippi Center for Obesity Research, University of Mississippi Medical Center
Co-Chair: Dr. Gabriel Navar, Professor & Chair of Physiology Department, Co-Director of Renal & Hypertension Center, Tulane University

1:30 p.m.

Highlighted Speaker: "A Link Between Autoimmunity and Hypertension"
Dr. Michael J. Ryan, Associate Professor of Physiology & Biophysics, Director of Graduate Studies in Physiology, Associate Dean, School of Graduate Studies in Health Sciences, Research Health Sciences in Research Service; University of Mississippi Medical Center, Jackson, MS

2:00 p.m.

"Acute Effects of Inhaled Iloprost on Exercise Capacity and Dynamic Hyperinflation an COPD"
Matthew R. Lammi, Assistant Professor, Department of Medicine, Louisiana State University Health Sciences Center, New Orleans, LA

2:15 p.m.

"Scientific Bumps in the Night: The Cross of Apoe and Treml1 Null Mice Unique Link Between Cardiovascular Disease, Diabetes, and Obesity"
A. Valance Washington, Assistant Professor, University of Puerto Rico, and University of Central del Caribe, Santa Juanita, Bayamon, PR

2:30 p.m.

"Differential Expression of Tumor Necrosis Factor-Alpha Type 1 and Type 2 Receptors in the Renal Tissue During Chronic High Salt Intake and Angiotensin II Treatment"
Dewan S. A. Majid, Professor, Department of Physiology, Hypertension & Renal Center of Excellence, Tulane University School of Medicine, New Orleans, LA

2:45 p.m.

"Decreased Survival Rate in Female Obese Leptin Receptor Mutant Dahl Salt-Sensitive Rats that Develop Chronic Kidney Disease"
Jan M. Williams, Assistant Professor, Department of Pharmacology, University of Mississippi Medical Center, Jackson, MS

Cell Signaling

Location: Azalea D
Co-Chair: Dr. Richard J. Roman, Billy Guyton Distinguished Professor and Chair, Department of Pharmacology & Toxicology, University of Mississippi Medical Center
Co-Chair: Dr. Emilia Galperin, Assistant Professor, Molecular & Cellular Biochemistry, University of Kentucky

1:30 p.m.

Highlighted Speaker: "Shoc2 Scaffold-Matchmaker of Intracellular Communications in Time and Space"
Dr. Emilia Galperin, Assistant Professor, Molecular & Cellular Biochemistry, University of Kentucky, Lexington, KY

2:00 p.m.

"Effective Gene Knockout of NPC1L1 in *Danio rerio* using a CRISPR/Cas9 Endonuclease System"
Benjamin Duncan, *Bluefield State College, Bluefield, WV*

2:15 p.m.

"Identification and Development of Casein Kinase 1 Inhibitors as Potential Therapeutics in the Treatment of Alzheimer's Disease"
Richard Schroeder, Xavier University of Louisiana, New Orleans, LA

2:30 p.m.

"A Proteomics Approach to Delineate the Role of C/Ebp δ in Ionizing Radiation-Induced Oxidative Stress"
Snehalata A. Pawar, Division of Radiation Health, Department of Pharmaceutical Sciences, Little Rock, AR

2:45 p.m.

"Polyaniline Coating on Borosilicate Glass as a Fluorescent Sensing Platform for Detection of DNA Mimic to miRNA *let-7*"
Partha Pratim Sengupta, Department of Biological Sciences, University of Southern Mississippi, Hattiesburg, MS

Grants Administrators'/Program Managers' Workshop

Location: Magnolia E, F, G, H

1:30-3:00 p.m.

"Uniform Guidance – For The Research Administrator And Researcher"

Dr. Denise Clark, Associate Vice President for Research Administration, University of Maryland

Dr. Ann Holmes, Assistant Dean, Finance & Administration for the College of Behavioral & Social Sciences, University of Maryland

This workshop will focus on the recently approved Uniform Guidance from the Office of Management and Budget, which pertains to Administrative Requirements, Cost Principles, and Audit Requirements for Federal Awards. This guidance was developed in response to the November 23, 2009 Executive Order 13520 on *Reducing Improper Payments* and the February 28, 2011 Presidential Memorandum, on *Administrative Flexibility, Lower Costs, and Better Results for State, Local, and Tribal Governments*. Federal agencies were required to implement these requirements by December 26, 2014. COBRE and INBRE PIs are strongly encouraged to have their grant administrator/program manager attend this workshop and become more familiar with the federal requirements and policies that oversee our NIH-IDeA awards. In addition, this workshop offers an opportunity for our administrators/managers to network and establish relationships that will serve or programs by opening lines of communications as we expand collaborations within the IDeA program.

Science Commercialization Workshop,
Thursday, November 12th, 3:15 p.m.-6:00 p.m.

Location: Magnolia E, F, G, H

Chair: Dr. Krishan Arora, Program Director, Center for Research Capacity Building, National Institute of General Medical Sciences

3:15 p.m.-3:25 p.m.

Opening Remarks

Dr. Krishan Arora, Program Director, Center for Research Capacity Building, National Institutes of Health

3:25p.m.- 3:55 p.m.

"Investing in Innovation: The HHS SBIR/STTR Programs"

Dr. Matthew Portnoy, NIH SBIR/STTR Program Manager

3:55 p.m.-4:15 p.m.

"Entrepreneurship And Commercialization: Building An Engine Of Innovation Translation In WV"

Mr. Richard Giersch, Director of Life Science Innovation, Entrepreneurship and Commercialization, West Virginia University

4:15 p.m.-4:35 p.m.

"SBIR And STTR Projects Carried Out By ElSohly Labs"

Dr. Mahmoud ElSohly, President & Laboratory Director of ELI, Research Professor in the Research Institute of Pharmaceutical Sciences, Professor of Pharmaceutics, University of Mississippi

4:35 p.m.-4:45 p.m.

Break

4:45 p.m.- 5:05 p.m.

"Technology Transfer and Science Commercialization at Tulane University: History, Lessons Learned, Plans For the Future"

Dr. John Christie, Executive Director, Technology Transfer and Intellectual Property Development, Tulane University

5:05 p.m.-5:25 p.m.

"The Road Towards Translation Of MTI-101 For The Treatment Of Relapsed Multiple Myeloma"

Dr. Lori Hazlehurst, Professor, Co-Leader of the Alexander B. Osborn Hematopoietic Malignancy & Transplantation Program, West Virginia University

5:25 p.m.-6:00 p.m.

Panel Discussion

Panelist: Dr. Mahmoud ElSohly, President & Laboratory Director of ELI, Research Professor in the Research Institute of Pharmaceutical Sciences, Professor of Pharmaceutics, University of Mississippi

Panelist: Mr. Richard Giersch, Director Life Science Innovation, Entrepreneurship and Commercialization West Virginia University

Panelist: Dr. Matthew Portnoy, Commercialization of Science Program Coordinator, National Institutes of Health

Panelist: Dr. John Christie, Executive Director, Office of Technology Transfer and Intellectual Property Development, Tulane University

Panelist: Dr. Lori Hazlehurst, Professor, Co-Leader of the Alexander B. Osborn Hematopoietic Malignancy & Transplantation Program

IDeA Program PI Dinner,
Thursday, November 12th, 6:30 p.m.-8:00 p.m.

Location: Azalea C, D
Hosted by: Dr. Stephen Cutler, Director, Center of Research Excellence in Natural Products Neuroscience, University of Mississippi

Alternative Careers & Innovation in Academia Breakfast Discussion Forum – Day 2
Friday, November 13th, 8:00 a.m.-9:00 a.m.

Location: Magnolia A, B, C, D
Chair: Dr. T. Ryan Withers, Principal Research Scientist, Progenesis Technologies, LLC

Panelist: Dr. Regine Douthard, Medical Officer in the Center for Research Capacity Building, National Institute of General Medical Sciences, National Institutes of Health
Panelist: Dr. Rafael Gorospe, Medical Officer in the Center for Research Capacity Building, National Institute of General Medical Sciences, National Institutes of Health
Panelist: Dr. Krishan Arora, Program Director, Center for Research Capacity Building, National Institute of General Medical Sciences
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Scientific Sessions,
Friday, November 13th, 9:00 a.m.-10:30 a.m.

General Biomedical Sciences

Location: Azalea A
Co-Chair: Dr. Alex Flynt, Assistant Professor, Biological Sciences, The University of Southern Mississippi
Co-Chair: Dr. Loyda Mendez, Associate Professor, School of Science & Technology, Universidad del Este

9:00 a.m.
Highlighted Speaker: "Chromatin Structural Changes In Gene Regulation"
Dr. Yvonne Fondufe-Mittendorf, Assistant Professor, Molecular & Cellular Biochemistry, University of Kentucky, Lexington, KY

9:30 a.m.
"Changes in Responses to Diesel Exhaust Particles from Bronchial Epithelial Cells of Asthmatic and Non-Asthmatic Subjects"
Dr. Loyda Mendez, Associate Professor, School of Science & Technology, Universidad del Este, Barrio, Carolina, PR

9:45 a.m.
"Role of Suppressor of Cytokines Signaling 3 (SOCS3) in Modulating Chronic Metabolic and Cardiovascular Effects of Leptin"
Jussara M. do Carmo, Assistant Professor, The University of Mississippi Medical Center, Jackson, MS

10:00 a.m.
"**Pathophysiology of Contrast-Induced Nephropathy and New Therapeutic Strategies"**
Altaf-M Khan, Research Instructor, Tulane University, School of Medicine, New Orleans, LA

10:15 a.m.
"**Dual Targeted Dual Responsive Nanoparticles for Brain Targeted Delivery"**
Eleni Markoutsa, Post Doctoral Research Associate, University of South Carolina, Columbia, SC

Infectious Disease/Immunology

Location: Azalea B
Co-Chair: Dr. Janet Donaldson, Associate Professor of Biological Sciences Mississippi State University
Co-Chair: Dr. Mark Smeltzer, Professor of Microbiology & Immunology, Department of Orthopedics University of Arkansas for Medical Sciences

9:00 a.m.
Highlighted Speaker: "The Balance Between Health And Disease In Chronic Gammaherpesvirus Infection Is Controlled By p53"
Dr. J. Craig Forrest, Assistant Professor, Department of Microbiology & Immunology, University of Arkansas for Medical Sciences, Little Rock, AR

9:30 a.m.

"Studying The Temperature-Dependent Gene Response in the Relapsing Fever Spirochete, *Borrelia turicatae*"

Dr. Jon Blevins, Associate Professor of Microbiology & Immunology, University of Arkansas for Medical Sciences, Little Rock, AR

9:45 a.m.

"Anaerobiosis: A Key Factor in Regulation of Virulence Factors in Listeria Monocytogenes"

Dr. Janet Donaldson, Associate Professor of Biological Sciences, Mississippi State University, Starkville, MS

10:00 a.m.

"*Francisella tularensis*, a Bacterium That's No-Bloody-Good"

Dr. Joseph Horzempa, Assistant Professor of Biology, West Liberty University, Wheeling, WV

10:15 a.m.

"Novel Grafting of Peptides onto Plant-Based Peptides for Modulation of CD2-CD58 Protein-Protein Interaction: Implications in Autoimmune Diseases"

Rushikesh V. Sable, University of Louisiana, Monroe, LA

Neuroscience

Location: Azalea C
Co-Chair: Dr. James K. Rowlett, Professor of Psychiatry & Human Behavior, University of Mississippi Medical Center
Co-Chair: Dr. Christopher R. McCurdy, Professor of Medicinal Chemistry and Pharmacology, School of Pharmacy, University of Mississippi

9:00 a.m.

"The Effects of Early-Life Risperidone Administration on Dopamine Research and Uptake during Adulthood"

Mark E. Bardgett, Northern Kentucky University, Highland Heights, KY

9:15 a.m.

Highlighted Speaker: "KNDy Neurons and the Control of Mammalian Reproduction"

Dr. Michael N. Lehman, Professor & Chair, Neurobiology & Anatomical Sciences, University of Mississippi Medical Center, Jackson, MS

9:45 a.m.

"The Modulatory Effects Of Caffeine On The Intrinsic Properties Of Spinal Lateral Motoneurons"

Marla Rivera-Oliver, Institute of Neurobiology, University of Puerto Rico, Medical Sciences Campus, San Juan, Puerto Rico, Department of Biology, University of Puerto Rico, Rio Piedras Campus, San Juan, PR

10:00 a.m.

"Design and Synthesis of Fluorinated Anthocyanins as Neuroprotective Antioxidants"

David A. Colby, The University of Mississippi, University, MS

10:15 a.m.

"Alternative Splicing in Photoreceptor Neurons"

Dr. Visvanathan Ramamurtny, West Virginia University

Genomics

Location: Azalea D
Co-Chair: Dr. Michael Garrett, Associate Professor of Pharmacology & Toxicology, Associate Professor of Medicine, Director, Institutional Molecular & Genomics Core Facility, University of Mississippi Medical Center
Co-Chair: Dr. Homayoun Valafar, Director, Bioinformatics Core, South Carolina INBRE; Associate Professor, Department of Computer Science & Engineering

9:00 a.m.
Highlighted Speaker: "Leveraging Rat Genetics to Understand Human Diseases of the Kidney"
Dr. Michael Garrett, Associate Professor of Pharmacology & Toxicology, Associate Professor of Medicine, Director, Institutional Molecular & Genomics Core Facility, University of Mississippi Medical Center, Jackson, MS

9:30 a.m.
"Histone Deacetylases 1 and 2 balance nephron progenitor renewal and differentiation during kidney organogenesis"
Hongbing Liu, Department of Pediatrics and Tulane Hypertenison & Renal Center of Excellence, Tulane University School of Medicine, New Orleans, LA

9:45 a.m.
"Unappreciated piRNA Biology of The Two-Spot Spider Mite"
Mosharrof Mondal, The University of Southern Mississippi, Hattiesburg, MS

10:00 a.m.
"Nutrition Intervention to Profile Metabolic, Microbiome and Vascular Health in Young Adults at Risk for Disease: FRUVEDomic Pilot Study"
Oluremi Famodu, MS, RDN,_ West Virginia University, Morgantown, WV

10:15 a.m.
"DNA Methylation In Healthy Aging Of Twins"
Sangkyu Kim, Tulane University Health Sciences Center, New Orleans, LA

Poster Session A,
Thursday, November 12th, 11:00 a.m.-12:30 p.m.

Location: Camellia Ballroom

Bioinformatics

A 1.1- "Superconvergence of Nonconforming Finite Element Approximation for the Second Order Elliptic Problems and Numerical Examples"
Anna Harris
University of Arkansas at Pine Bluff, Pine Bluff, AR

A 1.2- "Determining Potential Yeast Longevity Genes via PPI Networks and Microarray Data Clustering Analysis"
Bernard Chen[1], Roshan Doolabh[1], Fusheng Tang[2]
[1]Department of Computer Science, University of Central Arkansas, Conway, AR
[2]Department of Biology, University of Arkansas at Little Rock, Little Rock, AR

A 1.3- "microRNA-148/152 Family Members Concordantly Target Genes Important for Tumor Progression and Chemoresistance"
David Oliver[1], Hao "Emily" Ji[1], Samuel Lee[2], Ellen Gardiner[1], Mythreye Karthikeyan[3], Homayoun Valafar[4], Michael Shtutman[1]
[1]Drug Discovery and Biomedical Sciences, South Carolina College of Pharmacy, Columbia, SC
[2]Biology, University of South Carolina, Columbia, SC
[3]Chemistry and Biochemistry, University of South Carolina, Columbia, SC
[4]Computer Science and Engineering, University of South Carolina, Columbia, SC

A 1.4- "Genome-wide Distribution of G-quadruplex Forming Sequences and Their Role in Stability During Meiosis"
Julia H. Chariker, Donald M. Miller, Eric C. Rouchka
University of Louisville, Louisville, KY

A 1.5- "Computational Investigations of Enantiospecificity of Mutated CYP2C9"
Logan Bond[1], Grover P. Miller[2], Martin D. Perry Jr.[1]
[1]Ouachita Baptist University, Arkadelphia, AR
[2]University of Arkansas for Medical Sciences, Little Rock, AR

A 1.6- "Lower Level of Sestrin2 in Aging Sensitizes Heart to Ischemia and Reperfusion Injury"
Nanhu Quan, Wanqing Sun, Lin Wang, Xingchi Chen, Ji Li
University of Mississippi Medical Center, Jackson, MS

A 1.7- "A Framework to Feature Mining for Comorbidity Analysis: Age Related Macular Degeneration and Alzheimers Disease"
Pradeep Chowriappa[1], Sumeet Dua[1], Prerna Dua[2]
[1]Department of Computer Science, Louisiana Tech University, Ruston, LA
[2]Department of Health Informatics & Information Management, Ruston, LA

A 1.8- "The Effects of Residue Mutations on the Enantiospecificity of CYP2C9"
Trevor Meece[1], Grover P. Miller[2], Martin D. Perry, Jr.[1]
[1]Ouachita Baptist University, Arkadelphia, AR
[2]University of Arkansas for Medical Sciences, Little Rock, AR

A 1.9- "Does the Gut Microbiome Mediate Associations of Menarche with Cardiovascular Disease Risk in Postmenopausal Women?"
Yan Wang, Robert Delongchamp, Mohammed El Faramawi, Mohammed Orloff, Jordana Bell, Tim Spector, Barbara Fuhrman
The University of Arkansas at Little Rock, Little Rock, AR

A 1.10- "Discovery of Hidden Splice Junctions in Personal Reference Genomes"

Juw Won Park[1], Shayna Stein, Zhi-xiang Lu, Yi Xing
[1]The University of Louisville, Louisville, KY
[2]University of California, Los Angeles, CA

Cancer

A. 2.1- "MM-398 Accumulates in Metastatic Lesions and Prolongs Survival in an Experimental Model af Brain Metastases of Human Triple Negative Breast Cancer"

Afroz Shareef Mohammad, Chris E. Adkins, Emma Dolan, Emily Sechrest, Tori Terrell-hall, Paul Lockman
Department of Pharmaceutical Sciences, West Virginia University, Morgantown, WV

A. 2.2- "Effects of Curly kale *Brassica oleracea var. sabellica* on Viability of Cultured Mouse Melanoma Cells"

Bilal Qizilbash
Qizilbash, LLC, Clinton, MS

A 2.3- "Screening For Heterozygous Gene Mutations that Increase Loss of Heterozygosity in a Yeast System"

Cheyenne Hensley, Kellyn Hoffert, Erin Strome
Northern Kentucky University, Highland Heights, KY

A 2.4- "Folate-functionalized Polymeric Micelle for Combinatorial Therapy to Overcome Drug Resistant Breast Cancer"

Graham Temples[1], Wendy R. Cornett[2], Jeoung Soo Lee, PhD[1]
[1]Drug Design, Development and Delivery Laboratory, Department of Bioengineering, Clemson University, Clemson, SC
[2]Department of Surgery, USC Medical School, Greenville, SC

A 2.5- "Myxoma Virus Differentially Influences Human CD14$^+$ Myeloid Cells from Healthy Donors and Ovarian Cancer Patients"

Shana Chancellor[1], Jason Liem[1], Bernice Nounamo[1], Martin Cannon[1], Jia Liu[1,2]
[1]Department of Microbiology and Immunology, University of Arkansas for Medical Sciences (UAMS), Little Rock, AR
[2]Center for Microbial Pathogenesis and Host Inflammatory Response, College of Medicine, University of Arkansas for Medical Sciences (UAMS), Little Rock, AR

A 2.6- "Silver Nanoclusters as Luminescence Probes"

Lauryn Ashford, William Gladney, Bidisha Sengupta
Chemistry Department, Tougaloo College, Tougaloo, MS

A 2.7- DNA Templated Silver Nanoclusters as Fluorescence Probes for Oxidative Stress

Maria Muhammad, Corley Walters, Bidisha Sengupta
Chemistry Department, Tougaloo College, Tougaloo, MS

A 2.8- "Observations into the Role of the *MSH4-MSH5* Heterodimer in Yeast Genome Stability"

Olivia Davis, Miranda Combs, John Crum, Erin D. Strome
Northern Kentucky University, Highland Heights, KY

A 2.9- "Defining Mechanisms of Inhibin Action in Cancer"

P. Singh[1,2], P. Patel[1,2], N.Y. Lee[3], K. Mythreye[1,2]
[1]Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC
[2]Department of Drug Discovery and Biomedical Sciences, South Carolina College of Pharmacy, Columbia, SC
[3] Davis Heart Lung Institute, College of Pharmacy, Ohio State University, Columbus, OH

A 2.10- *"The Effects of Cannabinoids on Tumor Vasculature in Ewing Sarcoma"*

Rebekah Davis, RJ Quilao Jessie Little, Dr. Lori Hensley, Dr. Robert Griffin, Jessica Webber
Ouachita Baptist University, Arkadelphia, AR

A 2.11- "Cannabinoids' Effects on the Vasculature of Ewing's Sarcoma Tumors"

R.J. Quilao[1], Rebekah Davis[1], Sydney Heslep[1], Jessie Little[1], Jessica Webber[2], Klressa Barnes[1], Robert Griffin[3], Lori Hensley[1]
[1]Ouachita Baptist University Department of Biology, Arkadelphia, AR
[2]University of Arkansas for Medical Sciences Department of Pathology, Little Rock, AR
[3]University of Arkansas for Medical Sciences Department of Radiation Oncology, Little Rock, AR

A 2.12- "Association of Alcohol Consumption and Skin Allergy with Non-Melanoma Skin Cancer: Findings from the 2012 National Health Interview Survey"
Samuel Kakraba[1], Ke-Sheng Wang[2]
[1]UALR/UAMS Joint Graduate Program in Bioinformatics, University of Arkansas at Little Rock, Little Rock, AR
[2]Department of Biostatistics and Epidemiology, College of Public Health, East Tennessee State University, Johnson City, TN

A 2.13- "Using Antibody and Aptamer Modified Gold Nanomaterials for Detection and Destruction of Prostate Cancer Cells"
Santanu Banerjee
Tougaloo College, Tougaloo, MS

A 2.14- "Analysis of PARP1 Interactions with Cannabinoids"
Sydney Heslep, Lori L. Hensley, Martin D. Perry, Jr.
Ouachita Baptist University, Arkadelphia, AR

Cardiovascular Research

A 3.1- "Myocardial Glycolytic Rate Regulates Exercise Capacity and Cardiac Hypertrophy"
Andrew Gibb, Aruni Bhatnagar, Bradford G. Hill
University of Louisville, Louisville, KY

A 3.2- "A Novel Maternally Restricted Pro-angiogenic Therapeutic for Preeclampsia"
Gene L Bidwell III, Heather Chapman, Fakhri Mahdi, Omar Logue, Eric M George
The University of Mississippi Medical Center, Jackson, MS

A 3.3- "Sildenafil Treatment is Protective Against Progression of Renal Injury in the Preeclamptic Dahl Salt Sensitive Rat"
Ellen E. Gillis, Jennifer N. Mooney, Michael R. Garrett, Jennifer M. Sasser
Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS

A 3.4- "Cardiac SIRT1 Deficiency Causes an Impaired AMPK Signaling Response and Sensitizes Heart to Ischemia and Reperfusion Injury"
Lin Wang, Wanqing Sun, Nanhu Quan, Xingchi Chen,Courtney Cates, Ji Li
The University of Mississippi Medical Center, Jackson, MS

A 3.5- "Subcellular Control of Calcium Influx in the Diabetic Vasculature"
Matthew A. Nystoriak[1], Madeline Nieves-Cintron[2], Manuel F. Navedo[2]
[1]University of Louisville, Louisville, KY
[2]University of California, Davis, Davis, CA

A 3.6- "Protein Tyrosine Phosphatase 1B (PTP1B) Deficiency in Pro-Opiomelanocortin (POMC) Neurons Improves Glucose Tolerance but does Not Decrease Body Weight in Mice Fed a High Fat Diet"
Nicola Aberdein, Jussara M. do Carmo, John E. Hall
Department of Physiology and Biophysics, Mississippi Center for Obesity Research, University of Mississippi Medical Center, Jackson, MS

A 3.7- "Retinoic Acid and Sodium Butyrate Attenuate Renal Fibrosis and Inflammation in Guanylyl Cyclase-A/Natriuretic Peptide Receptor-A Gene-Targeted Mice"
Prerna Kumar, Ramu Periyasamy, Subhankar Das, Kailash N. Pandey
Physiology, Tulane University Health Sciences Center and School of Medicine, New Orleans, LA

A 3.8- "High Fat Diet Intake Differentially Affects Adipose Inflammation in Obesity-Prone and Obesity-Resistant Rats"
Stefany D. Primeaux[1,2], Flavia Souza-Smith[1], Jonquil Poret[1], Tony H. Tzeng[1], H. Douglas Braymer[2], Lisa M. Harrison-Bernard[1]
[1]Dept of Physiology, LSU-Health Sciences Ctr-New Orleans, LA
[2]Pennington Biomedical Research Ctr-Baton Rouge, LA

A 3.9- "Diabetes Alters the Molecular Mechanisms Underlying Plaque Rupture"
T. Cooper Woods
Tulane University School of Medicine, New Orleans, LA

A 3.10- "Role of Vimentin in Pluripotent Stem Cell Differentiation to the Endothelial Phenotype"
Taby Ahsan, Liana C Boraas, Julia Guidry
Tulane University, New Orleans, LA

A 3.11- "The Cardiac Deficiency of Pyruvate Dehydrogenase Complex Alters Cardiac Glucose Oxidation and Sensitizes Heart to Ischemic Insults"
Wanqing Sun, Nanhu Quan, Lin Wang, Xingchi Chen, Courtney Cates, Ji Li
The University of Mississippi Medical Center, Jackson, MS

Cell Signaling

A 4.1- "Creation of a NPC1L1 Knockout Using CRISPR Technology in Danio rerio"
Christian Pritchard, Benjamin Duncan, James Walters
Bluefield State College, Bluefield, WV

General Biomedical Sciences

A 5.1- "Regulation of Air Sac Primordium Development by a Cathepsin-L in *Drosophila melanogaster"*
Ajay Srivastava, Qian Dong, Breanna Brenneman, Christopher Fields
Department of Biology and Biotechnology Center, Western Kentucky University, Bowling Green, KY

A 5.2- "Dual Function Sers Active Nanoparticle Platform For Melamine Sensing"
Anant K. Singh, Yolanda Jones
Department of Chemistry and Physics, Alcorn State University, Lorman, MS

A 5.3- "Metabolic Dysfunction Due to a High Concentration of Dietary Fish Oil"
Alessandro Subauste, Simran Batth, Omonuwa Adah, Robert L. Hester, Angela Subauste
The University of Mississippi Medical Center, Jackson, MS

A 5.4- "Differential Effects of Sildenafil and Tadalafil in Human Penile Smooth Muscle Cells: New Insights for Old Mechanisms"
BM Rezk[1], AA Moustafa[2], P Sangkum[2], ZY Abd Elmageed[2], S Sikka[2], Abdel AB Mageed[2], WJG Hellstrom[2]
[1]Department of Natural Science, Southern University at New Orleans, New Orleans, LA
[2]Department of Urology, Tulane University, School of Medicine, New Orleans, LA

A 5.5- "Silver Clusters Inhibit the Biofilm Formed by *Bacillus Thuringiensis"*
Bidisha Sengupta[1], Christa Corley[1], Keith Cobb[1], Jr., Sudarson Sinha[2], Elrica Brown[3], Paresh Ray[2], Bianca Garner[3]
[1]Chemistry Department, Tougaloo College, Tougaloo, MS
[2]Chemistry Department, Jackson State University, Jackson, MS
[3]Biology Department, Tougaloo College, Tougaloo, MS

A 5.6- "Antibacterial Activity of the Novel C5-Curcumin-2-Hexadecynoic Acid Conjugate"
David J. Sanabria-Ríos, Yaritza Rivera-Torres, Joshua Rosario, Ricardo Gutierrez, Yeireliz Torres-García, Nashbly Montano, Gabriela Ortíz-Soto, Eddy Ríos-Olivares, José W. Rodríguez, Néstor M. Carballeira
The Inter American University of Puerto Rico, Metropolitan Campus, San Juan, PR

A 5.7- "Expanding the Repertoire of Amino Acid and Nucleic Acid Analogues for Use in the Synthesis of Novel Peptides"
Douglas Iverson, Crystal Serrano, Jessica Vital, Jonathan Long, John Adams, Anthony Bell
The University of Southern Mississippi, Hattiesburg, MS

A 5.8- "Impaired Glucose Metabolism in the TREML-1 null mice: A link to Obesity"
Elizabeth Castro-Rivera, A. Valance Washington
Universidad Central del Caribe, Bayamón, PR

A 5.9- "FEA of Orthopedic Implants towards the Development of Optical Strain Sensors for Fracture Fixation"
Hunter Pelham, Melissa M. Rogalski, Nakul Ravikumar, John D. DesJardins, Jeffrey N. Anker
Clemson University, Clemson, SC

A 5.10- *"Spatial Control of Shoc2-Mediated ERK1/2 Signaling Requires Remodeling Activity of the Atpase PSMC5"*
Eun Ryoung Jang, HyeIn Jang, Ping Shi, Gabriel Popa, Myoungkun Jeoung Emilia Galperin
Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY

A 5.11- "Transposable Element LINE-1 in Response to Ionizing Radiation"
Isabelle R. Miousse, Lijian Shao, Rupak Pathak, Jianhui Chan, Etienne Nzabarushimana, Sara Prior, Alan Tackett, Martin Hauer-Jensen, Daohong Zhou, Igor Koturbash
The University of Arkansas for Medical Sciences, Little Rock, AR

A 5.12- "Characterizing Modulators of the L1 Endonuclease"
Jade Meyers[1], Geraldine Servant[2], Vincent Streva[3], Cecily DeFreece[1]
[1]Xavier University of Louisiana, New Orleans, LA
[2]Tulane University of Louisiana, New Orleans, LA
[3]Children's Hospital, Boston, MA

A 5.13- "NHR-80 is Required for Oxygen-Deprivation Tolerance in Aging *Caenorhabditis elegans"*
James Mercado, Jo M. Goy
Harding University, Searcy, AR

A 5.14- "NaDC1 Knockout: Effects on Blood Pressure and Urine pH"
Federico Jose Teran[1,2], Weitao Huang[1], L. Lee Hamm[1,2,3], Kathleen S. Hering-Smith[1,2]
[1]Nephrology, Tulane University School of Medicine, New Orleans, LA
[2]Tulane Hypertension and Renal Center of Excellence, New Orleans, LA
[3]Research, SLVHCS, New Orleans, LA

A 5.15- "Spectral Rulers for Non-invasively Monitoring Load-Sharing Changes between Orthopedic Fixation Devices and Healing Bone"
Melissa M. Rogalski, Nakul Ravikumar, Hunter Pelham, Donald Benza, Joshua Lake, John D. DesJardins, Jeffrey N. Anker
Clemson University, Clemson, SC

A 5.16- "Sds-Page Buffer Can Be Reused For Multiple Times, But Not Towbin Transfer Buffer In Western Blotting"
Oluwabukola Omotola, Rajiv P. Heda, Jamie Avery-Holder, Ghanshyam D. Heda
Mississippi University for Women, Columbus, MS

A 5.17- *"Nuclease Stability of Intramolecular Four Way Junctions and DNA-PNA Four Way Junctions"*
Paul J. Stoulig III[1], Tamara Bell[2], Sana Solanja[1], Doug Iverson[1], Anthony Bell Jr.[1]
[1]The University of Southern Mississippi, Hattiesburg, MS
[2]Alcorn State University, Loreman, MS

A 5.18- "Stimulating Effect of Advanced Glycation End Products on Angiotensinogen Expression in Renal Proximal Tubule Cells"
Joseph M. Garagliano, Andrei Derbenev, Andrea Zsombok, L. Gabriel Navar, Ryosuke Sato
Department of Physiology and Hypertension and Renal Center of Excellence, Tulane University School of Medicine, New Orleans, LA

A 5.19- "Role of Conserved Components of Germ Cell Organelles in Germline Development in *Drosophila"*
Samuel J. Tindell, Jimiao Zheng, Nhan Huynh, Ming Gao, Tanyaradzwa Katanda, Bishal Shrestha, Hieu D. L. Vo, Alexey L. Arkov
Murray State University, Murray, KY

A 5.20- "Investigation of Phospholipids as Novel Reagents to Isolate Labile Protein-Nucleic Acid Complexes"
Vaniecia Wilson, Jalisa Keys, Alexis L.N. Sanders, Andrea Phang, Douglas Iverson, Anthony J. Bell Jr.
Department of Chemistry and Biochemistry, The University of Southern Mississippi, Hattiesburg, MS

A 5.21- "Total Synthesis of δ-Tocotrienol"
Xuan Zhang, Guangrong Zheng
Department of Pharmaceutical Sciences, College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, AR

Genomics

A 6.1- "Identification of Branch Points in Mirtrons"
Britton Strickland, Jaaved Mohammed, Matthew de Cruz, Mosharrof Hossain, Alex Flynt
The University of Southern Mississippi, Hattiesburg, MS

A 6.2- "The UMMC Molecular and Genomics Core Facility: A Resource for Genomic Technology throughout Mississippi and Beyond"
Ashley C. Johnson[1], Zhen Jia[1], Michael R. Garrett[1,2]
[1]Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS
[2]Director, UMMC Molecular and Genomics Core, University of Mississippi Medical Center, Jackson, MS

Infectious Disease/Immunology

A 7.1- "Knowledge and Awareness of HIV Facts, Infection Routes and Myths among the General Population in Jackson, MS and the Surrounding Metro-statistical Area"
Russell Baldwin[1], Amber Haymer[2], Dr. William K. Johnson[3]
[1]Belhaven University, Jackson, MS
[2]Mississippi Valley State University, Itta Bena, MS
[3]My Brother's Keeper, Inc., Jackson, MS

A 7.2- *"Staphylococcus aureus* Superantigen-induced T Regulatory Cells in Mouse Splenocytes"
Barbara L. F. Kaplan, Yianelly Rodríguez Ruiz, Nogi Park, Eun-Ju Yang, Jeffrey Eells, Keun-Seok Seo
Mississippi State University, Mississippi State, MS

A 7.3- "Role of Polyamine Transport in Pneumococcal Pneumonia"
Aswathy N. Rai, John Stokes, Justin A. Thornton, Edwin Swiatlo, Imran Sunesara, Bindu Nanduri.
Mississippi State University, Mississippi State, MS
University of Mississippi Medical Center, Jackson, MS

A 7.4- "Vaccination with a Live-Attenuated HSV-1 Vaccine VC2 Promotes Robust Immune Response and Complete Protection in Mice, Guinea Pigs, and Rhesus Macaques in Models of Ocular HSV-1 and Genital HSV-2 Infections"
Brent A. Stanfield[1], Rhonda Cardin[2], Bapi Pahar[3], Vladimir N. Chouljenko[1], Ronald Veazey[3], Konstantin G. Kousoulas[1]
[1]Department of Pathobiological Sciences and Division of Biotechnology and Molecular Medicine, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA
[2]Division of Infectious Diseases, Cincinnati Children's Hospital Medical Center, Cincinnati, OH
[3]Tulane National Primate Research Center Department of Comparative Pathology, Covington, LA

A 7.5- "Acyclovir Cannot Block HSV-1 DNA Accumulation in the Brain during Acute Viral Corneal Keratitis in ApoE e4/e4 Mice"
Christian Clement[1], Florence A. Clement[3], Alberto E. Musto[2], Timothy P. Foster[2], Partha S. Bhattacharjee[4], Harris E. McFerrin[4], Walter J. Lukiw[2], James M. Hill[2]
[1]Southern University at New Orleans, New Orleans, LA
[2]Louisiana State University Health Sciences Center, Baton Rouge, LA
[3]Loyola University New Orleans, New Orleans, LA
[4]Xavier University of Louisiana, New Orleans, LA

A 7.6- "Puma - A Critical Component of Innate Immunity Against Extracellular Pathogens"
Dan E. Kennedy II, Katie E. Heath, Katie Hill, Keun Seo, Tim Morgan, Jim Cooley, Justin A. Thornton
Mississippi State University. Mississippi State, MS

A 7.7- "Analyzing the Expression of *M46*, A Mold Phase- Specific Gene, In The Pathogenic Dimorphic Fungus *Histoplasma capsulatum"*
Davida Crossley
Department of Biological Sciences, Alcorn State University, Lorman, MS

A 7.8- "The Role of FTL_0129 in the Invasion of Erythrocytes by *Francisella tularensis"*
Devin Sindeldecker[1], Deanna Schmitt[1], Tricia Gilson[1], Alek Florjanczyk[1], Caleb Martin[1], Edward Beaumont[1], Jennifer Hickman[1], Donald Primerano[2], James Denvir[2], Joseph Horzempa[1]
[1]West Liberty University, West Liberty, WV
[2]Marshall University Genomics Core Facility, Huntington, WV

A 7.9- "Activated caspase-1 in the Colon and Small Bowel as a Marker for Barrier Dysfunction in Inflammatory Bowel Disease"
Elisabeth M. Davis, Katherine Wang, Yihong Kaufmann, Keith Lai, Hannah E. Goyne, Christian Jobin, Julia J. Liu
University of Arkansas for Medical Sciences, Little Rock, AR

A 7.10- "Th1/Th2 Imbalance by "Gain-Of-Function" Mutations Cause Fatal Theiler's Virus Infection in Mice"
Fumitaka Sato, Ph.D.[1], Eiichiro Kawai, M.D.[1], Nicholas E. Martinez, Ph.D.[1], Seiichi Omura, Ph.D.[1], Elaine Cliburn Stewart[1], Satoru Takahashi, M.D., Ph.D.[2], Keigyou Yoh, M.D., Ph.D.[2], Ikuo Tsunoda, M.D., Ph.D.[1]
[1]Louisiana State University Health Sciences Center, Shreveport, LA
[2]University of Tsukuba, Tsukuba, Ibaraki, Japan

A 7.11- "Characterization of Uninfected and *Rickettsia parkeri*-infected *Amblyomma maculatum* Tissues"
Gail M. Moraru, John V. Stokes, Andrea S. Varela-Stokes
College of Veterinary Medicine, Mississippi State University, Mississippi State, MS

A 7.12- "Role of 5'and 3' UTR Region to Regulate msaB Production in *Staphylococcus aureus"*
Gyan S. Sahukhal, Mohamed O. Elasri
Department of Biological Sciences, The University of Southern Mississippi, Hattiesburg, MS

A 7.13- "Assessing Differential Gene Regulation Triggered by Environmental Temperature in *Borrelia turicatae"*
Jacob Latham[1], Marissa Podgorski[1], Hannah Wilder[2], Job Lopez[2], Jon Blevins[1]
[1]University of Arkansas for Medical Sciences, Little Rock, AR,
[2]Baylor College of Medicine, Texas Children's Hospital, Houston, TX

A 7.14- "The *msaABCR* Operon Activates Capsule Production in *Staphylococcus aureus"*
Justin Batte, Mohamed O. Elasri
The University of Southern Mississippi, Hattiesburg, MS

A 7.15- "The Dose-Dependent Effect of Superantigens on Development of Immunosuppressive CD8+CD25+FOXP3+ T Cells"
Juyeun Lee, Nogi Park, Joo Youn Park, Keun Seok Seo
Mississippi State University, Mississippi State, MS

A 7.16- "Evaluation of Sex Education among Mississippi Students"
Kiana Moore, Jasmine Thompson, Mauda Mounger
[1]Jackson State University, Jackson, MS
[2]Alcorn State University Lorman, MS
[3]Delta Region AIDS Education Training Center, Jackson, MS

A 7.17- "High-throughput screening of CRISPR-Cas9 library for host factors essential for HSV-1 replication"
Kui Yang, Caitlin Anglin, Xiaoqun Dang, Joel Baines
Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Skip Bertman Drive, Baton Rouge, LA

A 7.18- "The Characterization of The Mold Specific Genes, *Ms88* and *Ms95*, In the Dimorphic, Pathogenic Fungi, *Histoplasma capsulatum"*
Mariah Lloyd, Glenmore Shearer Jr., Davida Crossley
Center of Biotechnology, Alcorn State University, Lorman, MS
Biological Sciences, University of Southern Mississippi, Hattiesburg, MS

A 7.19- "Non-structural Protein σ1s Enhances Reovirus Spread in Endothelial Cells"
Matthew B. Phillips, Karl W. Boehme
Department of Microbiology and Immunology and Center for Microbial Pathogenesis and Host Inflammatory Responses, University of Arkansas for Medical Sciences, Little Rock, AR

A 7.20- "Autoantibody Profiling and Immunosuppressive Medications in Systemic Lupus Erythematosus and Incomplete Syndromes"
Michael R. Eledge, Corbett S. Hall, Benjamin F. Bruner, Ph.D., James JA, Ph.D.
[1]Harding University, Searcy, AR
[2]Oklahoma Medical Research Foundation, Oklahoma City, OK

A 7.21- "The Dose-Dependent Effect of staphylococcal superantigens on Development of Functional CD4+CD25+FOXP3+ Regulatory T Cells"
Nogi Park, Juyeun Lee, Joo Youn Park, Keun Seok Seo
Mississippi State University, Mississippi State, MS

A 7.22- "Cigarette Smoke Mediated Regulation of Autophagy in RAW264.7 Macrophages"
Prathyusha Bagam, Sanjay Batra
Laboratory of Pulmonary Immunotoxicology, Environmental Toxicology Ph.D. Program, Health Research Center, Southern University and A&M College, Baton Rouge, LA

A 7.23- "The Role of the Erythrocyte Cytoskeleton Protein Spectrin During Invasion by *Francisella tularensis*"
Rebecca Barnes, Deanna M. Schmitt, Tricia L. Gilson, Joseph Horzempa
Department of Natural Sciences and Mathematics, West Liberty University, West Liberty, WV

A 7.24- "Modualtion of anti-*Mycobacterium tuberculosis* Adaptive Immune Responses-Role of IDO"
S. Mehra[1], U. S. Gautam[2], T. Foreman[2], D. Kaushal[2]
[1]LSU-SVM,PBS, Baton Rouge, LA
[2]TNPRC, Covington, LA

A 7.25- "Promoter Analysis of *M46* gene, in the Pathogenic Dimorphic Fungus *Histoplasma capsulatum"*
Tamara Bell, Ashly Claiborne, Glenmore Shearer Jr., Davida Crossley
[1]Alcorn State University, Lorman, MS
[2]The University of Southern Mississippi, Hattiesburg, MS

A 7.26- "Role of *msaABCR* operon in Biofilm Development of *Staphylococcus epidermidis"*
Victoria Reid, M. Erin Riggins
Mississippi Gulf Coast Community College, Gulfport, MS

Neuroscience

A 8.1- "Leptin Inhibits Presympathetic Neurons in the Rostral Ventrolateral Medulla"
Hong Gao[1], Heike Muenzberg-Gruening[2], Andrea Zsombok[1], Andrei V. Derbenev[1]
[1]Department of Physiology, Tulane University Health Sciences Center, New Orleans, LA
[2]Pennington Biomedical Research Center, LSU, Baton Rouge, LA

A 8.2- "Control of Cortactin Levels by Wg/Wnt Regulates Activity Dependent Synaptic Plasticity"
Daniel Alicea, Carihann Dominicci, Marizabeth Perez, Carolina Maldonado, Bruno Marie
Institute of Neurobiology, University of Puerto Rico, Medical Sciences Campus, Rio Piedras, PR

A 8.3- "The Postmortem Brain Core of the COBRE Center for Psychiatric Neuroscience"
Craig A. Stockmeier, Gouri Mahajan
The University of Mississippi Medical Center, Jackson, MS

A 8.4- "Pharmacoresistant Epilepsy: Methods for Measuring Efficacy vs. Toxicity of Cannabidiol and Structural Analogues"

Dennis R. Carty, Stephen J. Cutler, Mahmoud A. ElSohly, Ikhlas A. Khan, Kristine L. Willett
Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS

A 8.5- "Low Intensity Pulsed Focused Ultrasound Alters Long-Term Excitability Properties of Cortical Cells in Rats"

Leonardo Rodríguez-Negrón[1], Rafael Vázquez-Torres[2], Bermary Santos-Vera[2], Carlos A. Jiménez-Rivera[2], Eduardo J. Juan-García[1]
[1]Department of Electrical & Computer Engineering, University of Puerto Rico at Mayagüez, Mayagüez, PR
[2]Department of Physiology, Medical Sciences Campus, University of Puerto Rico, San Juan, PR

A 8.6- "The Imaging Core Facility of the COBRE Center for Psychiatric Neuroscience"

Grazyna Rajkowska, Mohadetheh Moulana, Craig A. Stockmeier
The University of Mississippi Medical Center, Jackson, MS

A 8.7- "Astrocyte Pathology in the Ventral Prefrontal White Matter in Major Depressive Disorder"

Rajkowska G, Legutko B, Stockmeier CA, Miguel-Hidalgo JJ
The University of Mississippi Medical Center, Jackson, MS

A 8.8- "Polymeric Micelle as a RhoA siRNA Carrier for Axonal Regeneration in Rat SCI Model"

So Jung Gwak[1], Christia Macks[1], Ken Webb[1], Michael Lynn[3], Mark Kindy[2], Jeoung Soo Lee[1]
[1]Deptartment of Bioengineering, Clemson University, Clemson, SC
[2]Department of Neurology, USC Medical School, Greenville, SC
[3]Dept of Pharmaceutical Sciences, College of Pharmacy, University of South Florida, Tampa, FL

A 8.9- "Measuring Mitochondrial Size and Movement in Primary Mouse Neurons"

John Z. Cavendish, Saumyendra Sarkar, Sujung Jun, James W. Simpkins
West Virginia University, Morgantown, WV

A 8.10- "ShRNA Based Suppression of Cx43 in Vivo and In Vitro to Determine Depression-Related Cellular and Behavioral Abnormalitie"

José Javier Miguel-Hidalgo, Katherine Hall, Zhen Jia, Natalie J. Booker
The University of Mississippi Medical Center, Jackson, MS

A 8.11- "Peripheral Inflammation During Pregnancy Leads to Increased BBB Leakage in the Post-Partum Period"

Kedra Wallace, Teylor Bowles, Shauna-Kay Spencer, Cynthia Bean
The University of Mississippi Medical Center, Jackson, MS

A 8.12- "The Functional Roles of miR-1017"

Matthew de Cruz
The University of Southern Mississippi, Hattiesburg, MS

A 8.13- "Evaluation of Berberine as an Inhibitor of Human Monoamine Oxidase A and B: Potential Application in Treatment of Neurological Disorders"

Deependra Singh[1], Narayan D Chaurasiya[1], Stephen J. Cutler[2], Larry A Walker[1,2], Babu L. Tekwani[1,2]
[1]National Center for Natural Products Research, The University of Mississippi, University, MS
[2]Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS

A 8.14- "High Throughput Screening for the Investigation of Blood-Brain Barrier Integrity Enhancers"

Quoc-Viet Duong, Ashley DePaula, Courtney Flick, Trista LeBeouf, Youssef Mousa, Jeffrey N. Keller, Amal Kaddoumi
The University of Louisiana at Monroe, LA

A 8.15- "The National Center of Neuromodulation for Rehabilitation (NC NM4R)"

Steven A Kautz, PhD
Medical University of South Carolina, Charleston, SC

A 8.16- "Oleocanthal Enhances Amyloid− β Clearance and Reduce Inflammation in the Brains of TgSwDI Mice"

Yazan S. Batarseh, Hisham Qosa, Amal Kaddoumi
The University of Louisiana at Monroe, Monroe, LA

Poster Session B,
Friday, November 13th, 10:45 a.m.-12:15 p.m.

Location: Camellia Ballroom

Bioinformatics

B 1.1- "Assessment of mRNA 3'Untranslated Region (3'UTR) Dynamics During Neuroplasticity"
Benjamin Harrison, Jeffrey Petruska, Eric Rouchka
University of Louisville, Louisville, KY

B 1.2- "Assessment of Major RNA-Seq Assembly Approaches for Identification of Long Noncoding Rnas in Different Tissue Types"
Dan Li, Carolyn Arthur, Mary Yang
The University of Arkansas at Little Rock, Little Rock, AR

B 1.3- "Combinatorial Therapy of Rolipram and pNGF for Traumatic Brain Injury"
Christian Macks[1], So Jung Gwak[1], Michael Lynn[2], Mark Kindy[3], Jeoung Soo Lee[1]
[1]Department of Bioengineering, Clemson University, Clemson, SC
[2]Department of Neurology, USC Medical School, Greenville, SC
[3]Dept of Pharmaceutical Sciences, College of Pharmacy, University of South Florida, Tampa, FL

B 1.4- "Identification of Successful Mentoring Communities Using Network-based Analysis of Mentor-Mentee Relationships Across Nobel Laureates"
Julia H. Chariker, Yihang Zhang, John R. Pani, Eric C. Rouchka
University of Louisville, Louisville, KY

B 1.5- "Integrated Modeling for an Enhanced Predictive DILI Model"
Kristin McEuen[1,2], Leihong Wu[1,2], Minjun Chen[1,2], Shradda Thakkar[1,2], Weida Tong[1,2]
[1]The University of Arkansas at Little Rock, Little Rock, AR
[2]The University of Arkansas for Medical Sciences, Little Rock, AR

B 1.6- "Sequencing and Analyzing Ribosomal Frameshifting of *Trichomonas vaginalis* virus 4 Isolates along with Treatment of Ribavirin on *T. vaginalis* Cells"
Lizhuo Ai, Hirni Patel, Cory Toyota
Millsaps College, Jackson, MS

B 1.7- "From *In silico* to *in vitro*: Rapid Verification of Bioinformatically Identified Promoters and Terminators"
Jace Bradshaw, AlleaBelle Gongola, Nathan Reyna
Ouachita Baptist University, Arkadelphia, AR

B 1.9- "Surface Signature Analysis of the Binding Pattern of Sesquiterpene Lactones to Nf-Kb"
Ujwani Nukala[1,2], Paola E. Ordóñez[1,3,4], Shraddha Thakkar[1,2], Darin E. Jones[4], Monica L. Guzmán[5], Cesar M. Compadre[1]
[1]Department of Pharmaceutical Sciences, University of Arkansas for Medical Sciences, Little Rock, AR
[2]Joint Bioinformatics Graduate Program, University of Arkansas at Little Rock and University of Arkansas for Medical Sciences, Little Rock, AR
[3]Departamento de Química Aplicada, Universidad Técnica Particular de Loja, Loja Ecuador
[4]Department of Chemistry, University of Arkansas at Little Rock, AR
[5]Division of Hematology/Oncology, Department of Medicine, Weill Cornell Medical College, New York, NY

B 1.10- "Performance Evaluation for Gene Set Analysis Approaches for RNA-seq Data"
Yasir Rahmatallah, Galina Glazko
University of Arkansas for Medical Sciences, Little Rock, AR

B 1.11- "Autophagy Regulates Muscle Contraction Induced Myokine Synthesis

Simran Batth, Tarek Abd-elhamid, Jose Subauste, Angela Subauste
University of Mississippi Medical Center, Jackson, MS

Cancer

B 2.1- "Design and *In Vitro* Evaluation of modified Tocosol™ Paclitaxel Nanodispersion Using a Quality-by-Design Approach"

Ahmed Abu-Fayyad[1], Paul W. Sylvester[1], Jennifer L. Carroll[2,3], James A. Cardelli[2,3], Sami Nazzal[1]
[1]College of Health and Pharmaceutical Sciences, School of Pharmacy, University of Louisiana at Monroe, Monroe, LA
[2]Department of Microbiology and Immunology, Louisiana State University Health Sciences Center, Shreveport, LA
[5]Feist-Weiller Cancer Center, Louisiana State University Health Sciences Center, Shreveport, LA

B 2.2- "Pharmacological Clearance of Senescent Cells by ABT263 Mitigates Total Body Irradiation-Induced Premature Aging of the Hematopoietic System in Mice"

Yingying Wang[1,2,], Jianhui Chang[1,*], Lijian Shao[1], Wei Feng[1], Remi-Martin Laberge[3], Marco Demaria[3], Judith Campisi[3,4], Yi Luo[1], Nukhet Aykin-Burns[1], Kimberly Krager[1], Martin Hauer-Jensen[1], Aimin Meng[2], Daohong Zhou[1]*
[1]Division of Radiation Health, Department of Pharmaceutical Sciences and Winthrop P. Rockefeller Cancer Institute, University of Arkansas for Medical Sciences, Little Rock, AR
[2]Institute of Radiation Medicine, Peking Union Medical College & Chinese Academy of Medical Sciences (PUMC & CAMS), Tianjin, China
[3]Buck Institute for Research on Aging, Novato, CA
[4]Lawrence Berkeley National Laboratory, Berkeley, CA
[*]These authors contributed equally to this work

B 2.3- "CDK8 Inhibition Potentiates Anti-ER and Anti-HER2 Therapies and Prevents Targeted Drug Resistance in Breast Cancer"

McDermott MS[1], Lim C[1], Ivers L[2], O'Donovan N[2], Altilia S[1], Chen M[1], Chumanevich A[1], Catroppo JF[3], Gyorffy B[4], Oliver D[1], Shtutman M.[1], Roninson IB[1], Broude EV[1]
[1]Department of Drug Discovery and Biomedical Sciences, South Carolina College of Pharmacy, University of South Carolina, Columbia, SC
[2]National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin
[3]Department of Pathology, Microbiology and Immunology, University of South Carolina School of Medicine, Columbia, SC
[4]Research Laboratory for Pediatrics and Nephrology, Hungarian Academy of Sciences, Budapest H-1083, Hungary

B 2.4- "Polymeric Micelle for pTK and GCV Delivery to Spinal Cord Tumor"

So-Jung Gwak, Justin Nice, Christian Macks, Jeoung Soo Lee
Drug Design, Development and Delivery Laboratory, Department of Bioengineering, Clemson University, Clemson, SC

B 2.5- "Tetrahydrobiopterin (BH4) – Mitochondria Crosstalk in Radiation-induced Skin Injury"

Kimberly J. Krager, Rupak Pathak, Qiang Fu, Martin Hauer-Jensen, Nukhet Aykin-Burns
Division of Radiation Health, University of Arkansas for Medical Sciences, Little Rock, AR

B 2.6- "Bone Marrow-Derived Mesenchymal Stem Cells and Preadipocytes Cultured with Breast Tumor Cells Favor Cytokine Secretion"

Leah Figurski, Yuya Kudo, Linda Vona-Davis
West Virginia University, Morgantown, WV

B 2.7- "Analysis of the DNA-Cleaving Efficiencies and Mechanism of Bifunctional DNA-Cleaving Reagents"

Leon H. Karekezi, Marcus E. Powell, Courtney B. Mullins, Gurjit Kaur, Lauren M. Hoth, B. Woods Curry, Emily H. Stewart, Jonathan P. Giurintano, Wolfgang H. Kramer
Millsaps College, Jackson, MS

B. 2.8- "Alternate Protein Targets & Drug Designing for Breast and Prostate Cancers"
Pradip K Biswas
Laboratory of Computational Biophysics & Bioengineering, Department of Physics, Tougaloo College, Tougaloo, MS

B. 2.9- "A Mixed Immuno-Chemotherapy Against Colon Cancer: Modeling and Simulations"
Qing Wang[1], Zhijun Wang[1], David J Klinke[2]
[1]Shepherd University, Shepherdstown, WV
[2]West Virginia University, Morgantown, WV

B 2.10- "Reactions of Oxaliplatin Analogs with DNA Nucleotides at Variable pH and Concentration"
Rippa Sehgal, Dr. Kevin Williams
Western Kentucky University, Bowling Green, KY

B 2.11- "Dysbiosis Induced Impairment of Immune Surveillance in Non-Gastrointestinal Tumors"
Samir Jenkins, Carl Cerniglia, Kieng B. Vang-Dings, Robert J. Griffin, Ruud P.M. Dings
The University of Arkansas for Medical Sciences, Little Rock, AR

B 2.12- "Design, Synthesis and Evaluation of a Novel Doxorubicin-Peptidomimetic Conjugate for the Specific Delivery of Doxorubicin to HER2 Positive Breast Cancer Cells"
Sandeep Pallerla[1], Ted Gauthier[2], Rushikesh Sable[1], Seetharama D. Jois[1]
[1]University of Louisiana at Monroe, Monroe, LA
[2]Louisiana State University, Baton Rouge, LA

B 2.13- "BVES and BCAR3: A Potential Tight Junction/Focal Adhesion Signaling Pathway"
Shenika Poindexter
Alcorn State University, Alcorn State, MS

B 2.14- "Xanthohumol Inhibits the Proliferation of Neuroblastoma NG-108"
Xingchi Chen, Wanqing Sun, Nanhu Quan, Lin Wang, Courtney Cates, Ji Li
Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS

Cardiovascular Research

B 3.1- "An Insight into the Antioxidant Responses to Exogenous Oxidative Stress Agents and a Glimpse into the Role of Catalase in the Reproductive Fitness of tahe Gulf-Coast Tick (*Amblyomma Maculatum*)"
Deepak Kumar, Shahid Karim
Department of Biological Sciences, The University of Southern Mississippi, Hattiesburg, MS

B 3.2- "Disruption in PKC Mediated K Channel Regulation Contributes to Enhanced Serotonin Mediated Carotid Vessel Constriction from the Fawn Hooded Hypertensive Rat"
Gurjit Kaur, Mallikarjuna R Pabbidi
Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS

B 3.3- "Diabetes Resistant Tissue Engineered Vascular Grafts"
Jhilmil Dhulekar, M.S., Eric Wright, Zoltan Hajdu, M.D., Dan Simionescu, Ph.D., Agneta Simionescu, Ph.D.
Department of Bioengineering, Clemson University, Clemson, SC

B 3.4- "Effects of Radiation and Sunitinib in the Rat Heart"
Marjan Boerma, Vijayalakshmi Sridharan, Martin Hauer-Jensen
The University of Arkansas for Medical Sciences, Little Rock, AR

B 3.5- "Epicardial Fat and Markers of Hypertension"
Janae Jackson[1], Chris Adams[2], Nepal Chowdhury[3], Todd Gress[2], Carla Cook[1], Paulette Wehner[2], Ellen Thompson[2], Mohammed Waqas[2], Caitlin Kocher[1], Nalini Santanam[1,2]
[1]Department of Pharmacology, Physiology Toxicology, Joan C Edwards School of Medicine, Marshall University, Huntington, WV
[2]Department of Cardiology, Joan C Edwards School of Medicine, Marshall University, Huntington, WV
[3]Department of Thoracic Surgery, Joan C Edwards School of Medicine, Marshall University, Huntington, WV

B 3.6- "COBRE Translational Research in Hypertension & Renal Biology"
Nina R. Majid
Tulane University School of Medicine, New Orleans, LA

B 3.7- "Localizing Cardiac Magnetic Dipole Sources in Fetus Using Inverse and Forward Solutions on Fetal Magnetocardiography"
Recep Avci[1], Hari Eswaran[2], James D Wilson[1,2]
[1]University of Arkansas Little Rock, Little Rock, AR
[2]University of Arkansas for Medical Sciences, Little Rock, AR

B 3.8- "Role of Sphingosine-1-phosphate in High Blood Pressure Programmed by Intrauterine Growth Restriction in Mouse"
Suttira Intapad, Ph.D.
Department of Physiology & Biophysics, University of Mississippi Medical Center, Jackson, MS

B 3.9- "The Efficacy of Soluble Guanylyl Cyclase (Sgc) Stimulators and Activators in a Rat Model of Preeclampsia"
Ta'Shariah Robinson, Ellen Gillis, Joey Granger, Eric George, Michael Garrett, Jennifer Sasser
Departments of Pharmacology and Toxicology and Physiology and Biophysics, The University of Mississippi Medical Center, Jackson MS

B 3.10- "Pericyte Fate Depends on Stem Cell Type and Age in Cultured Microvascular Networks"
Walter L. Murfee, Mohammad S. Azimi, Amy L. Strong, Bruce A. Bunnell
Tulane University, New Orleans, LA

B 3.11- "Role of ER Stress in Development of Hypertensive-Diabetic Nephropathy"
Zhen Wang, Jussara M. do Carmo, John E. Hall
Department of Physiology and Biophysics, Mississippi Center for Obesity Research, University of Mississippi Medical Center, Jackson, MS

Cell Signaling

B 4.1- "The Effects of Oleic Acid Concentration on Cholesterol Absorption in the Larval Zebrafish Enterocyte"
Laura Faith Stevens, Jennie Spencer, James Walters
Bluefield State College, Bluefield, WV

B 4.2- "C/EBPδ Modulates Oxidative Stress and Mitochondrial Dysfunction to Promote Post-Radiation Survival"
Sudip Banerjee[1], Nukhet Aykin-Burns[1], Kimberly J. Krager[1], Stepan B. Melnyk[2], Martin Hauer-Jensen[1 3], Snehalata A. Pawar[1]
[1]Division of Radiation Health, College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, AR
[2] Arkansas Children's Hospital Research Institute, Little Rock, AR
[3]Surgical Services, Central Arkansas Veterans Healthcare System, Little Rock, AR

General Biomedical Sciences

B 5.1- "The *Trichomonas vaginalis Virus* Among Mississippi and ATCC *T. vaginalis* Isolates"
Allison K. Judge, John. C. Meade, Cory G. Toyota
Millsaps College, Jackson, MS

B 5.2- "Leptin Stimulation of GnRHr in Gonadotropes: Is Musashi involved?"
Angela K. Odle, Melody Allensworth-James, Melanie MacNicol, Angus MacNicol, Gwen V. Childs
University of Arkansas for Medical Sciences, Little Rock, AR

B 5.3- "Development of Four-Way Junctions (4wjs) as Novel Therapeutic Inhibitors of the DNA-Binding Cytokine High Mobility Group B1 (HMGB1)"

Douglas Iverson[1], Paul Stoulig[1], Tamara Bell[2], Crystal Serrano[1], Sana Salongi[1], Anushobha Regmi[1], Dexter Dean[1], Filbert Totsingan[3], Anthony J. Bell Jr.[1]
[1]The University of Southern Mississippi, Hattiesburg, MS
[2]Alcorn State University, Lormon, MS
[3]New York University, New York, NY

B 5.4- "Regulation of Biofilm Formation in the Symbiotic Bacterium *Xenorhabdus nematophila"*

Tilak Patel, Elizabeth Hussa
Millsaps College, Jackson, MS

B 5.5- "Molecular Dynamics Simulation Study of VECAR in Water: Data Analysis"

Bijay Shrestha, Binaya Bajgain, Hye-Young Kim
Southeastern Louisiana University, Hammond, LA

B 5.6- "Decarboxylative Photocyclization of Pyromellitimide w-Carboxylic Acids"

Donya Razinoubakht, Gurjit Kaur, Eli A. Smith, David S. Sandlin, Andrew S. Olinger, Axel G. Griesbeck, Wolfgang H. Kramer
Millsaps College, Jackson, MS
Universitaet zu Koeln, Cologne, GER

B 5.7- "Inhibition of Retinoic Acid Receptor Beta Enhances the Neuronal Differentiation of Mouse Embryonic Stem Cells"

Diamond Moses, Eduardo Martinez-Ceballos
Southern University and A&M College, Baton Rouge, LA

B 5.8- "Synthesis and Antioxidant Activity Studies of Tocotrienol Analogues with Variable Tail Length"

Satheesh Gujarathi, Lijian Shao, Xingui Liu, Daohong Zhou, Guangrong Zheng
Department of Pharmaceutical Sciences, College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, AR

B 5.9- "The Role of Bolaamphiphilic Character in Self-Assembly of VECAR Molecules: A Molecular Dynamics Simulation Study"

Hye-Young Kim[1], Brian Novak[2], Dorel Moldovan[2], Bijay Shrestha[1]
[1]Southeastern Louisiana University, Hammond, LA
[2]Louisiana State University, Baton Rouge, LA

B 5.10- "Increased Expression of Dkk-1 in Human Bone Marrow Stromal Cell Populations Following Chemotherapy Exposure"

Ian Hare, Debra Piktel, Rebecca Evans, Jim Fortney, Laura F Gibson
West Virginia University, Morgantown, WV

B 5.11- "Short- and Long-Term Effects of Exposure to Low Doses of High-LET Radiation in the Mouse Lung"

Isabelle R. Miousse[1], Sara Prior[1], Etienne Nzabarushimana[1], Rupak Pathak[2], Lijian Shao[2], Jianhui Chang[2], Antiño R. Allen[2], John R. Latendresse[3], J. Marjan Boerma[2], Martin Hauer-Jensen[2], Gregory Nelson[4], Igor Koturbash[1]
[1]Department of Environmental & Occupational Health, University of Arkansas for Medical Sciences, Little Rock, AR
[2]Division of Radiation Health, Department of Pharmaceutical Sciences, University of Arkansas for Medical Sciences, Little Rock, AR
[3]Departments of Behavioral Neuroscience, Toxicologic Pathology Associates, Jefferson, AR
[4]Loma Linda University, Loma Linda, CA

B 5.12- "Biochemical And Molecular Methods For The Detection Of Endogenous Cftr Expression"

Jamie Avery-Holder, Oluwabukola Omotola, Ghanshyam D. Heda
Mississippi University for Women, Columbus, MS

B 5.13- "MC4R in Forebrain Neurons Contributes to Leptin's Acute Effects to Stimulate Peripheral Glucose Uptake"
Mark A. Pinkerton, Alexandre A. da Silva, Jussara M. do Carmo
Department of Physiology and Biophysics, Mississippi Center for Obesity Research, University of Mississippi Medical Center, Jackson, MS

B 5.14- "Tiny Cells for a Tiny Animal"
Kody Paul Mansfield
The University of Southern Mississippi, Hattiesburg, MS

B 5.15- "Role of SIRT3 in the Pathobiology of Pulmonary Fibrosis"
Sosulski ML, Gongora R, Sanchez CG
Tulane University Health Sciences Center, New Orleans, LA

B 5.16- "Saturated Fat-Dependent Ubiquitination: Potential Role in Obesity-Associated Non-Alcoholic Fatty Liver Disease"
Paul Kim[1], Andrea Estrada[2], Claire Stewart[2], Dong Wang[2], Yuren Wei[2], Michael Pagliassotti[2]
[1]Grambling State University, Grambling, LA
[2]Colorado State University, Fort Collins, CO

B 5.17- "SC BioCRAFT Bioengineering and Bioimaging Core"
Robert A. Latour[1], Guzeliya Korneva[1], Ken Webb[1], Jiro Nagatomi[1], John H. Parrish[1], Anne-Marie Broom[2]
[1]Clemson University, Clemson, SC
[2]Medical University of South Carolina, Charleston, SC

B 5.18- "A Potential Role for TtSNX4 in macronuclear Degradation in *Tetrahymena thermophila* Conjugation"
Allison Ariatti[1], Marcella Cervantes[2], Jeff Kapler[2], Sabrice Guerrier[1]
[1]Millsaps College, Jackson, MS
[2]Texas A and M, College Station, TX

B 5.19- "Electrospinning Natural Polymer Fibers"
Lucas Veide[2], Robert Alongi[2], C. Cameron Skinner[2], Sharon K. Hamilton[1], Gisela Buschle-Diller[2]
[1]Department of Chemistry and Physics, Delta State University, Cleveland, MS
[2]Department of Biosystems Engineering at Auburn University, Auburn, AL

B 5.20- "Synthesis and Antioxidant Activity of δ-tocoflexol, a δ-tocotrienol Analogue Designed to have Improved Pharmacokinetic Profile"
Xingui Liu, Lijian Shao, Satheesh Gujarathi, Cesar Compadre, Martin Hauer-Jensen, Peter Crooks, Daohong Zhou, Guangrong Zheng
Department of Pharmaceutical Sciences, College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, AR

B 5.21- "Autophagy Regulates Muscle Contraction Induced Myokine Synthesis"
Simran Batth, Tarek Abd-elhamid, Jose Subauste, Angela Subauste
University of Mississippi Medical Center, Jackson, MS

Genomics

B 6.1- "Telomere Shortening is Associated with Cartilage Transcriptome of Patients with Osteoarthritis"
Malwina Czarny-Ratajczak, James Eastwood, Vinod Dasa, John Zhang, Melody Baddoo, Tiffany Chynces, S. Michal Jazwinski
Tulane Center for Aging, Department of Medicine, Tulane School of Medicine, New Orleans, LA

B 6.2- "Chromatin Structural Changes in Gene Regulation"
Yvonne Fondufe-Mittendorf
University of Kentucky, Lexington, KY

Infectious Disease/Immunology

B 7.1- "Specific Mucin Response to Human Paramyxovirus Infections"
Banos-Lara, A. Guerrero-Plata
Louisiana State University, Baton Rouge, LA

B 7.2- "The *msaABCR* Operon Plays a Role in Antibiotic Susceptibility in *Staphylococcus aureus* Biofilm"
Bina L. Jayana, Latoyia Downs, Gyan S. Sahukhal, Mohamed O. Elasri
Department of Biological Sciences, The University of Southern Mississippi, Hattiesburg, MS

B 7.3- "Role of the SOS Response in the Resistance of *Listeria monocytogenes* to Conditions Encountered within the Gastrointestinal Tract"
Brandy Roberts[1], Amber Thompson[1], Morgan L. Wright[1], Hossam Abdelhamed[2], Jessica G. Wilson[1], Janet R. Donaldson[1]
[1]Mississippi State University, Mississippi State, MS
[2]College of Veterinary Medicine, Mississippi State University, Mississippi State, MS

B 7.4- "An Investigation of Nitrogen Metabolism in the Dimorphic Fungus *Histoplasma capsulatum"*
Logan Blancett, Caitlin Cooksey, Thomas Buford, Glen Shearer
Department of Biological Sciences, The University of Southern Mississippi, Hattiesburg, MS

B 7.5- "Identifying Enriched CDR3 Sequences in Systemic Lupus Erythematous Patients"
Corbett S. Hall[1], Michael R. Eledge[1], Dr. Judith A. James[2], Dr. Benjamin F. Bruner[1]
[1]Harding University, Searcy, AR
[2]Oklahoma Medical Research Foundation, Oklahoma City, OK

B 7.6- "Memory Tfh cells promote rapid antibody production after secondary *Plasmodium yoelii* infection"
Daniel J. Wikenheiser, Jason S. Stumhofer
University of Arkansas for Medical Sciences, Little Rock, AR

B 7.7- "Induction of an Atypical Lymphoid Progenitor Lin$^-$Sca-1$^+$c-kit$^-$ Cells in Spleen During Acute Infection with *Plasmodium yoelii"*
Debopam Ghosh, Jason S. Stumhofer
Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR

B 7.8- "Recruitment of NLRP10 and NLRP12 in Lipid Raft Entities Following Cigarette Smoke Challenge in Murine Macrophages"
Dhirendra P. Singh[1], Kevin Shen[1], Sanjay Kumar[2], Xiaochu Wu[3] Sanjay Batra[1]
[1]Laboratory of Pulmonary Immunotoxicology, Environmental Toxicology Ph.D. Program, Southern University and A&M College, Baton Rouge, LA
[2]Department of Computer Science, Jamia Millia Islamia, New Delhi, India
[3]Departments of Comparative Biomedical Sciences, Louisiana State University, Baton Rouge, LA

B 7.9- Optimal Antibody-Mediated Immunity to Chlamydia Genital Infection Requires Interferon-γ Activation of an Effector Cell Population
Elizabeth K. Naglak, Sandra G. Morrison, Richard P. Morrison
University of Arkansas for Medical Sciences, Little Rock, AR

B 7.10- "Cigarette Smoke Mediated Regulation of Epigenetic Signatures on *NF-κB* Proximal Promoter Region in Murine Macrophages"
Gagandeep Kaur[1], Arvind Panday[2], Anne Grove[2] Sanjay Batra[1]
[1]Laboratory of Pulmonary Immunotoxicology, Environmental Toxicology Ph.D. Program, Southern University and A&M College, Baton Rouge, LA

B 7.11- *"Rickettsia Parkeri* Modulates Tick Superoxide Dismutase to Survive Within the Tick Vector"
Gary Crispell, Khemraj BC, Shahid Karim
Department of Biological Sciences, The University of Southern Mississippi, Hattiesburg, MS

B 7.12- "Inhibition of HSV-1-Associated Ocular Neovascularization by Cyclin-Dependent Kinase Inhibitors"
Harris E. McFerrin, Thomas Vu, Elise I. LeMelle, Tatyana T. Santoke, Bria Carmichael, Hasahn Conway, Sydnie Turner, Briana Jarrett, Kevin Lam, Usman Chaudrey, Moamen Ismail, Willie Sparkman, Ashley N. Sankey, Monique N. Westley, Eric J. Fontenot, Eric Stewart, Heba A. Sarhan, Fiyinfolu T. Mustapha, Kenneth F. Swan, Partha S. Bhattacharjee, Cindy B. Morris, Konstantin G. Kousoulas, Deborah E. Sullivan
Xavier University of Louisiana, New Orleans, LA

B 7.13- "Rhinovirus Infection Induces Th2-Promoting Innate Cytokines in an *Ex Vivo* Precision Cut Lung Slice Model"
Joshua L Kennedy, Emily Brown, Megan Kurten, Stacie M Jones, Richard C Kurten
University of Arkansas for Medical Sciences, Department of Pediatrics, Arkansas Children's Hospital Research Institute, Little Rock, AR

B 7.14- "The Contribution of Pyruvate Oxidase to Pneumolysin Release in *Streptococcus pneumonia"*
Joseph C. Bryant[1], Ridge C. Dabbs[1], Jason W. Rosch[2], Larry S. McDaniel[3], Justin A. Thornton[1]
[1]Mississippi State University. Mississippi State, MS
[2]St. Jude Children's Research Hospital. Memphis, TN
[3]University of Mississippi Medical Center, Jackson, MS

B 7.15- "Role of Ttick Antioxidants in *Rickettsia parkeri* Colonization in the Gulf-Coast tick (*Amblyomma maculatum)"*
Khemraj Budachetri, Shahid Karim
Department of Biological Sciences, The University of Southern Mississippi, Hattiesburg, MS

B 7.16- "Improved Immune Function and Reduced Chlamydia Genital Infection in a Murine Stress Model Fed with Active Hexose Correlated Compound"
Kristin Brown, Tesfaye Belay
Bluefield State College, Bluefield, WV

B 7.17- "Tracking Antigen-Specific CD4 T Cell Responses to *Chlamydia* Female Reproductive Tract Infection"
Lin-Xi Li[1], Stephen J McSorley[2]
[1]University of Arkansas for Medical Sciences (UAMS), Little Rock, AR
[2]University of California Davis, Davis, CA

B 7.18- "Effect of Parasitism on the Distribution of Serotonin in the Nervous Tissues of *Biomphalaria alexandrina*, an Intermediate Host for Intestinal Schistosomiasis"
Solymar Rolon-Martinez[1], Mohamed R. Habib[2], Lee O. Vaasjo[1], Roger P. Croll[3], Mark W. Miller[1]
[1]University of Puerto Rico, San Juan, PR
[2]Theodor Bilharz Research Institute, Cairo, Egypt
[3]Dalhousie University, Halifax, NS, Canada

B 7.19- *"msaA* and *msaC* Plays Role in MsaB Production to Regulates Virulence and Biofilm Development in *Staphylococcus aureus"*
Megan Mullis, Sarah Simmons, Gyan S. Sahukhal, Mohamed O. Elasri
Department of Biological Sciences, The University of Southern Mississippi, Hattiesburg, MS

B 7.20- "Regulation of Respiratory Paramyxovirus-induced Disease by Neutrophils"
Nagarjuna R. Cheemarla, Rocio Banos-Lara, Antonieta Guerrero-Plata
Louisiana State University, Baton Rouge, LA

B 7.21- "Bile Induced Membrane Damage Varies Dependent Upon Oxygen Availability in *Listeria monocytogenes"*
Oindrila Paul[1], Jessica G. Wilson[1], Dominique N. Clark[2], Amber Thompson[1], Janet R. Donaldson[1]
[1]Department of Biological Sciences, Mississippi State University, Mississippi State, MS
[2]Tougaloo College, Tougaloo, MS

B 7.22- *"Mycobacterium Tuberculosis* Cell Wall Fractions Induce Inflammatory Cytokines in Primary Mouse Macrophages and Fibroblasts"
Quinton L. Anderson, Jo M. Goy
Harding University, Searcy, AR

B 7.23- "Molecular Characterization of Glycine Rich Proteins in *Amblyomma americanum*, the Lone Star Tick"
Rebekah Bullard, Shahid Karim
University of Southern Mississippi, Hattiesburg, MS

B 7.24- "Administrative Core Functions at the Mississippi State University COBRE"
Stephen B. Pruett, Mark Lawrence, Giselle Thibaudeau
Mississippi State University, Mississippi State, MS

B 7.25- "Investigating the Role of Mouse Sca1^{+} Lung Mesenchymal Stem Cells in Bacterial Pneumonia"
Tirumalai Rangasamy, Shanshan Cai, Sagar Paudel, Laxman I Ghimire, Samithamby Jeyaseelan
Pathobiological Sciences, Louisiana State University, Baton Rouge, LA

B 7.26- "Elucidating the Functional Role of the H_2O_2-Generating Dual Oxidase (Duox) in the Gulf Coast Tick, *Amblyomma maculatum*"
Virginia C. Meyers, Shahid Karim
The University of Southern Mississippi, Hattiesburg, MS

B 7.27- "Characterization of Host Receptor Profiling and Viral Mutations Determining Influenza Virus Host Tropisms"
Xiu-Feng Wan, Chun-Kai Yang, Minhui Guan, Feng Wen, Lei Zhong
Influenza Systems Biology Laboratory, Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS

Neuroscience

B 8.1- "Developmental Lead (Pb^{+2}) Disrupts Transcriptional Response of Ecdysone-Responsive Genes in the Fruit Fly"
Zoé Márquez, Wilfredo Meléndez, Ángel Rivera Collazo, José Agosto Rivera, Humberto Ortiz-Zuazaga, Adrinel Vázquez-Montes
Universidad del Turabo, Ana G. Méndez System, Gurabo, PR

B 8.2- "Intracellular Mechanisms Modulating Gamma Band Activity in the Pedunculopontine Nucleus"
B. Luster[1], F.J. Urbano[2], E. Garcia-Rill[1]
[1]Center for Translational Neuroscience, University of Arkansas for Medical Sciences, Little Rock, AR
[2]IFIBYNE-CONICET, University of Buenos Aires, Argentina

B 8.3- "The Effects of Aerobic Exercise on Post-stroke Depression, Functional Mobility, and Metabolic Capacity in Individuals with Chronic Stroke"
Catherine Vanderwerker, Aaron Embry, Brian Cence, Chris Gregory
Medical University of South Carolina, Charleston, SC

B 8.4- "Consequences of Self-Administered Methamphetamine Throughout Pregnancy on Rat Dams and Their Offspring"
Daniela Rüedi-Bettschen, Simran Chawla, Camille S. Washington, Donna M. Platt
University of Mississippi Medical Center, Jackson, MS

B 8.5- "Conformational Dynamics in the Propagation of Amyloid-β Oligomers in Alzheimer Disease"
Dexter N. Dean[1], Kayla M. Pate[2], Melissa A. Moss[2], Vijayaraghavan Rangachari[1]
[1]Department of Chemistry and Biochemistry, The University of Southern Mississippi, Hattiesburg, MS
[2]Department of Chemical Engineering, The University of South Carolina, Columbia, SC

B 8.6- "Design, Synthesis and Biological Evaluation of Novel Benzofuran Cannabinoid Ligands"
Eric W. Bow, John M. Rimoldi
Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS

B 8.7- "Protein Succination is Increased on Select Proteins in the Brainstem of the Ndufs4 Knockout Mouse"
Gerardo G. Piroli, Allison M. Manuel, Anna C. Capps, Michael D. Walla, John E. Baatz, Richard D. Palmiter, Albert Quintana, Norma Frizzell
University of South Carolina School of Medicine, Columbia, SC

B 8.8- "Glutamatergic Output from the Medial Prefrontal Cortex Modulates the Daily Rhythm in Amphetamine Reward"

Nedah N. Nemati, Ron Cassada, Ian C. Webb
The University of Mississippi Medical Center, Jackson, MS

B 8.9- "Allosteric Modulation of the Cannabinoid Receptor Type 1 (CB_1) by Org 27569"

Carolina Deifelt Streese, Sara A. Pettaway, Tracy A. Brooks, Janet A. Lambert, Samuel H. Hans, Meredith E. Stocks, Stephen J. Cutler
The University of Mississippi, University, MS

B 8.10- "The COBRE *In Vitro* Core of the University of Mississippi"

Janet A. Lambert, Samuel H. Hans, Meredith E. Stocks, Narayan D. Chaurasiya, Babu L. Tekwani, Sara A. Pettaway, Stephen J. Cutler
The University of Mississippi, University, MS

B 8.11- "The Role of Acid Sensing Ion Channel 2 in Modulating Seizure Activity and Cerebral Edema"

Junie P. Warrington
University of Mississippi Medical Center, Jackson, MS

B 8.12- "Excitatory and Inhibitory rTMS as Mechanistic Contributors to Walking Recovery"

Mark G. Bowden, PhD, PT, Elizabeth C. Wonsetler, DPT, Charalambos C. Charalambous, MS, Mark S. George, MD, Steven A. Kautz, PhD
The Medical University of South Carolina, Charleston, SC

B 8.13- *"In vitro* Phenotypic Cell-Based and Molecular Target-Based Neuropharmacological Assays for Determining Neuroactive Properties of Natural Products"

Narayan D. Chaurasiya[1], Ilias Muhammad[1], Stephen J. Cutler[2], Larry A. Walker[1,2], Babu L. Tekwani[1,2]
[1]National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences The University of Mississippi, University, MS
[2]Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS

B 8.14- "Sleep Disturbance and Inflammation: A Role for Stress Hormones?"

Noah T. Ashley, Audrey C. Brown, David W. Sams, Jennie E. Dumaine
Western Kentucky University, Bowling Green, KY

B 8.15- "Lithium Decreases the Effects of Neuronal Calcium Sensor Protein 1 in Pedunculopontine Neurons"

Stasia D'Onofrio, Francisco J. Urbano, Edgar Garcia-Rill
The University of Arkansas for Medical Sciences, Little Rock, AR

B 8.16- "Alterations in Kinesin Catalytic Activity Resulting from Mutations that Cause Hereditary Spastic Paraplegia"

Davon Carter, Scott Jennings, David Nathan, Chelsea Kelland, Liautaud Prophete, Khari Gilmore, Mahusoodanan Mottamal, Thomas Huckaba
Xavier University of Louisiana, New Orleans, LA

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Highlighted Speakers
Thursday, November 12, 1:30-3:00 p.m.

Bioinformatics, Azalea A

"Fully Mining RNA-Seq Data For Noncoding RNA Analyses"
Dr. Glen Borchert, Assistant Professor, Biology Department & Pharmacology Department, University of South Alabama, Mobile, AL

Cancer, Azalea B

"The Mechanism of Cancer Cell Death Mediated by Knockdown of COPZ1 Gene Encoding Coatomer Protein Complex Subunit Z 1 – New Target Gene for Cancer Therapy"
Michael Shtutman, Assistant Professor, The University of South Carolina, Columbia, SC

Cardiovascular Research, Azalea C

"A Link Between Autoimmunity and Hypertension"
Dr. Michael J. Ryan, Associate Professor of Physiology & Biophysics, Director of Graduate Studies in Physiology, Associate Dean of the School of Graduate Studies in Health Sciences, Research Health Scientist in Research Service, University of Mississippi Medical Center, Jackson, MS

Cell Signaling, Azalea D

"Shoc2 Scaffold-Matchmaker of Intracellular Communications in Time and Space"
Dr. Emilia Galperin, Assistant Professor, Molecular & Cellular Biochemistry, University of Kentucky, Lexington, KY

Grants Administrators Workshop, Magnolia E, F, G, H

"Uniform Guidance – For The Research Administrator And Researcher"
Dr. Denise Clark, Associate Vice President for Research Administration, University of Maryland; Dr. Ann Holmes, Assistant Dean, Finance & Administration for the College of Behavioral & Social Sciences, University of Maryland

Highlighted Speakers
Friday, November 13, 9:00-10:30 a.m.

General Biomedical Sciences, Azalea A

"Chromatin Structural Changes In Gene Regulation"
Dr. Yvonne Fondufe-Mittendorf, Assistant Professor, Molecular & Cellular Biochemistry, University of Kentucky

Infectious Disease/Immunology, Azalea B

"The Balance Between Health And Disease In Chronic Gammaherpesvirus Infection Is Controlled By p53"
Dr. J. Craig Forrest, Assistant Professor, Department of Microbiology & Immunology, University of Arkansas for Medical Sciences

Neuroscience, Azalea C

"KNDy Neurons and the Control of Mammalian Reproduction"
Dr. Michael N. Lehman, Professor & Chair, Neurobiology & Anatomical Sciences,
University of Mississippi Medical Center

Genomics, Azalea D

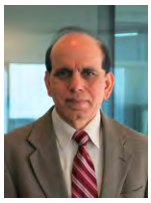
"Leveraging Rat Genetics to Understand Human Diseases of the Kidney"
Dr. Michael Garrett, Associate Professor of Pharmacology & Toxicology, Associate Professor of Medicine, Director, Institutional Molecular & Genomics Core Facility, University of Mississippi Medical Center

Science Commercialization Workshop, Speakers

Thursday, November 12, 3:15-6:00 p.m.

Opening Remarks

Dr. Krishan Arora, Program Director, Center for Research Capacity Building, National Institutes of Health



Krishan Arora, Ph.D., is a program director in the Center for Research Capacity Building, where he administers the IDeA Networks of Biomedical Research (INBRE) component of the Institutional Development Award (IDeA) program. He also manages a portfolio of Centers of Biomedical Research Excellence (COBRE) grants. Before joining NIGMS, Arora served as an NIH program director for 14 years, first in the Division of Research Infrastructure at the former National Center for Research Resources and later in the Division of Scientific Programs at the National Institute on Minority Health and Health Disparities. Arora earned a B.Sc. in biology from Punjab University, an M.Sc. in biochemistry from Punjab Agricultural University and a Ph.D. in biochemistry from the Australian National University. He conducted postdoctoral research at Johns Hopkins University School of Medicine.

"Investing in Innovation: The HHS SBIR/STTR Programs"

Dr. Matthew Portnoy, NIH SBIR/STTR Program Manager

Dr. Matthew Portnoy is the NIH SBIR/STTR Program Manager and Director, Division of Special Programs, Office of Extramural Programs, Office of Extramural Research, Office of the Director, NIH. In this role, he manages the SBIR/STTR programs at NIH and coordinates the 24 NIH Institutes/Centers that receive funding for the programs. Additionally, as the Director, Division of Special Programs, Dr. Portnoy and his staff provide scientific program management and oversight of the Academic Research Enhancement Award (AREA) Program, and support for conferences and scientific meetings (R13/U13), ensures that NIH extramural staff are trained to meet the ever-changing demands of their job, and communicates funding opportunities and critical information concerning NIH's programs, policies, and procedures to the biomedical research and training community through the NIH Guide for Grants and Contracts. Dr. Portnoy received his B.S. in molecular and cell biology from Penn State University. He received his Ph.D. in biochemistry and molecular biology from Johns Hopkins University School of Public Health. Matt then joined the Intramural Program of NIH National Human Genome Research Institute as a post-doctoral fellow. Dr. Portnoy made the leap to the extramural side of NIH in 2005 and joined the NIH's National Institute of General Medical Sciences (NIGMS) as a program director. Over his time at NIGMS, he managed R01 grant portfolios in DNA repair, recombination and replication, SBIR/STTR grants, F32 post-doctoral fellowships, cooperative agreements, and R25 education grants. Dr. Portnoy also served as SBIR/STTR program lead for NIGMS for 6 years prior to his current post.

"Entrepreneurship And Commercialization: Building An Engine Of Innovation Translation In WV"

Mr. Richard Giersch, Director of Life Science Innovation, Entrepreneurship and Commercialization, West Virginia University

Rich Giersch is the Director of Health Sciences Innovation, Entrepreneurship and Commercialization for West Virginia University (WVU). After graduating from WVU he attended UNC-Chapel Hill for graduate school. He has held Director level positions at two venture capital firms, was the chief operating officer of a biotech company in Research Triangle Park, North Carolina and the Chief Science Officer for the New Jersey Center for Biomaterials. He has helped companies secure over $100 million in federal funding, tax, and relocation incentives and is a founder of

CereDx a company developing precision diagnostics for brain injury.

"SBIR And STTR Projects Carried Out By ElSohly Labs"

Dr. Mahmoud ElSohly, President & Laboratory Director of ELI, Research Professor in the Research Institute of Pharmaceutical Sciences, Professor of Pharmaceutics, University of Mississippi

Dr. Mahmoud A. ElSohly is a Research Professor at The National Center for Natural Products Research, and Professor of Pharmaceutics, School of Pharmacy, University of Mississippi and is the Director of the NIDA Marijuana Project. He is also the President and Laboratory Director of ElSohly Laboratories Incorporated, an analytical forensic drug testing and a product development laboratory.

He received his undergrad and Masters from Cairo University, Cairo, Egypt and his Ph.D. from the University of Pittsburgh, School of Pharmacy, Pittsburgh, PA. He has over 40 years' experience working with the isolation of natural products, synthetic, analytical and forensic chemistry. He has more than 30 patents and approximately 300 publications in these areas of science. He has been working on cannabis and cannabinoids for 37 years. He has published extensively in the areas of cannabis analysis and cannabinoids isolation as well as cannabinoids formulations.

Dr. ElSohly is a member of many professional organizations such as American Society of Pharmacognosy; American Pharmaceutical Association; Fellow of the American College of Forensic Examiners; Fellow of the American Academy of Forensic Sciences; Society of Forensic Toxicologists; American Chemical Society; Fellow of the American Institute of Chemists; Society of Toxicology, Southeast Chapter; International Cannabis Research Society. He is also a member of several honorary societies such as Rho Chi; Phi Kappa Phi; Sigma Xi; American Association for the Advancement of Science.

Some of his honors are: IACM Special Award for his Major Contributions to the Re-introduction of Cannabis as a Medicine from the International Association for Cannabinoids Medicine (2015), The Lifetime Achievement Award from the International Cannabinoid Research Society (2013), The University of Mississippi's Distinguished Research and Creative Achievement Award (2013), The University of Pittsburgh Legacy Laureate Award (2011), The University of Mississippi's School of Pharmacy Researcher of the Year Award (2011), and The University of Pittsburg, School of Pharmacy Distinguished Alumnus Award (2002).

"Technology Transfer and Science Commercialization at Tulane University: History, Lessons Learned, Plans For the Future"

Dr. John Christie, Executive Director, Technology Transfer and Intellectual Property Development, Tulane University

John Christie is the Executive Director of the Office of Technology Transfer and Intellectual Property Development (OTT) at Tulane University and is responsible for the overall management, supervision, and planning of activities within the office. Tulane's OTT strives to develop effective means of moving groundbreaking research from the laboratory to the commercial sphere where it can provide a meaningful public benefit, most often in the form of improved healthcare outcomes.

The OTT's activities include development and implementation of programs to create a comprehensive inventory of Tulane's intellectual property; identifying promising technological developments and encouraging their disclosure; protection of Tulane's intellectual property, either directly or in cooperation with outside counsel; and commercializing intellectual property through an aggressive and innovative licensing program. The OTT is responsible for crafting commercial value from inventions that arise from all Tulane research, both medical and non-medical. In this capacity, John is responsible for developing intellectual property strategy, marketing, and licensing of all university technology, and for fostering industry sponsored research collaborations. Tulane's share of revenue from intellectual property supports research and other activities at the university.

John is active in the Association of University Technology Managers (AUTM) and the Licensing Executives Society (LES), and also participates in

numerous initiatives in New Orleans and at the state level to promote the development and implementation of technology transfer policies and practices.
John has been affiliated with Tulane for seventeen years. He has a Bachelor of Art's degree from the University of Texas at Austin and was awarded the Master of Business Administration degree from Tulane.

"The Road Towards Translation Of MTI-101 For The Treatment Of Relapsed Multiple Myeloma"

Dr. Lori Hazlehurst, Professor, Co-Leader of the Alexander B. Osborn Hematopoietic Malignancy & Transplantation Program, West Virginia University

Lori Hazlehurst, Ph.D., is a co-program leader of the Co-Leader of MBRCC Alexander B. Osborn Hematopoietic Malignancy and Transplantation Program at West Virginia University
Dr. Hazlehurst's research interest focuses on developing novel strategies for the treatment of tumors that home or metastasize to the bone. One of the major focus of her laboratory is the development of a novel cyclic peptide coined MT-101 that Modulation Therapeutics (co-founded by Dr. Hazlehurst) has licensed. Dr. Hazlehurst has experience obtaining successful SBIR and STTR funding to further advance the lead molecule. She received her Ph.D. from the University of Vermont and did her post-doctoral training at University of Arizona and the Moffitt Cancer Center. She joined the faculty at the Moffitt Cancer Center in 2002. She recently joined the faculty of West Virginia University where she is currently Professor of Pharmaceutical Sciences in the School of Pharmacy.

Alternative Careers & Innovation in Academia Breakfast Discussion Forum

Dr. T. Ryan Withers,
Principal Research Scientist, Progenesis Technologies, LLC

T. Ryan Withers, Ph.D. serves as the Principal Research Scientist for Progenesis Technologies, a small biotechnology start-up originating at Marshall University. Prior to joining Progenesis, Dr. Withers held various research and management positions at Celera Genomics, MRIGlobal and the National Institutes of Health. He earned a B.S. in Biology from West Virginia University, a M.S. in Biotechnology from Johns Hopkins University, and a Ph.D. in Biomedical Sciences from Marshall University. Dr. Withers' was recently awarded a Small Business Innovation Research (SBIR) grant from the National Institute of General Medical Sciences to develop a non-pathogenic bacterial strain for the production of medically-relevant biopolymers. He has authored several peer-reviewed articles in various research journals and has previously received funding from the National Aeronautics and Space Administration.

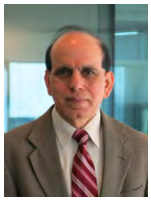
Dr. Regine Douthard, Medical Officer in the Center for Research Capacity Building, National Institute of General Medical Sciences, National Institutes of Health

Régine A. Douthard, M.D., M.P.H., is a medical officer in the Center for Research Capacity Building, where she administers Institutional Development Award (IDeA) program grants. In addition, she oversees the dual degree, individual predoctoral F30 fellowship program. Before coming to NIGMS, Douthard was a medical officer in the Division of Research Infrastructure at the former National Center for Research Resources. During her tenure there, she oversaw Research Centers in Minority Institutions (RCMI) program grants. Douthard earned an M.D. from the School of Medicine and Health Sciences in Libreville, Gabon, and an M.P.H. from Emory University. She completed a residency in family practice and a fellowship in environmental and occupational medicine at the George Washington University Medical Center in Washington, D.C.

Dr. Rafael Gorospe, Medical Officer in the Center for Research Capacity Building, National Institute of General Medical Sciences, National Institutes of Health

J. Rafael Gorospé M.D., Ph.D., is a medical officer in the Center for Research Capacity Building, where he administers Institutional Development Award (IDeA) program grants. Before joining NIGMS, he was a medical officer in the Division of Research Infrastructure at the former National Center for Research Resources. Prior to that, Gorospé was a faculty member in the department of pediatrics at George Washington University and at the Children's National Medical Center, where he conducted research on childhood genetic brain disorders. He earned an A.B. in humanities from the University of the Philippines, an M.D. from Virgen Milagrosa University in the Philippines and a Ph.D. in molecular genetics from the University of Pittsburgh.

Dr. Krishan Arora, Program Director, Center for Research Capacity Building, National Institutes of Health



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Dr. Matthew Portnoy, NIH SBIR/STTR Program Manager

Dr. Matthew Portnoy is the NIH SBIR/STTR Program Manager and Director, Division of Special Programs, Office of Extramural Programs, Office of Extramural Research, Office of the Director, NIH. In this role, he manages the SBIR/STTR programs at NIH and coordinates the 24 NIH Institutes/Centers that receive funding for the programs. Additionally, as the Director, Division of Special Programs, Dr. Portnoy and his staff provide scientific program management and oversight of the Academic Research Enhancement Award (AREA) Program, and support for conferences and scientific meetings (R13/U13), ensures that NIH extramural staff are trained to meet the ever-changing demands of their job, and communicates funding opportunities and critical information concerning NIH's programs, policies, and procedures to the biomedical research and training community through the NIH Guide for Grants and Contracts.

Dr. Portnoy received his B.S. in molecular and cell biology from Penn State University. He received his Ph.D. in biochemistry and molecular biology from Johns Hopkins University School of Public Health. Matt then joined the Intramural Program of NIH National Human Genome Research Institute as a post-doctoral fellow. Dr. Portnoy made the leap to the extramural side of NIH in 2005 and joined the NIH's National Institute of General Medical Sciences (NIGMS) as a program director. Over his time at NIGMS, he managed R01 grant portfolios in DNA repair, recombination and replication, SBIR/STTR grants, F32 post-doctoral fellowships, cooperative agreements, and R25 education grants. Dr. Portnoy also served as SBIR/STTR program lead for NIGMS for 6 years prior to his current post.

Chase Kasper, Assistant Vice President for Research, Technology Transfer & Corporate Relations

Chase Kasper is the Assistant Vice President for Research, Technology Transfer & Corporate Relations at the University of Southern Mississippi. He directs the Office of Technology Development and has been involved with technology licensing, intellectual property management, and technology transfer in a university setting for over 12 years. He has also overseen programs and projects related to incubators, start-ups, entrepreneurship, and compliance.

Prior to working in academia, Mr. Kasper has spent over 13 years in the private sector. He has been involved with successfully managing multi-million dollar product lines, product planning and execution, assessing alliance/partnership opportunities, designing corporate identity guidelines, developing international pricing structures, launching new products, and assessing customer requirements and meeting customer satisfaction goals.

Mr. Kasper is active in the Association of University Technology Managers (AUTM) and has been a member of AUTM for over 10 years. He has presented on a wide variety of topics related to university technology transfer including technology transfer operations, technology assessment, economic development, and intellectual property.

Mr. Kasper received his B.B.A and M.B.A. from Mississippi State University and has been active in several professional organizations including Association of University Technology Managers, the Licensing Executives Society, the University-Industry Demonstration Partnership and the Network of Academic Corporate Relations Officers.

Conference Abstracts

Student Research/Career Development Luncheon, Azalea A

Thursday, November 12, 12 p.m.-1:30 p.m.

"Expanding And Enhancing Undergraduate Research As A Primary Strategy To Increase Research Capacity: Lessons Learned From The Council On Undergraduate Research (CUR)"
Dr. Elizabeth Ambos, Executive Officer, Council on Undergraduate Research, Washington, D.C.

In this session, participants will gain insights as to how systems and consortia of higher education institutions can most effectively link and leverage undergraduate research, scholarship, and creative activities (URSCA) initiatives at individual institutions within systems/consortia. As substantive evidence exists that URSCA is a primary high-impact practice that demonstrably increases student success across the disciplines, and especially for students from groups underrepresented in higher education, URSCA has become essential to most institutions' work to "embrace and advance the equity imperative," and thus is a focus for most NIH-funded IDeA INBRE projects that seek to build a well-qualified STEM workforce. For over two decades, the Council on Undergraduate Research (CUR) has worked with more than 500 institutions to develop and successfully implement a series of professional development institutes devoted to expanding and enhancing URSCA within individual departments, schools/colleges, and entire institutions. The organization has recently completed a four-year National Science Foundation-funded project (09-20275) working with six systems/consortia and 80 of their institutions. Lessons learned from work with these six systems/consortia provide insights into both the successes and challenges associated with scaling-up and scaffolding URSCA within the system/consortium construct, and thus have applicability to IDeA consortia.

Mentoring Workshop Luncheon, Azalea B

Thursday, November 12, 12 p.m.-1:30 p.m.

"Mentoring for an Evolving Academic Career"
Dr. Ian Paul, Professor & Director, Medical Student Education, Assistant Director, Division of Neurobiology & Behavior Research, Department of Psychiatry & Human Behavior, The University of Mississippi Medical Center

This seminar will describe and provide a rationale for ongoing, organized mentoring throughout an academic career. It will discuss principles, styles, and the cycle of mentoring as well as needed skills and resources to avoid common mentoring pitfalls.

Bioinformatics, Azalea A

Thursday, November 12, 1:30 p.m.-3 p.m.

1:30 p.m.- Highlighted Speaker: "Fully Mining RNA-Seq data for Noncoding RNA Analyses"

Glen Mark Borchert, Ph.D.
University of South Alabama, Mobile, AL

Breast cancer is the leading cause of female cancer mortality. Strikingly, the two most widely utilized breast cancer cell lines, primary MCF-7 and metastatic MDA-MB-231, have been used in over half of all the breast cancer studies in the primary literature. Since these cell lines differ in several well established ways in terms of morphology, invasiveness and physiological responses, we recently performed a RNA-seq analysis examining both their total RNA and small RNA populations in order to identify novel gene candidates responsible for their phenotypic differences. Having successfully generated over 150 million transcript sequencing reads from these cells, we now have extensive coverage of the mRNA and small RNA transcriptomes for each of these lines allowing us to identify specific regulations responsible for characteristic differences between them. To our surprise, distinct examinations of this data have generated three major new lines of investigation for our research:
(1) While we find little to no change in the expressions of over 2,500 human microRNAs (miRNAs) between these cell lines, we identify 25 miRNAs significantly overexpressed in MDA-MB-231 cells as well as 19 miRNAs overexpressed in MCF-7s. Strongly corroborating the importance of these miRNAs in breast cancer, 39 of these 45 miRNAs have been previously reported as being directly involved with breast cancer pathology and/or the modulation of breast cancer cellular response to chemotherapeutic agents. As such, we are now actively engaged in determining cellular functions for the six new miRNAs we find differentially expressed between MCF-7 and MDA-MB-231 cells likely playing uncharacterized roles in breast cancer pathology.
(2) Further computational analyses of our RNA-Seq data identified over 250,000 A-to-I edit sites primarily located in mRNA 3' UTRs. When these locations were screened against the list of currently annotated miRNAs we discovered that these A-to-I editing events caused a subset (~5%) of human miRNAs to have significantly altered mRNA complementarities leading us to propose that modulating the targets of miRNAs via mRNA editing plays a direct role in the pathology of many carcinomas.
And (3), in a more recent analysis of our RNA-Seq data we compared the snoRNA derived RNA (sdRNA) expression profiles of MCF-7 and MBA-MD-231 cell lines. Excitingly, we find 13 snoRNAs significantly overexpressed (≥10 fold) in MBA-MD-231 cells as compared to MCF-7s. To our surprise, we find microRNA-like fragments derived from all 13 snoRNAs are expressed in MBA-MD-231s. Moreover, additional experimentation finds small RNA reads from 10 of 13 small RNA-generating snoRNAs are complexed with Ago following immunoprecipitation suggesting their active involvement in RNAi and potential relevance to breast cancer pathology.
In summary, we find RNA-Seq provides a comprehensive, quantitative, and unbiased view of RNA sequences allowing for the ready discovery of novel observations unobtainable with previous technologies, and that the data generated by a single RNA-Seq can lead to numerous new relevant lines of investigation.

2:00 p.m.- "HPIDB: A Curated Database for Host-Pathogen Interactions"

Mais Ammari, Cathy R Gresham, Fiona McCarthy, Bindu Nanduri[1]
[1]Mississippi State University, Mississippi State, MS
[2]University of Arizona, Tucson, AZ

Background: Identification and analysis of host-pathogen interactions (HPI) is essential to understand infectious diseases. The HPIDB database provides a unified query interface for HPI information and visualization.
Methods: HPIDB provides comprehensive HPI annotations by mining available HPI from multiple external bioinformatics databases and complementing this by targeted biocuration of HPI from literature that is consistent with the International Molecular Exchange (IMEx) consortium standards. HPIDB also provides a platform for community annotations to support network analysis of functional genomics data in the context of HPI. HPIDB allows users to transfer homologous HPI identified in HPIDB (by BLAST) to their host-pathogen systems and visualize the HPI network in Cytoscape.
Results: Since the first description of the database in 2010, infrastructural updates include an improved query response time, ability to search by multiple IDs, enhanced taxon search, revised method for loading external interaction data into HPIDB, displaying additional interaction information and associated host and/or pathogen Gene Ontology (GO) functions in HPIDB results, and a newer version of BLAST for sequence and homology searches, and visualization capability. In its current release, HPIDB has 43,276 manually curated entries that can be used as a gold standard to transfer HPI information across multiple host-pathogen systems. All data in HPIDB can be accessed through a series of dynamic charts that allow a simple "point and click" download in a tab-delimited format at http://www.agbase.msstate.edu/hpi/main.html.
Conclusion: HPIDB content and interface ensure that

researchers are able to easily access and analyze high quality, comprehensive host-pathogen interaction data. All HPIDB data are updated regularly and are publically available for download directly from HPIDB and disseminated to other MI resources.
Acknowledgment: Grant # P20GM103646 (Center for Biomedical Research Excellence in Pathogen Host Interactions) from the National Institute for General Medical Sciences supported Nanduri. This work was funded by Agriculture and Food Research Initiative Competitive Grant no. 2015-67015-23271 from the USDA National Institute of Food and Agriculture.

2:15 p.m.- "An Automated Pipeline for Whole Genome Sequencing Data Analysis"
Chad Hayden, Mary Yang
The University of Arkansas at Little Rock, Little Rock, AR

Background: Whole-genome sequencing data analysis is commonly used in revealing disease related SNPs, INDELs and CNVs. Despite a number of software tools have been developed to process and analyze whole-sequencing data, presently no single variant identification tool can capture comprehensive genomic variations. We developed an automated pipeline for whole-genome sequencing data analysis, allowing easily adopting algorithms into the pipeline and consensus variant callings.
Methods: The pipeline was built in python and is able to handle both paired-end and single-end reads. The pipeline includes quality control (QC), read alignment, variant calling and functional annotation. After QC, reads are aligned to the reference genome using BWA. Picard is used to mark and remove PCR duplicate reads. Read re-alignment and re-calibration, as well as variant calling, are performed using GATK. FreeBayes and Platypus variant callers are used along with GATK to provide consensus results. The ANNOVAR package is used for functional annotation of the genetic variants.
Results: We used datasets from the 1000 Genome Project to test and validate our pipeline. Our results can be compared with the variant calling results provided by the 1000 Genome Project. Hence we are able to adjust software components and parameters of the pipeline based on benchmark results. Furthermore, MuTect program is used to call somatic mutations in the analysis of sequencing data from paired cancer and health samples.
Conclusion: Our automated pipeline can facilitate whole-genome sequencing data analysis and foster biomedical research collaboration. The pipeline serves as a flexible tool to allow users with minimal programming knowledge to easily run NGS data analysis.
Acknowledgment: This work was supported by grants from NCRR (P20RR016460), NIGMS (P20 GM103429), 1R15GM114739 and Arkansas Science and Technology Authority (ASTA)

2:30 p.m.- "An Assessment of TMAP for Host-Pathogen Genomic Pipelines"
John Caskey[1,2], Pushpendra Singh[3], Ramesh Subramanian[1,2], Rahul Sharma[3], Gus Kousoulas[1], Ram Ramanujam[2]
[1]Division of Biotechnology & Molecular Medicine, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA
[2]Center for Computation and Technology, Louisiana State University, Baton Rouge, LA
[3]Hansen's Disease Laboratory, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA

Background: Alignment algorithms are very important in obtaining the highest accuracy possible for the alignment or mapping step in bioinformatics pipelines especially since this function directly affects downstream analyses. The TMAP algorithm is a multi-step mapping program, which can be customized to handle different lengths of reads and different experimental conditions. The dataset of interest in this experiment was a *M. leprae*-infected *M. musculus* sample, which had been subjected to different treatment conditions to remove as much mouse genomic DNA as possible, while preserving *M. leprae* DNA. After DNA sequencing, three pipelines were used to assess the samples: Partek®, DNASTAR®, and a custom in-house built pipeline.
Methods: DNA from mouse-infected tissue was treated either with NaOH, immuno-magnetic separation, or immuno-magnetic separation with Whole Genome Amplification. Next, the DNA was extracted, then sequenced using the Ion Proton® Sequencer. Following sequencing, the reads were processed using the Partek® genomics pipeline, the DNASTAR® pipeline, and with a custom in-house pipeline that used several tools including fastx_trimmer, BWA, and Bowtie2. All scripts and workflows are available upon request.
Results: We found that immunomagnetic separation provided the best coverage and the best overall alignment for *M. leprae.* Pipelines that used either TMAP, Bowtie2, or BWA required additional time or prior knowledge when isolating reads from bacterial host-pathogen datasets. For wet lab work, these results suggest that immuno-magnetic separation can provide a good method to isolate bacterial pathogen DNA. Comparative bionformatics analysis shows that certain alignment programs may ambiguously map pathogen and host reads.
Conclusions: DNASTAR®'s SeqManNGEN required no additional customization, unlike the other alignment programs. A thorough knowledge of the biological hypotheses, facts, and assumptions are needed to

fully take advantage of bionformatics analysis of a given data set.

2:45 p.m.- "The Implications of the Presence of Lycotoxin in the Venom of the Wolf Spider *Rabidosa rabida* (Areanae Lycosidae)"

Sara Wilmsen, Ryan Stork, Dennis Province
Harding University, Searcy, AR

Background: *Rabidosa rabida* is a large, lycosid spider that is common across much of the eastern half of North America. Because of its large size and availability, this spider is a good candidate for venom and digestive fluid collection. Both venom and digestive fluid are protein-rich substances that have not been studied in this spider before. However, two antimicrobial proteins have been described in a spider of the same family as *rabida*. These are known as lycotoxin I and lycotoxin II.
Methods: We performed reverse-phase high performance liquid chromatography (RP-HPLC) and mass spectroscopy (MS) analysis on *Rabidosa rabida's* venom and digestive fluid looking for lycotoxin I and II; and also similarities between the two substances. We also plan to send samples of venom and digestive fluid to UAMS to have them analyzed by matrix assisted laser desorption ionization - time of flight mass spectrometry (MALDI-TOF MS). We then hope to be able to isolate different proteins in these two substances.
Results: Lycotoxin I seems to be present in both the venom and digestive fluid. Other than the toxin, these two substances do not seem to be very similar.
Conclusion: Lycotoxin I has been shown to have antimicrobial properties associated with them. Having found this toxin in both digestive fluid and venom in *Rabidosa rabida* we may find others with similar properties.

Cancer, Azalea B

Thursday, November 12, 1:30 p.m.-3 p.m.

1:30 p.m.- Highlighted Speaker: The Mechanism of Cancer Cell Death Mediated by Knockdown of COPZ1 Gene Encoding Coatomer Protein Complex Subunit ζ1 – New Target Gene for Cancer Therapy

Alexander V. Gasparian, David Oliver, Hao Ji, Elina Levina, Chang-Uk Lim, Eugenia Broude, Michael Shtutman
University of South Carolina, Columbia, SC

Background: Function-based genomic screening identified the coatomer protein complex ζ1 (COPZ1) gene as essential for survival of tumor cells of many origins. Depletion of COPZ1 is absolutely detrimental for both dividing and quiescent tumor cells but does not affect growth of normal cells. COPZ1 encodes a subunit of coatomer protein complex 1 (COPI) involved in intracellular protein traffic. The knockdown of COPZ1, but not of COPZ2 encoding isoform coatomer protein complex ζ2, caused Golgi apparatus collapse, blocked autophagy, and induced apoptosis in both proliferating and nondividing tumor cells. In contrast, inhibition of normal cell growth required simultaneous knockdown of both COPZ1 and COPZ2. Therefore COPZ1 depletion becomes an attractive therapeutic approach for the targeted anti-cancer therapy. Here we studied the upstream molecular mechanism of apoptosis induced by COPZ1 knockdown.
Methods: Cell lines were purchased from American Type Culture Collection (Manassas, VA). siRNA assays for all the genes were first conducted using siRNAs from Qiagen (Valencia, CA); subsequent assays incorporated additional siRNAs from Dharmacon (Lafayette, CO). Gene expression was analyzed by immunoblotting and or by qRT-*PCR. Cell death was evaluated by confocal microscopy. Apoptosis was evaluated by immunoblotting using anti-PARP antibodies (Cell Signaling Technology, Danvers, MA, cat # 9542P). JNK phosphorylation was studied by Immunoblotting using anti-P-SAPK/JNK T183/Y185 antibody (Cell Signaling Technology, cat # 4668P). Chemical inhibitors of JNK activity were purchased from Tocris Bioscience (*Bristol, BS11 0QL, UK*).* Fluorescence microscopy was used for chromatin staining with DAPI, and immunofluorescence analysis of GM130 (BD Biosciences, San Jose, CA, cat # 610822).
Results: We showed that COPZ1 knockdown-mediated COPI dysfunction leads to unfolded protein response (UPR). We hypothesized that UPR is an important step in the apoptosis induction. To test this hypothesis, we studied if down regulation of the individual molecular elements of UPR (IRE1, PERK-CHOP1/GADD153, ATF6) attenuates apoptosis induced by COPZ1 knockdown. Surprisingly, no UPR knockdowns survived better. Therefore COPZ1 knockdown induces UPR-independent apoptosis in cancer cells. We also found that COPZ1 knockdown-mediated apoptosis is attenuated by inhibition of JNK activity and connected to the mitochondria dysfunction in two different cancer cell lines.
Conclusions: COPZ1 knockdown induces UPR-independent apoptosis in cancer cells. It is attenuated by inhibition of JNK activity and connected to the mitochondria dysfunction in two different cancer cell lines. These findings open new directions in our study of the molecular mechanism of COPZ1 knockdown-mediated apoptosis. Understanding of that mechanism should help with the development of efficient anti-cancer therapy targeting both proliferating and quiescent tumor cells.
Acknowledgment: This work was funded by NIGMS grant 1P20GM109091-01, NIH grant P30 GM103336 to the Center for Colon Cancer Research at the University of South Carolina and SC INBRE pilot award.

2:00 p.m.- "Drug Designing for Prostate Cancer"

Raquema Williams, Pradip Biswas
Department of Physics, Tougaloo College, Tougaloo, MS

Prostate cancer affected 2,975,970 (43%) men in the United States this year, and killed 29,480. Nuclear hormone receptor, Androgen Receptor (AR) is the main protein responsible for prostate cancer progressions and the main targets for hormonal therapies. Based on the de-novo and acquired resistances of hormone therapy, our objective is to 1) elucidate ligand selective responses of the protein based on differences in AR-ligand hydrogen bonding contacts, 2) study protein-protein and protein-DNA interfaces of AR and identify and validate alternate targets for hormone-independent drug designing. Using protein crystal structures, molecular modeling (VMD, Swiss PDB-Viewer), molecular dynamics simulation (GROMACS), and bioinformatics (BLAST) we have explored unique sequence motifs for dimerization and DNA recognition suitable for drug designing.
For the wild type and mutated (THR-877 to ALA) AR, the hydrogen bonding pattern of DHT (an AR agonist), Flutamide (an AR antagonist), and Resveratrol analogues clearly provide the distinction between agonist and antagonist actions.
For AR ligand binding domain, the exceptionally short dimerization contact found in our modeling and simulation technique was not sufficient to act as a drug designing target. From AR-DNA complex, we found the LCAXRXD motif (578-584) is of particular interest as provides both AR-AR and AR-DNA contacts. This motif has been grafted on alanine and glutamine helices and in-silico and in-vitro testings are

in progress.
The discovery of novel protein targets and ligands from protein-protein and protein-DNA interfaces would open avenues for the development of a new generation of small molecule therapeutics for prostate cancers.
This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

2:15 p.m.- "The Cancer CURE: A Course-embedded Undergraduate Research Experience"
Lori Hensley, Jessie Little, Amy Eubanks, Rebekah Davis, RJ Quilao, Klressa Barnes, Sydney Heslep, Nathan Reyna
Ouachita Baptist University, Arkadelphia, AR

Background: A major goal of the INBRE program is to provide research opportunities for undergraduate students and serve as a springboard for these students to continue in health research careers within IDeA states. At Ouachita Baptist University, the Biology Department is committed to providing all students with opportunities to participate in authentic research experiences that teach them to evaluate complex biology problems from a variety of perspectives; we believe students must do science to learn science. Although enrollment numbers in our department prevent every biology major from performing research in a one-on-one faculty-mentored research experience, we offer each of our students the opportunity to complete a genuine research experience though course-embedded research.
Methods: We optimized protocols, made instructional videos, and expanded INBRE project leader research to create a course-embedded undergraduate research experience (CURE) that will allow thirty-eight students enrolled in our Cell Biology class to design and carry out their own cancer research projects. Course curriculum is coordinated to enhance the impact of the lab.
Results: Students chose from seven tumor types and fifteen experimental cancer drugs to design cell viability and migration assays. Using primary literature, they identified proteins likely to be differentially expressed between control and experimental groups and then assessed expression with western blotting. Cleary stated hypotheses, experimental plans with detailed lab notebooks, and data and statistical analysis were completed for each set of experiments. Some students expanded their projects by applying for funding through a student INBRE voucher system in conjunction with CORE facilities at the lead institution.
Conclusion: Assessment data from CURE labs at OBU demonstrate that students participating in these labs show significant learning gains over non-CURE students in areas such as reading scientific literature, engaging in lab projects where the outcome is unknown, collecting and analyzing data, maintaining lab notebooks, working in small groups, presenting research, and critiquing scientific work of others. Faculty and students are excited about the cancer CURE; students are expected to present their research at local and state meetings this year.
Acknowledgment: This work was funded in part by NCRR grant P20RR016460 and NIGMS grant P20 GM 103429 from the National Institutes of Health.

2:30 p.m.- "Development of New Patient-Derived Xenograft (Pdxs) Models for Accurate Assessment of New Therapeutics in Pre-Clinical Settings for Breast Cancer Patients"
Ryan J. Ice[1], Anna A. Kiseleva[2,7], Brandon C. Jones[2], Yuriy V. Loskutov[1], Mathew B. Smolkin[3], Mohamad A. Salkeni[1,6], Hannah W. Hazard[1,4], Ginger P. Layne[1,5], Sricharan Mahavadi[7], Elena N. Pugacheva[1,2]
[1]Mary Babb Randolph Cancer Center, West Virginia University School of Medicine, Morgantown, WV
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[5]Department of Radiology, West Virginia University School of Medicine, Morgantown, WV
[6]Department of Medicine, West Virginia University School of Medicine, Morgantown, WV
[7]INBRE Program, West Virginia University School of Medicine, Morgantown, WV

Background: Although advances in treating early stage breast cancers have increased the overall survival rate, once the disease has metastasized treatment options subside to palliative care. The limited access to metastatic biopsies and disease-relevant pre-clinical models to test new therapeutics targeted against advanced metastatic cancers limited progress and translation of investigational therapeutics to the clinic.
Methods: To address this deficiency we developed a collection of metastatic patient derived xenograft models via direct transplantation of metastatic biopsy or residual surgical material procured at the MBR Cancer Center, WVU or NIH/NCI supported Cooperative Human Tissue Network (CHTN). We successfully collected and established triple negative as well as ER/PR positive patient xenografts which are available for collaborative research. We further characterized and utilized the PDXs to assess the efficacy of new combination therapy to treat distant metastases.
Results: The efficacy of Aurora A kinase inhibition by small molecule inhibitor Alisertib as monotherapy and in combination with microtubule targeting drug,

eribulin, on different stages of metastasis and potential mechanisms of its action was defined. Our work using PDX models indicates that Alisertib does not limit growth of the primary tumor growth. These findings are similar to the results of clinical trials with Alisertib in breast cancer. Importantly, we found that Alisertib nevertheless dramatically decreases growth of the established metastases and prevents further dissemination via inactivation of AKT and activation of cytotoxic autophagy. Combination of Alisertib with eribulin led to a synergistic decrease in growth of metastases and mammary tumor.
Conclusion: These preclinical findings obtained using PDX models provide a new, clinically relevant understanding of the mechanisms by which Alisertib mediates its anti-metastatic effects and advocate for combination with eribulin in the future clinical trials in metastatic breast cancer and early stage solid tumors.
Acknowledgment: This work was supported by a grants from the NIH/NCI, CA148671 (to E.N.P); IDeA CTR NIH/NIGMS, U54GM104942 pilot award to E.N.P and by a NIH/NCRR (5 P20 RR016440-09 to the Mary Babb Randolph Cancer Center) award. M.S was supported by the WV-INBRE grant 5P20GM103434-13. The Animal Models & Imaging Facility was supported by the NIH grants P20 RR016440, P30 RR032138/GM103488 and S10RR026378. Some of the tissue samples for PDXs were provided by the Cooperative Human Tissue Network which is funded by the National Cancer Institute.

2:45 p.m.- Patient Advocate Video

Martin Hauer-Jensen
University of Arkansas for Medical Sciences, Little Rock, AR

While the number of cancer cases and cancer deaths in the US has remained relatively stable over the past 40 years, the number of cancer survivors has increased exponentially. Cancer survivors may be cured of their original malignancy, but many suffer from treatment-related secondary problems ranging from mild psychosocial disabilities to life-threatening physical sequelae.
This video presentation by Tranette Ledford is a powerful statement. Tranette is a writer, journalist and communications consultant with a background in print and broadcast media. She has worked in production at ABC News' Good Morning America, as a news anchor, political correspondent and reporter for CNN, and for many local television stations. Her work has been featured in the New York Times, Fox Radio News Network, Women Magazine, and elsewhere. She was diagnosed with cervical cancer in 2012 and underwent surgery and chemo-radiation therapy. She describes, from a patient's point of view, how debilitating suffering from pelvic radiation disease can be.
Acknowledgement: Supported by NIGMS of the National Institutes of Health under award number P20 GM109005.

Cardiovascular Research, Azalea C

Thursday, November 12, 1:30 p.m.-3 p.m.

1:30- Highlighted Speaker: Highlighted Speaker: "A Link Between Autoimmunity and Hypertension"

Dr. Michael J. Ryan, Associate Professor of Physiology & Biophysics, Director of Graduate Studies in Physiology, Associate Dean, School of Graduate Studies in Health Sciences, Research Health Sciences in Research Service; University of Mississippi Medical Center, Jackson, MS

Greater than 75 million in the United States have primary hypertension, or hypertension for no identifiable cause. For a variety of reasons, reducing blood pressure to prescribed clinical goals is not achieved in approximately 50% of these patients. One contributing reason is that the underlying factors causing hypertension in different populations remain incompletely understood. A growing body of literature suggests that immune and inflammatory responses are activated in hypertensive patients. More specifically, several studies show that patients with primary hypertension have increased circulating autoantibodies, thus suggesting the possibility that hypertension may have its origins in autoimmune disease. Interestingly, the prevalence of hypertension is markedly increased in patients with autoimmune disorders like systemic lupus erythematosus (SLE) and rheumatoid arthritis. Based on the association between immune system activation and hypertension, and the prevalent hypertension in patients with autoimmune disease, we have been utilizing an experimental model of SLE with hypertension to better understand the immune mechanisms that promote hypertension. This presentation will focus on the impact that immunosuppression has on the development of hypertension during SLE.

2:00 p.m.- "Acute Effects of Inhaled Iloprost on Exercise Capacity and Dynamic Hyperinflation in COPD"

Matthew R. Lammi, Brianne Aiello, Jessica Johnson PharmD BCBS, Mohamed Ghonim, Kusma Pyakurel, Johnny D'Aquin, John B. Zamjahn PhD RRT RPFT, Andrew Pellett PhD, Connie Romaine APRN, NP-C, Marie C. Sandi FNP-BC, Judd Shellito, A. Hamid Boulares, Bennett deBoisblanc
The University of Mississippi Medical Center, Jackson, MS

Rationale: Dynamic hyperinflation (DH) is the primary cause of exercise intolerance in COPD. Inhaled prostanoids have the potential to improve V/Q matching and thereby reduce dead space, minute ventilation and DH. We hypothesized that inhaled iloprost would decrease DH and improve exercise capacity in COPD patients, irrespective of concomitant pulmonary hypertension.
Methods: Patients with moderate to severe COPD were randomized in a blinded crossover design to receive inhaled iloprost (5μg) or matching saline placebo by AeroNeb 30 minutes prior to a symptom-limited, maximal cardiopulmonary exercise test (CPET). Arterial blood gases and inspiratory capacity (IC) were measured every 2 minutes. End-expiratory lung volume (EELV) ÷ total lung capacity (TLC), a measure of DH, was derived and compared between the iloprost and placebo CPETs at matched metabolic isotimes (based on $\%VCO_2max$). Alveolar dead space fraction (VD_{alv}/VT) was calculated as ($PaCO_2$-$PETCO_2$)/$PaCO_2$. Treatment variables were compared using paired t-tests and are expressed as mean+SD.
Results: Seven patients were enrolled (age 56±8 years, 62.5% female). FEV_1 and residual volume were 45.5±9.3% and 170±43% predicted, respectively. Estimated echocardiographic pulmonary artery systolic pressure was 31±11mmHg. EELV/TLC was similar following iloprost versus placebo at rest but was significantly lower following iloprost at $75\%VCO_2max$ (0.67±0.06 vs. 0.71±0.07, respectively p=0.03). Furthermore, symptom-limited exercise duration was significantly longer (682±149 vs. 626±177 seconds, p=0.03) and peak VO_2 was significantly higher (20.3±10.3 vs. 17.6±9.0 mL/kg/min, p=0.003) following iloprost versus placebo. There was no significant difference in Borg dyspnea score or oxygenation at rest. However, the change in oxygenation during exercise was improved after iloprost compared to placebo (change in PaO_2 from pre-exercise to end-of-exercise: 22.6±17.6 vs. 12.3±5.5mmHg, p=0.004; change in A-a gradient: -22.2±15.2 vs. -11.6±7.4mmHg, p=0.06). VD_{alv}/VT was significantly lower after iloprost at $50\%VCO_2max$ (0.04±0.08 vs. 0.13±0.08, p=0.03) and $75\%VCO_2max$ (0.05±0.07 vs. 0.10±0.08, p=0.03).
Conclusions: In this preliminary study of COPD patients, inhaled iloprost led to a reduction in DH and alveolar dead space, increased exercise time and peak VO_2, and improved oxygenation. If supported by subsequent data, inhaled prostanoids could become a novel treatment strategy for patients with COPD independent of the presence of pulmonary hypertension.

2:15 p.m.- "Scientific Bumps in the Night: The Cross of *Apoe* and *Treml1* Null Mice Unique Link Between Cardiovascular Disease, Diabetes, and Obesity"

A. Valance Washington, Marieli González-Cotto, Carlos J. Collado, Elizabeth Castro-Rivera, Amanda Pacheco, Fiorella Reyes, Mayra Báez, Adriana Rivera-Dompencial, Deborah Marrero, Gerónimo Maldonado-Martínez, Robert Hunter
[1]University of Puerto Rico, Rio Piedras, PR
[2]University of Central del Caribe, Santa Juanita, Bayamon, PR

Background: Atherosclerosis is a chronic inflammatory process of the vessel wall driven by inflammation. While the function of macrophages in atherosclerosis is established, the role of platelets is less defined. The receptor Triggering Receptor Expressed on Myeloid Cells (TREM)-like transcript (TLT)-1 is prepacked in platelet a-granules and brought to the surface upon activation. TLT-1 has both membrane-bound and soluble forms (sTLT-1) and the latter has been found to enhance platelet activation as well as platelet-endothelial cell interactions. High levels of sTLT-1 found in sepsis patients led to our demonstration that TLT-1 is a key regulatory molecule in the interface between the hemostatic and inflammatory systems. Patients with coronary artery disease also have elevated sTLT-1 levels, and in light of this, we hypothesized that TLT-1 plays an important role in atherosclerosis progression.
Methods: To address our hypothesis, we generated apoe$^{-/-}$/*treml1*$^{-/-}$ double knockout mice [DKO] and evaluated atherosclerotic progression in these mice compared to control mice. We used combined techniques of histology, immunohistochemistry, flow cytometry, ELISA, Single Nucleotide Polymorphism analysis, and collagen flow chambers.
Results: We found that DKO mice fed an atherogenic diet (HFD) showed significantly increased weight gain when compared to apoe$^{-/-}$. Accordingly, DKO mice showed increased triglycerides compared to controls. Surprisingly, assessment of lesion size revealed that DKO mice have significantly smaller lesions in the aortic sinus at four and 12 weeks after HFD compared to apoe$^{-/-}$ mice. These surprising differences opened up the possibility of differences in the microbiome and to address this question we evaluated the development of atherosclerosis in 8 week old littermates. We found that DKO mice maintained the high cholesterol levels and smaller lesion sizes compared to their littermate *treml1* heterozygote counterparts thus lowering the possibility of any role for microbiome differences. Interestingly, diet seems to play a role in TLT-1 regulation of cholesterol levels. Mice fed a chow diet for 26 weeks also had smaller lesion sizes, however had significantly lower levels of cholesterol defining a role for TLT-1 in mediating dietary control on fatty acid metabolism. Evaluation of platelet activation in the periphery of these mice demonstrated lower platelet activation in the DKO mouse which may account for the differences in lesion progression and suggests that blockage of TLT-1 may inhibit platelet binding to the endothelial cell surface. We tested a TLT-1 antibody and demonstrate that the anti-TLT-1 significantly inhibits platelet adhesion to a collagen-coated surface under flow.
Conclusions: Using an *apoe/treml1*$^{-/-}$ mouse model we uncovered a novel connection of TLT-1 to obesity and through single nucleotide polymorphism (SNP) analysis identified glucose regulation as the potential underlying mechanism of obesity. We further demonstrate that our obese mice have smaller aortic lesions and used a single chain antibody against TLT-1 to demonstrate that TLT-1 is a viable therapeutic target in cardiovascular disease.
Acknowledgment: Support for this project was obtained through grants 2P20GM103475, G12RR-03035, 8U54MD007587-03, and 1R01HL090933-01A2.

2:30 p.m.- "Differential Expression of Tumor Necrosis Factor-Alpha Type 1 and Type 2 Receptors in the Renal Tissue During Chronic High Salt Intake and Angiotensin II Treatment"

Dewan S. A. Majid, Minolfa C. Prieto, Alexander Castillo
Department of Physiology, Hypertension & Renal Center of Excellence, Tulane University School of Medicine, New Orleans, LA

Background: Tumor necrosis factor-alpha (TNF-α) production, stimulated by angiotensin II (AngII) and high salt (HS) intake, has been implicated in salt sensitive hypertension (SSH) though the mechanism is not yet clearly defined. TNF-α exerts natriuresis that is mediated by its' receptor type 1 (TNFR1) while its' receptor type 2 (TNFR2) is involved in mediating inflammatory renal injury. In this study, we examined a differential role for TNFR1 and TNFR2 in AngII induced SSH and associated renal injury.
Methods: We have assessed protein expressions of TNFR1 and TNFR2 in mice (n=6-7 in each group) chronically treated with or without AngII (25 ng/min; implanted minipump) for 4 weeks which were fed either normal (NS; 0.4% NaCl) or high salt (HS; 4% NaCl) diets. Systemic blood pressure (SBP) in these mice was measured by tail-cuff plethysmography and 24 hour urine collections were made using metabolic cages at the start and at the end of treatment period when the kidneys were harvested after sacrificing the mice with euthanasia. Immuno-histochemical analysis of TNFR1 and TNFR2 proteins in renal slices was performed by measuring the staining area as well as the intensity of receptors' immunoreactivities using NIS Elements Software (Nikon), which allowed the semi-quantitation of positive staining and the intensity of these proteins. The finding in protein expressions

are expressed in percent area of positive staining and the relative intensity.
Results: HS intake alone did not alter mean SBP (HS; 77±1 vs NS; 76±3 vs mmHg) but it caused an exaggeration of AngII induced increases in mean SBP (AngII+HS; 104±2 vs AngII+NS; 95±2 mmHg). The area of TNFR1 staining was higher in HS (6.0± 0.9 vs 3.2 ±0.7%; P<0.05) than NS group. However, such TNFR1 staining was rather slightly lower in HS group (5.0± 0.7%) than that in NS group (6.3±0.7%) during chronic AngII treatment. Similar qualitative differences were also observed in relative intensity in protein expressions. The visual score of TNFR2 immunoreactivity was minimal in NS (1.5± 0.2%) and HS (1.5± 0.1%) groups but it was high in AngII+NS group (2.2± 0.1%; P<0.001 vs NS group) and even greater in AngII + HS group (2.7± 0.2%; P<0.01 vs AngII+ NS group).
Conclusions: These data suggest that the increases in TNFR1 activity due to HS alone facilitate salt excretion that results no change in SBP in response to HS intake. However, such HS induced increases in TNFR1 activity was compromised in elevated AngII condition causing more salt retention and thus, an exaggerated hypertensive response. On the other hand, HS induced increases in TNFR2 activity in elevated AngII condition facilitates an enhancement in renal injury response.
Acknowledgment: This work was funded by an Institutional Development Award (IDeA) from the NIGMS under grant number P30GM103337 and Tulane University Bridge Fund (to D.S.A.M.)

2:45 p.m.- "Decreased Survival Rate in Female Obese Leptin Receptor Mutant Dahl Salt-Sensitive Rats that Develop Chronic Kidney Disease"
Jan M. Williams, Kasi McPherson, Devin Guillory, Lateia Taylor, Denisha Spires, Ashley C. Johnson, Michael R. Garrett
Department of Pharmacology, University of Mississippi Medical Center, Jackson, MS

Background: Obesity contributes to sex differences in the risk for chronic kidney disease (CKD) in which males tend to develop CKD earlier in life than females. Therefore, in the current study, we examined whether there were sex differences in the development of CKD in the obese leptin receptor mutant Dahl salt-sensitive (SS^{LepR}mutant) strain which was derived from Zinc-finger nucleases.
Methods: Experiments were performed on 6 week-old SS and SS^{LepR}mutant rats. Arterial pressure (via tail-cuff) and protein excretion were collected every 4 weeks until the rats reached 18 weeks of age. To determine insulin resistance we performed intraperitoneal glucose tolerance tests (IPGTT) using 2g/kg dose of glucose at 6, 12, and 18 weeks of age. At the end of the protocol, measurements of plasma concentrations of leptin, cholesterol, triglycerides, and creatinine were determined.
Results: We observed an increase in body weight in both female and male SS^{LepR}mutant rats when compared to SS rats throughout the study. Glucose tolerance was impaired significantly in female and male SS^{LepR}mutant rats versus SS rats by 18 weeks of age. The SS^{LepR}mutant strain also developed hyperinsulinemia in comparison to their lean SS counterparts (6.86±0.83 vs 0.72±0.04 ng/mL, respectively, n=6). However, blood glucose in the SS^{LepR}mutant strain remained within normal range throughout the course of the study regardless of sex. Female and male SS^{LepR}mutant rats developed severe systolic hypertension by 18 weeks of age when compared to the values measured in SS rats (199±7 and 201±10 vs. 159±5 and 155±4 mmHg, respectively, n=6). Yet, the rise in arterial pressure occurred earlier in female SS^{LepR}mutant rats than males. Protein excretion was significantly higher in the SS^{LepR}mutant strain as opposed to the values observed in SS rats at 18 weeks of age regardless of sex (488±61 and 631±86 vs. 50±17 and 149±23 mg/day, respectively, n=6). At the end of study, kidneys from the SS^{LepR}mutant strain displayed increased glomerulosclerosis and interstitial fibrosis than SS rats. Female and male SS^{LepR}mutant rats had a significant increase in plasma creatinine levels and averaged 2.1±0.4 mg/dL (n=6) compared to the normal value of 0.5±0.1 mg/dL (n=6) observed in the SS strain suggesting the presence of severe CKD. While both, female and male, SS rats survived the length of study, the survival rate of female SS^{LepR}mutant rats was markedly reduced compared to their male counterparts (62%, 21 of 34 vs. 25%, 6 of 24, respectively).
Conclusion: Overall, these data indicate that the SS^{LepR}mutant strain may be a useful model to study sex differences during the development of CKD associated with obesity.
Acknowledgement: This research supported by NIGMS NIH P20GM104357 and AHA 12SDG9440034 (to J.M.W).

Cell Signaling, Azalea D

Thursday, November 12, 1:30 p.m.-3 p.m.

1:30 p.m. Highlighted Speaker: "Shoc2 Scaffold-Matchmaker of Intracellular Communications in Time and Space"
Emilia Galperin
University of Kentucky, Lexington, KY

Enzyme-binding scaffolds organizing the macro-molecular signaling assemblies represent a considerable portion of proteins in cells. These scaffolding proteins guide the spatial organization of the signaling enzymes and the flow of molecular information. However, mechanisms that control assembly and dynamics within scaffolding complexes remain largely unknown. We have unraveled a novel, multi-level paradigm in which allosteric modifications alter the ability of the scaffold protein Shoc2 to actively accelerate transmission of ERK1/2 signals.
Shoc2 is the scaffold protein that accelerates the ERK1/2 signaling pathway in response to growth factors. This evolutionarily well-conserved protein is a critical regulator of the ERK1/2 signaling pathway. Mutations in Shoc2 result in Noonan-like RASopathy, a developmental disorder with a wide spectrum of symptoms. Shoc2 integrates the Ras and RAF-1 components of the ERK1/2 pathway into a multi-protein complex. The amplitude of the ERK1/2 signals transduced through the complex is fine-tuned by the E3 ligase HUWE1. HUWE1-mediated inducible ubiquitination of Shoc2 and its signaling partner RAF-1is utilized as a posttranslational modification to attenuate the amplitude of the ERK1/2 signaling. The mechanism that controls ubiquitination of Shoc2 and RAF-1 involve the (AAA+) ATPase PSMC5. PSMC5 is an integral part of the Shoc2 complex that triggers translocation of the Shoc2-Ras-RAF-1 signaling complexes to endosomes. There PSMC5 displaces the E3-ligase HUWE1 from the scaffolding complex to attenuate ubiquitination of Shoc2 and RAF-1. Noonan-like Rasopathy mutation that changes the subcellular distribution of Shoc2 leads to alterations in Shoc2 ubiquitination due to the loss of accessibility to PSMC5. In summary, our studies demonstrate that proteins of the ubiquitin machinery are intimately involved in regulating the ERK1/2 signal transmission through the remodeling of the Shoc2 scaffold complex in a spatially-defined manner. Our studies describe a novel mechanism of how scaffolds can regulate specificity and dynamics of cellular networks through remodeling mechanisms and make a significant advancement in our understanding of how the ERK1/2 signaling pathway is governed by the critical scaffold Shoc2.
Acknowledgments: This project was supported by funds from NCI (R00CA126161 to EG), NIGMS (P20GM103486) (formally supported by the Center for Research Resources), NIGMS (R01GM113087 to EG), the American Cancer Society (RSG-14-172-01-CSM to EG) and from the American Heart Association (15PRE25090207 to HJ).

2:00 p.m.- "Effective Gene Knockout of NPC1L1 in *Danio rerio* using a CRISPR/Cas9 Endonuclease System"
Benjamin Duncan, Christian Pritchard, James Walters
Bluefield State College, Bluefield, WV

Background: How dyslipidemias such as obesity and diabetes are impacted by intestinal absorption of lipids is currently under debate. While dietary lipids impact whole-body lipid homeostasis, how NPC1L1, the main cholesterol transporter of intestinal enterocytes, functions in this process is unclear. To study how NPC1L1 variants impact enterocyte regulation of cholesterol uptake we are establishing an *npc1l1-/-* line in zebrafish using CRISPR. We hypothesize that a subsequently introduced high absorption variant of NPC1L1 will show increased cholesterol uptake when compared to wild-type larvae.
Methods: The CRISPR targeting plasmid was assembled in a one-step digestion/ligation reaction and produced the Cas9 mRNA. Two plasmids containing the Cas9 protein and an *npc1l1* gRNA target sequence were injected into single cell embryos.
Results: After injection, the gRNA and newly made Cas9 protein should combine to form the Cas9 duplex. We will screen for frame shift mutations resulting in early stop codons within the *npc1l1* coding sequence.
Conclusion: The CRISPR method of producing frame shift mutations is an established and efficient method for creating knock-outs in zebrafish and can be utilized to establish a line of *npc1l1-/-* (null background) zebrafish. Future experiments will expose *npc1L1-/-* and TL (wild-type) strains to compare how cholesterol absorption is affected by low and high fat diets.
Acknowledgements: Supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence.

2:15- "Identification and Development of Casein Kinase 1 Inhibitors as Potential Therapeutics in the Treatment of Alzheimer's Disease"
Richard Schroeder, Navneet Goyal, Phan Tram, Treasure Stone, Kathy Nguyen, Melyssa Bratton, Elena Skripnikova, Jayalakshmi Sridhar
Xavier University of Louisiana, New Orleans, LA

Background: Alzheimer's disease (AD) is a progressive neurodegenerative disorder associated with the accumulation of the neurotoxic peptide Amyloid β (Aβ). The casein kinase 1 family comprises eight isozymes, two of which (CK1δ and CK1ε) are predominantly expressed in the brain. CK1δ plays a

critical role in AD through phosphorylation of tau, a protein associated with microtubules, which precedes neuritic lesion formation, implicating CK1δ in the tau fibrillization reaction pathway. CK1δ has been reported to be associated with pathological accumulation of tau in several neurodegenerative diseases including AD, Down syndrome, progressive supranuclear palsy, and parkinsonism dementia complex of Guam.
Methods: Initial computational screenings and molecular docking studies were performed using the kinase domain of the CK1δ isozyme. The top candidates from the docking studies were subjected to preliminary high-throughput screenings. A lead structure was identified and several promising novel compounds containing the lead scaffold were synthesized.
Results: Our investigation of quinones as kinase inhibitors revealed a quinone compound that inhibited CK1δ and Pim1 kinase preferentially over CK1γ2 and 98 other human protein kinases. Similarity searches and preliminary in-vitro CK1δ kinase inhibition assays yielded a few compounds with good potency. We have identified several additional naphthoquinone structures exhibiting significant kinase inhibition potency in CK1δ.
Conclusion: Inhibition of CK1δ has been shown to reduce fibrillar lesions and to inhibit Aβ production associated with Alzheimer's disease. Initial in-vitro CK1δ kinase inhibition assays identified a few compounds with good inhibition potency. We have developed several additional compounds exhibiting excellent kinase inhibition potency in CK1δ.
Acknowledgment: Support from the National Institutes of Health through the LBRN/NIGMS pilot grant [8P20GM103424] is gratefully acknowledged. Additional support through the RCMI core facilities and startup grant is provided in part by the Louisiana Cancer Research Consortium (LCRC), grant [8G12MD007595] from the NIH/NIMHD/ DHS and DoD comprehensive grant [BC102922] is acknowledged. We also thank the Center for Undergraduate Research program at Xavier University of Louisiana for student funding of Pham Tram.

2:30 p.m. "A Proteomics Approach to Delineate the Role of C/Ebpδ in Ionizing Radiation-Induced Oxidative Stress"

Snehalata A. Pawar[1], Sudip Banerjee[1], Stephanie D. Byrum[2], Lisa Orr[2], Alan J. Tackett[2], Usha Ponnappan[3], Martin Hauer-Jensen[1, 4]
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[2]Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR
[3]Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR
[4]Surgical Services, Central Arkansas Veterans Healthcare System, Little Rock, AR

Background: CCAAT enhancer binding protein delta (*Cebpd*, C/EBPδ) is a transcription factor implicated in the regulation of oxidative stress, DNA damage response and inflammation. Although these processes are implicated in response to ionizing radiation (IR), the involvement and role of C/EBPδ in this context, remains to be delineated. The involvement of C/EBPδ in IR, was first demonstrated by studies from our laboratory where in *Cebpd*-deficiency sensitized mice to IR-induced lethality. The molecular mechanism of C/EBPδ-mediated protection against radiation injury and lethality are yet to be elucidated.
Our preliminary studies reveal that C/EBPδ modulates oxidative stress and IR-induced mitochondrial dysfunction. Using a proteomics approach we have now identified alterations in protein expression that are dependent on C/EBPδ and downstream targets that may play a role in protection from-IR-induced oxidative stress.
Methods: *Cebpd*-knockout (KO) and *Cebpd*-wild type (WT) mouse embryonic fibroblasts were exposed to 2 Gy and harvested at 0 and 24h post-irradiation and whole cell lysates were prepared (n=1/ treatment/timepoint). Proteins from total cellular lysates were normalized, resolved by SDS-PAGE, and visualized with Coomassie blue. Each of the 4 gel lanes was entirely sliced into 17 equal bands, subjected to trypsin digestion, and peptides were analyzed by LC-MS/MS with a Thermo LTQ Orbitrap Velos mass spectrometer equipped with a Waters nanoACQUITY LC system. Proteins and post-translational modifications were identified by searching Mascot (95% confidence threshold). To determine significantly differential levels of protein between the KO and WT MEFs, a label-free approach based on spectral counting was used. Proteins that showed up or down regulation (> 2-fold) in expression following IR from WT or mutant mice were subjected to IPA analyses (Ingenuity Pathway Analysis) and further validated using Western blotting and or real-

time PCR.
Results & Conclusions: We identified a total of 2666 proteins (0.45% false discovery rate using a decoy database from 135,904 spectra). Of note targets involved in glutathione biosynthesis and antioxidant enzymes were significantly down-regulated in irradiated KO MEFs. Results from these studies will be presented, identifying the novel C/EBPδ targets and pathways following exposure to IR and those involved in modulating IR-induced oxidative stress.
Acknowledgment: This work was funded by Centers of Biomedical Research Excellence (COBRE)-Center for Host Responses to Cancer Therapy at UAMS from NIGMS under grant number P20GM109005.

2:45 p.m.- "Polyaniline Coating on Borosilicate Glass as a Fluorescent Sensing Platform for Detection of DNA Mimic to miRNA *let-7*"
Partha Pratim Sengupta, Beddhu Murali, Alex S. Flynt
Department of Biological Sciences, University of Southern Mississippi, Hattiesburg, MS

Micro-RNAs (miRNA) are an exciting focus of clinical study not only for targeted therapies but also as biomarkers. Among potential miRNA cancer biomarkers the most broadly useful is *let-7*. Conducting polymers (CPs) are polyconjugated, charged species which are highly sensitive to changes in the polymeric chain environment and other perturbations in the chain conformation that could be caused by nucleic acid chemistry. Here we used changes in the delocalized electronic structure are manifested in altered optoelectronic properties of CPs to sense miRNA molecules. In this research work we have synthesized processable CP polyaniline (PANI) in situ blended with polyethylene glycol (PEG) as a transducer element for miRNA sensing. PEG encapsulates the PANI molecule as observed in Scanning Electron Microscopy (SEM). 22 nucleotide DNA which complements *let-7* is immobilized to PANI coated on borosilicate glass cover slip and then subjected to UV irradiation to enhance the electrostatic bonding between PANI and 5′phosphate group of DNA. The borosilicate glass was irradiated with H_2O-UV radiation to lower the surface contact angle and having a uniform coating with PANI-PEG blend. The change in fluorescence property of PANI by oligo immobilization and hybridization with mimic let-7 is measured by fluorescence microscope and the image analyzed by MATLAB. A heuristic algorithm determines color threshold of the fluorescent active image. This image segmentation helps to determine the average pixel intensity representing the active image foreground of PANI fluorescence triggered by DNA immobilization and hybridization process. This would help us to quantify response of PANI based biosensor for detecting miRNA *let-7*.
Keywords: PANI-PEG blend, fluorescence property, 22 nucleotide DNA, H_2O-UV irradiation, hybridization

Grants Administrators'/ Program Managers' Workshop, Magnolia E, F, G, H

Thursday, November 12, 1:30 p.m.-3 p.m.

1:30 p.m."Uniform Guidance – For The Research Administrator And Researcher"
Dr. Denise Clark, Associate Vice President for Research Administration; Dr. Ann Holmes, Assistant Dean, Finance & Administration for the College of Behavioral & Social Sciences, University of Maryland, College Park, MD

This workshop will focus on the recently approved Uniform Guidance from the Office of Management and Budget, which pertains to Administrative Requirements, Cost Principles, and Audit Requirements for Federal Awards. This guidance was developed in response to the November 23, 2009 Executive Order 13520 on *Reducing Improper Payments* and the February 28, 2011 Presidential Memorandum, on *Administrative Flexibility, Lower Costs, and Better Results for State, Local, and Tribal Governments*. Federal agencies were required to implement these requirements by December 26, 2014. COBRE and INBRE PIs are strongly encouraged to have their grant administrator/program manager attend this workshop and become more familiar with the federal requirements and policies that oversee our NIH-IDeA awards. In addition, this workshop offers an opportunity for our administrators/managers to network and establish relationships that will serve or programs by opening lines of communications as we expand collaborations within the IDeA program.

Science Commercialization Workshop, Magnolia E, F, G, H

Thursday, November 12, 3:15 p.m.-6 p.m.

3:20-4:05 p.m.- "Investing in Innovation: The HHS SBIR/STTR Programs"
Dr. Matthew Portnoy
NIH SBIR/STTR, Office of Extramural Programs, NIH, Bethesda, MD

The NIH SBIR and STTR programs offer many opportunities for small companies to do innovative research in the life and health sciences. In this session, Dr. Matthew Portnoy, NIH SBIR/STTR Program Manager, will provide a general overview of the programs touching on topics such as eligibility requirements, the NIH SBIR/STTR budget, submission, review, when and how to communicate with the NIH staff, Phase III assistance programs, etc. Flexibility is key to the success of NIH's programs and along with that comes many nuances (both programmatically and procedurally) for which applicants must be aware. The times are ever changing and NIH is no exception as it implements new policies and procedures, so don't miss out on the latest updates.

4:05-4:20 p.m.- "Entrepreneurship And Commercialization: Building An Engine Of Innovation Translation In WV"
Mr. Richard Giersch, Director of Life Science Innovation, Entrepreneurship and Commercialization, West Virginia University, Morgantown, WV

The path forward for innovation, entrepreneurship, and commercialization at West Virginia University is collaborative, regional, and networked. Over the past two years WVU has evaluated its internal resources, regional strengths, and statewide needs to build a pool of resources to translate academic invention and innovation to the populations who need it the most. The reasons we began this process, our current state of development, and path forward will be presented as well as thoughts for how to replicate this model in other areas.

4:20-4:35 p.m.- "SBIR and STTR Projects Carried Out By ElSohly Labs"
Mahmoud A. ElSohly
ElSohly Laboratories, Inc., Oxford, MS

Background**:** ElSohly Laboratories, Incorporated (ELI) is a privately held Mississippi Corporation certified by DHHS and the College of American Pathologists and registered with DEA and FDA, which has been offering analytical and advisory services to the drug-testing community since 1985. Company is actively working on drug development and has more than 20 issued patents, many of which are currently licensed to different companies.
Methods: National Institute of Health (NIH) granted Phase I and II to complete these projects. Below are two projects to describe as examples.
Results: Development of a product for desensitization to contact dermatitis caused by poison ivy and other plants of the family Anacardiaceae. We have developed compositions that when administered parentally produced a high degree of desensitization. These products are currently in clinical development. The development of these agents will address a major health problem in the United States where contact dermatitis causes significant problems to outdoor workers and forest service individuals causing loss of productivity.
Products of Δ^9–tetrahydrocannabinol (THC) for enhanced bioavailability has been developed for use as suppositories, transmucosal, delivery systems (oral mucosal patches) and eye drops for the treatment of glaucoma. These products are now licensed to a pharmaceutical company for development.
Conclusion: These examples prove that by securing the grants from NIH for phase I, and II enable small companies to advance in drug development and proceed to clinical development of their products.
Acknowledgment: This work was funded by National Institutes of Health, grants No. 1R41AR053395-01, 2R42AR053395-02A1, 5R42GM067304-03 and 1R41EY020042-01.

4:45-5:00 p.m.- "Technology Transfer and Science Commercialization at Tulane University: History, Lessons Learned, Plans for the Future"
John M. Christie
Tulane University, New Orleans, LA

Founded in 1834, Tulane University is one of the most highly regarded and selective independent research universities in the United States. A member of the prestigious Association of American Universities, we take pride in being a part of this select group of universities with "pre-eminent programs of graduate and professional education and scholarly research." Our schools and colleges offer undergraduate, graduate and professional degrees in the liberal arts, science and engineering, architecture, business, law, social work, medicine and public health and tropical medicine. The university is ranked by the Carnegie Foundation for the Advancement of Teaching as a university with "very high research activity." Of more than 4,300 higher educational institutions rated by the foundation, Tulane remains in a prestigious category that includes only 2 percent of universities nationwide. Technology transfer at Tulane University moves cutting edge research into the broader community, educates external constituencies about the development and implications of the research and sees that the products of Tulane research are

deployed to the broadest possible public benefit. Technology transfer, while viewed simply as a potential revenue source at some research institutions, has long been considered an important extension of Tulane University's core missions of education, research and service.
Technology transfer began in earnest at Tulane well before the formal establishment of an office dedicated to the function. Key researchers in the School of Medicine sought patent protection for their inventions beginning in the early 1980s, and worked proactively with industry to develop these inventions and bring them to market. Since that time, the Office of Technology Transfer and Intellectual Property Development has successfully moved the fruit of Tulane researchers into industrial collaborations aimed at bringing new technologies to market to deliver meaningful public benefit.
Today's Office of Technology Transfer and Intellectual Property Development is developing inventions from faculty and trainees across the university, at present including inventions from the School of Medicine, the School of Science and Engineering, the School of Public Health and Tropical Medicine, and the School of Law. Broad vision, a wealth of experience and institutional support at the highest levels ensure that Tulane will remain a leader in university technology transfer well into the future.
The Office of Technology Transfer is working diligently with faculty and students to develop their own small companies around their innovations. Tulane launched its inaugural Novel Technology Challenge in the fall of 2014 and the second Challenge is underway now. The School of Science and Engineering is developing a MakerSpace to support the Challenge and similar efforts. Additionally, the Office of Technology Transfer is working with the School of Medicine to develop its own internal business plan competition, in response to strong student interest.
The depth and breadth of Tulane's achievements in this area is rare for a university of this size. Tulane's technology transfer success falls into several broad areas: Peptide chemistry, Diagnostics for Infectious Diseases, Vaccines for Infectious Diseases, and Medical Devices.

5:00-5:15 p.m.- "The Road Towards Translation of MTI-101 for the Treatment of Relapsed Myeloma"
M. F. Emmons, N. Anreddy, M. L. McLaughlin, L. A. Hazlehurst
The University of West Virginia Morgantown, WV

Background: Multiple myeloma is a disease that initially responds to standard of care agents. However, unfortunately the emergence of drug resistance continues to limit the success of chemotherapy. MTI-101 is a first-in-class peptidomimetic that binds a CD44 containing complex and triggers necrotic cell death in multiple myeloma cell lines. Importantly, MTI-101 is more active in specimens obtained from relapsed myeloma patients compared to those obtained from newly diagnosed patients. We previously showed that MTI-101 has activity as a single agent and is synergistic with the standard of care agent bortezomib using *in vitro* and *in vivo* models. Here we report that MTI-101 provokes a robust and sustained increases in intracellular Ca^{2+} levels in myeloma cell lines leading to necrotic cell death.
Methods: We used an isogenic drug resistant model, coupled to gene expression profiling (GEP) to identify genes that may contribute to MTI-101 sensitivity. We observed significant changes in genes predicted to regulate Ca^{2+} homeostasis. These findings were repeated using real time RT-PCR. Pharmacological inhibition of Ca^{2+} channels was used to probe the MTI-101 induced increase in intracellular Ca^{2+} levels
Results: MTI-101-resistant myeloma cells express markedly reduced levels of the IP3 receptor isoform 3 (IP3R), phospholipase C (PLC)-β, and the plasma membrane Ca^{2+} channels TRPC1 and TRPM7. Consistent with a role for both Ca^{2+} release and Ca^{2+} entry in mediating cell death pre-treatment with either the pan PLC (U73122) or the store operated channels (2-APB) blocks the robust increase in intracellular Ca^{2+} levels and cell death. Additionally, treatment with MTI-101 induces oligomerization of Stim1 a prerequisite for Ca^{2+} entry via store operated channels.
Conclusion: MTI-101 induced cell death is in part mediated by inducing Ca^{2+} overload via induction of a PLC-IP3R-TRPC1/ORAI1 induced increase in intracellular Ca^{2+} levels. Previous reports have indicated that Ca^{2+} entry is important for progression of cancer. We proposed that targeting this same pathway using an agonist is a tractable strategy for inducing Ca^{2+} overload in aggressive tumors.
Acknowledgment: This work was funded in part by GM104942 West Virginia IDEA-CTR, RO1 CA195727-01 (LAH), Miles for Moffitt (LAH)

General Biomedical Science, Azalea A

Friday, November 13, 9 a.m.- 10:30 p.m.

9:00 a.m.- Highlighted Speaker: "Chromatin Structural Changes In Gene Regulation"
Dr. Yvonne Fondufe-Mittendorf, Assistant Professor, Molecular & Cellular Biochemistry, University of Kentucky, Lexington, KY

Background: The positioning of nucleosomes with respect to the DNA sequence plays an important role in regulating transcription. Studies in yeast reveal that the overwhelming majority of nucleosome positions are encoded in the genome by specific signals that favor or disfavor nucleosome positions. We recently showed that this code exist in archaeal genome and must have evolved together with the packaging of the DNA into the nucleus. However, the extent to which nucleosome occupancy and dynamics is modulated to regulate specific gene expression patterns is limited. Moreover, the extent to which chromatin architectural proteins binding at the entry/exit of the nucleosome dyad, regulate nucleosome positioning and stability is not known.
Methods: We study how chromatin architectural proteins, H1, MeCP2, HMGD1 and PARP1 regulate chromatin dynamics in transcription reg
Results: We show that CAPs bind to specific genomic locations correlating with their functional transcriptional outcomes. We also show that CAPs control specific chromatin structures that play a role in in not only transcription regulation but also how the genes are ultimately spliced.
Conclusion: This study shows that the CAPs regulate chromatin structures, and thus are active participants in regulating chromatin-mediated gene regulation. Such information is critical in the functional understanding of chromatin structural organization during gene regulatory processes.
Acknowledgment: This work was funded by NIEHS Grant **1R01ES024478-01A1**; NSF **1517986**, IRSF (to Y.N.F-M) and by an Institutional Development Award (IDeA) from the NIGMS under grant number 8P20GM103486-09.

9:30 p.m.- "Changes in Responses to Diesel Exhaust Particles from Bronchial Epithelial Cells of Asthmatic and Non-Asthmatic Subjects"
Loyda B. Méndez, Rochely Luna, Wilmarie Torres, Ceidy Torres
Universidad del Este, Carolina, PR

Background: It is well known that asthmatic individuals are at a greater risk for the respiratory effects of particle pollution. However the molecular mechanisms that render asthmatic individuals more susceptible to the adverse effects of particle pollution have not been fully elucidated. Therefore the main objective of this study is to determine exposure-dose relationships of PM cytotoxic effects in asthmatic and non-asthmatic bronchial epithelial cells in order to gain insights about possible mechanisms of susceptibility. Our data shows that asthmatic cells are more prone to undergo into apoptosis than non-asthmatic cells.
Methods: Primary human bronchial epithelial cells derived from asthmatic (AHBE) and non-asthmatic (NHBE) individuals were acutely exposed (20 min to 480 min) to different concentrations of diesel exhaust particles (DEP; 0 to 25 µg/mL). Physiologically relevant concentrations were calculated with the multiple-path particle dosimetry model using the human age-specific symmetric model for ages 8 to 9. Multiple endpoints of cell viability and cytotoxicity, including metabolic activity and apoptosis, were measured within the same well 24 hours after initial exposure.
Results: Dose-response analysis revealed that (AHBE) cells were more prone to undergo into apoptosis than NHBE cells. In addition, AHBE cells were more metabolically active than NHBE, as assessed by the alamar blue assay. A positive correlation (r = 0.84 to 0.94) was observed between metabolic activity and caspase-3 activity in AHBE cells suggesting that cellular metabolism impairment might render AHBE more susceptible to the effects of DEP.
Conclusion: This study shows that asthmatic cells respond different than non-asthmatic cells when exposed to the same doses of DEP, a main constituent of urban particle pollution. Differences in cellular responses might explain the susceptibility of asthmatic individuals to the adverse respiratory effects of particle pollution. Overall, our results provide information about the biological mechanisms underlying the adverse effects of particle pollution in asthmatic individuals.
Acknowledgment: This work was funded by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM103475.

9:45 p.m.- "Role of Suppressor of Cytokines Signaling 3 (SOCS3) in Modulating Chronic Metabolic and Cardiovascular Effects of Leptin"
Jussara M. do Carmo, J. Nathan Freeman, Alexandre A. da Silva, Zhen Wang, John E. Hall
Department of Physiology & Biophysics, Mississippi Center for Obesity Research, University of Mississippi Medical Center, Jackson, MS

Background: Suppressor of cytokine signaling 3 (SOCS3) is a negative regulator of leptin signaling. Hypothalamic SOCS3 is upregulated in obesity and has been suggested to contribute to development of resistance to leptin's anorexic effects. In this study we determined whether deletion of SOCS3 in the entire central nervous system (CNS) amplifies the chronic anorexic and blood pressure (BP) effects of physiological increases in plasma leptin in mice fed a normal diet. Methods: $SOCS3^{flox/flox}$–Nestin-cre mice

were generated by breeding SOCS3$^{flox/flox}$ with Nestin-cre mice. BP and heart rate (HR) were recorded by telemetry, and oxygen consumption (VO_2) was monitored by indirect calorimetry in 22-week-old SOCS3$^{flox/flox}$–Nestin-cre (n=4) and control mice (SOCS3$^{flox/flox}$, n=4). Results: Compared to controls SOCS3$^{flox/flox}$–Nestin-cre mice were lighter (30±1 vs 33±1 g) and normoglycemic (124±7 vs 146±10 mg/dl), consumed less food (3.0±0.4 vs 3.6±0.2 g/day) and had similar VO_2 (77±6 vs 73±3 ml/kg/min). SOCS3$^{flox/flox}$–Nestin-cre mice had similar MAP (103±3 vs 107±3 mmHg) but higher HR (666±15 vs 602±17 bpm) compared to control mice. Chronic leptin infusion reduced food intake and increased MAP more (15±3 vs 7±2 mmHg) in SOCS3$^{flox/flox}$–Nestin-cre (46±3 vs 35±4%) compared to control mice. No significant changes were observed in HR in either group. Leptin infusion significantly reduced blood glucose levels in both groups (124±7 to 97±7 vs 146±10 to 105±7 mg/dl). These results indicate that SOCS3 deletion in the entire CNS reduces body weight and food intake, and amplifies leptin's effect on appetite and blood pressure. Conclusion: Thus, SOCS3 signaling attenuates the chronic actions of leptin on blood pressure as well as appetite regulation even in non-obese mice fed a normal diet. Acknowledgment: This work was funded by NHLBI-PO1HL51971 (to J.E.H.), an Institutional Development Award (IDeA) from the NIGMS P20GM104357 and by AHA SDG5680016 (to J.M.C)

10:00 a.m.- "Pathophysiology of Contrast-Induced Nephropathy and New Therapeutic Strategies"

Altaf-M Khan[1,2], Federico J. Teran[1,2], Kathleen S. Hering-Smith[1,2,3,4], Dewan S.A. Majid[2,3], Vecihi Batuman[1,2,4]
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Background: Contrast media (CM)-induced nephropathy (CIN) is associated with significant clinical and economic consequences. However, an effective and reliable strategy to prevent CIN remains elusive due to lack of a reliable CIN mouse model. Innate immunity mediated by Toll-like receptors (TLRs) and reactive oxygen species (ROS) are involved in the pathophysiology of CIN. We studied pathogenesis of CIN using ionic and nonionic CM in human renal proximal epithelial tubule cells (RPTECs), endothelial nitric oxide synthase deficient (eNOS$^{-/-}$)[1] and aged diabetic (db/db) mice to explore novel therapeutic strategies.
Methods: For *in vitro* studies, RPTECs were exposed to both ionic (Urografin) and nonionic (iohexol or iodixanol) CM 50 mg iodine/ml for 24 hr. To develop an ionic CIN mouse model, 8-10 wk old male eNOS$^{-/-}$ mice were i.v. given Urografin (1.85 g iodine/kg). Pituitary adenylate cyclase-activating polypeptide 38 (PACAP38) was used as a therapeutic agent both *in vitro* and *in vivo* studies. To develop a nonionic CIN model, *24-wk-old male db/db mice were given nonionic CM (3 g of iodine/kg bw) via jugular vein catheter. For a hypertensive and diabetic mouse model, male* eNOS$^{-/-}$ were fed on high fat diet and diabetes were induced using low doses of streptozotocin (50 mg/kg) for 3 days.
Results: Both ionic and nonionic CM caused significant injury in RPTECs by inducing cytotoxicity, inflammatory cytokines, apoptosis, kidney injury biomarkers (KIB), suppressing cell growth and hyper activating TLR2. Both Urografin and iohexol induced significant kidney injury in eNOS$^{-/-}$ and aged db/db mice respectively, as measured by serum creatinine, KIB, apoptosis, neutrophil infiltration and tubular cell damage. PACAP38 significantly reversed this damage both in RPTECs and eNOS$^{-/-}$ mice. In both models, CM also hyper activated ROS and TLRs specifically TLR2 was the most prominent in CIN. After i*ohexol injection, db/db mice showed a significant decrease in GFR, metabolic acidosis and significant increases in serum creatinine, urine and kidney KIM-1 levels, macrophage/monocytes expression and kidney damage histologically compared to control db/db mice. Aged d*iabetic eNOS$^{-/-}$ mice showed significant increase in polyuria, glucosuria, and significantly lower urine pH values compared to non-diabetic eNOS$^{-/-}$ mice. Diabetic eNOS$^{-/-}$ mice showed significantly higher systolic blood pressure and blood hemoglobin A1C. Aged diabetic eNOS$^{-/-}$ mice also developed nephropathy as confirmed by significant increase in KIB and are prone to CIN.
Conclusions: The eNOS deficient and db/db mice may be good animal models for contrast nephropathy. Urografin was the most toxic radiocontrast dye followed by iohexol or iodixanol to induce CIN. *Innate immunity mediated by TLR2 plays a major role in the pathogenesis of CIN as demonstrated by in vitro and in vivo studies. TLR2 may prove to be a promising drug target for the development of new therapeutics against CIN.*
Acknowledgment: Tulane Department of Physiology for providing metabolic cages. "Research reported in this publication was partly supported by the National Institute of General Medical Sciences of the NIH under Award Number P30GM103337. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH."
References:
1. Khan, AM, Maderdrut, JL, Li, M, Toliver, HL, Coy, DH, Simon, EE, Batuman, V: Pituitary adenylate cyclase-activating polypeptide prevents contrast-

induced nephropathy in a novel mouse model. *Physiol Rep,* 1**:** e00163, 2013.

10:15 a.m.- "Dual Targeted Dual Responsive Nanoparticles for Brain Targeted Delivery"

Eleni Markoutsa, Peisheng Xu
University of South Carolina, Columbia, SC

Background: The treatment of neurological diseases remains a challenge due to the existence of the blood-brain barrier (BBB), which prevents the passage of most molecules from the blood to the brain. Many drug delivery systems such as liposomes, micelles, polymers, and inorganic nanoparticles (NPs) have been developed for brain targeting, but only very few of them had the ability to penetrate to the BBB in efficient levels and to release their payload inside the brain controllably.
Method: We developed a Dual Targeted and Dual Responsive Nanoparticle (DTDRN), which can cross the BBB with high efficiency and selectivity. The glutathione (GSH) and pH responsive nanoparticles are highly stable in circulation, while quickly releasing ligands and payloads once within the brain due to the high intracellular level of glutathione (2-3 mM) and low pH (around 6.5) in brain tissue. Nanoparticles were prepared by crosslinking reaction of polymer (PDA-PEG) via disulfide bonds. DTDRNs were decorated with scopine and GSH through glutathione-responsive disulfide bonds that facilitate nanoparticles penetration through the BBB. The efficacy of DTDRN to penetrate the BBB was evaluated by both *in vitro* Transwell model and *in vivo* mouse model.
Experimental Results: *In vitro* experiments revealed that uptake and transcytosis of mono-targeted-NPs by cell monolayers were substantially higher compared to those of control NPs, while dual-targeted nanoparticles exhibited much higher cellular uptake and transcytosis than the mono-targeted ones. When acidic and/or reductive conditions were applied on the lower compartment of the Transwell system, the transcytosis of DTDRNs was substantially enhanced, due to the fact that DTDRNs are labile in environment with low pH or high redox potential like the brain. *In vivo* and *ex vivo* results confirmed increased brain targeting of mono and dual decorated nanoparticles as compared with non-targeted nanoparticles. As expected, the DTDRN showed better brain targeting effect than both mono targeting counterparts. Immunohistochemistry studies further confirmed the capability of DTDRNs to penetrate into the brain and revealed their distribution pattern in the brain tissue.
Conclusion: Due to the unique dual targeting and dual responsive properties, DTDRN effectively passed through the BBB and exhibited brain specific targeting effect, suggesting it is a very promising drug carrier to transport therapeutics to the brain for the treatment and diagnosis of brain diseases.

Acknowledgment: This work was funded by the National Institutes of Health (5P20GM109091-02).

Infectious Disease/Immunology, Azalea B

Friday, November 13, 9 a.m.- 10:30 p.m.

9:00 a.m.- Highlighted Speaker: "The Balance Between Health and Disease in Chronic Gammaherpesvirus Infection Is Controlled by p53"

Jeffrey M. Sifford, Debopam Ghosh, Eduardo Salinas, Jason Stumhofer, J. Craig Forrest
University of Arkansas for Medical Sciences, Little Rock, AR

Background: Tumor suppressor p53 responds to numerous cellular stresses, including viral oncogene expression, by enforcing cell-cycle arrest and cell death. Gammaherpesviruses (GHVs) express proteins that manipulate cell cycle and normal cellular differentiation pathways during latency establishment, maintenance, and reactivation. While p53 activity limits cellular proliferation following expression of GHV oncogenes in cultured cells, whether p53 functions in vivo to limit latency and disease during GHV infection is not known.
Methods And Results: To determine if p53 enforces an intrinsic barrier to GHV chronic infection in vivo, we performed experimental infections of mice with murine gammaherpesvirus-68 (MHV68), a natural rodent pathogen that provides a powerful small animal model to define mechanisms of GHV pathogenesis. Following intranasal infection of wild-type (WT) C57BL/6 mice with a YFP-expressing recombinant MHV68 to mark infected cells in vivo, we detected potent induction of p53 in YFP+ splenocytes, but not YFP- cells, during latency establishment. To determine if p53 activation influenced viral latency, we next infected p53-null mice with MHV68. While lytic replication in the lungs was equivalent in WT and p53-null mice, we observed in p53-null mice a 5-10-fold increase in the number of latently-infected cells compared to WT mice on day 16 post-infection, a time point corresponding to latency establishment and virus-driven cellular proliferation. This correlated with enhanced infection of germinal center and plasma cells in spleens. The increase in latently-infected cells, however, was not maintained over time, as we found that MHV68 latent burdens in p53-null mice crashed below those of WT mice by day 42 post-infection. Remarkably, MHV68 infection of p53-null mice decreased the period of disease free existence when compared to mock-infected animals, with diseased MHV68-infected mice exhibiting frank tumors of lymphocyte origin.
Conclusions: These data indicate that p53 is activated during MHV68 infection and functions to restrict latency establishment in vivo. These data also demonstrate that p53 limits MHV68-driven tumorigenesis. Given the reduction in latent infection at day 42 post-infection, these data further suggest that p53, perhaps by maintaining genetic stability in infected cells, facilitates homeostatic maintenance of MHV68 latency.
Acknowledgments: This work was supported by R01-CA167065 from the National Cancer Institute and Center for Biomedical Research Excellence grant P20-GM103625 from the National Institute for General Medical Sciences.

9:30 p.m.- "Studying the Temperature-Dependent Gene Response in the Relapsing Fever Spirochete, *Borrelia turicatae*"

Jon Blevins[1], Jacob Latham[1], Hannah Wilder[2], Job Lopez[2]
[1]The University of Arkansas for Medical Sciences, Little Rock, AR
[2]Baylor College of Medicine, Texas Children's Hospital, Houston, TX

Background: Relapsing fever is caused by vector-borne *Borrelia* spirochetes that are transmitted to humans via the bite of ticks or body lice. During their enzootic cycle, vector-borne spirochetes exist in the two distinct niches found within the arthropod vector and vertebrate. Lyme disease spirochetes undergo significant changes in global gene expression to allow them to adapt to these diverse environments, but the correlate that occurs in tick-borne relapsing fever spirochetes, such as *Borrelia turicatae,* remains undefined.
Methods: We used large-scale proteomic analyses to define regulatory changes that occur in *B. turicatae* in response to temperature alteration. To study the molecular mechanisms contributing to temperature-dependent gene regulation, a temperature-responsive gene, *bta121*, was selected for further characterization. *Trans* expression experiments and a luciferase-based transcriptional reporter are being used to identify *cis* regulatory sequences contributing to temperature-dependent regulation in *B. turicatae*.
Results: 223 proteins were differentially produced between bacteria grown at 37°C and 23°C; the plasmid-encoded *bta121* was one of these temperature-regulated genes. *Trans* expression studies showed that BTA121 was produced from a construct carrying *bta121* expressed from its adjacent 95-bp upstream region, but BTA121 production was no longer responsive to temperature. Luciferase reporter experiments using fusions with the same 95-bp upstream region confirmed these findings.
Conclusion: *B. turicatae* undergoes global changes in protein production in response to temperature and a region of DNA upstream of the *bta121* promoter contains a site that represses transcription at 37°C. Experiments are underway to define the sequences involved in regulation of *bta121*. Once the site is identified, *trans*-acting factors contributing to *bta121* regulation will be identified, and we will also survey the *B. turicatae* genome for other temperature-

responsive genes with this putative regulatory sequence.
Acknowledgment: This work was supported by a pilot grant through a Centers of Biomedical Research Excellence (COBRE) award from the NIGMS (P20-GM103625).

9:45 p.m.- "Anaerobiosis: A Key Factor in Regulation of Virulence Factors in *Listeria monocytogenes"*

Morgan Wright[1], Sally White[1], Daniel McClung[1], Haley Jenkins[1], Bindu Nanduri[2], Mariola Edelmann[2,3], Jessica G. Wilson[1], Janet R. Donaldson[1]
[1]Mississippi State University, Mississippi State, MS
[2]College of Veterinary Medicine, Mississippi State University, Mississippi State, MS
[3]University of Florida, Gainesville, FL

Background: *Listeria monocytogenes* is a Gram positive, facultative intracellular organism responsible for the foodborne disease listeriosis, the third leading cause of foodborne related deaths in the United States. Recent work from our laboratory has revealed a link between anaerobic conditions and virulence capability. Here, we present novel information on the impact of oxygen on survival and pathogenesis of *Listeria monocytogenes* in conditions that mimic the human gastrointestinal tract.
Methods: *Listeria monocytogenes* strains representing various serovars were treated with porcine bile extract under aerobic or anaerobic conditions and survival was assessed by viable plate counts. Proteomes expressed in the presence of bile under aerobic or anaerobic conditions were characterized using an LTQ-orbitrap mass spectrometer and gene expression was analyzed by qPCR.
Results: Strains that were related to outbreaks of listeriosis had an increase in resistance to bile under anaerobic conditions in comparison to aerobic conditions. This same impact was not observed under aerobic conditions. Proteomic comparisons revealed differences in metabolic shifts under anaerobic conditions that may impact bile resistance. This was further characterized by an increase in bile salt hydrolase activity and invasive potential. However, this increase in resistance and invasion varied between strains.
Conclusion: These results suggest that metabolic shifts during anaerobic conditions increase the stress response of *Listeria monocytogenes.* However, this response varies between virulent and avirulent strains. The shift in metabolic pathways during anaerobic exposure may therefore influence survival within the gastrointestinal tract. Further research is needed to characterize the role these key proteins have in virulence *in vivo*.

Acknowledgement: This work was funded by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM103646.

10:00 a.m.- *"Francisella tularensis*, a Bacterium that's No-Bloody-Good"

Deanna M. Schmitt, Tricia Gilson, Leanne Mazzella, Matthew Ford, Rebecca Barnes, Taylor Rogerson, Ashley Haught, James Birch, Joseph Horzempa
West Liberty University, West Liberty, WV

Francisella tularensis is among the most virulent organisms on the entire planet. Fewer than 10 of these bacteria are capable of initiating a disease that causes death in 60% of untreated patients. *F. tularensis* bacteria are notorious for replicating in host macrophages and neutrophils – cells that normally phagocytose and kill squatting bacteria. Unlike phagocytic cells, erythrocytes (red blood cells) are normally incapable of engulfing extracellular material, including bacteria. At a low frequency, *F. tularensis* can invade erythrocytes, suggesting that these bacteria induce a change in non-endocytosing cells to allow for translocation into the red blood cell cytosol. This manipulation of the erythrocyte is mediated by effector molecules of two distinct protein secretion systems. These effectors likely modulate spectrin, a major component of the erythrocyte cytoskeleton, to facilitate bacterial entry. *F. tularensis* bacteria residing within erythrocytes are more resistant to antibiotics. Moreover, intraerythrocytic bacteria are better at colonizing *Amblyomma americanum* and *Ixodes* sp. ticks – blood sucking arthropods capable of transmitting *F. tularensis* to humans. Erythrocyte invasion seemingly protects or allows for *F. tularensis* bacteria to better adapt to the low pH associated with the tick gut. Interestingly, other bacteria recently discovered to invade erythrocytes also use ticks as arthropod vectors for transmission. We speculate that, like *Francisella*, these other bacteria gain access to the intracellular space of erythrocytes to enhance tick colonization.
Acknowledgment: This work was supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence, funding from the WV Research Challenge Fund (HEPC.dsr.14.13), and a grant from the National Institute of Allergy and Infectious Diseases 5K22AI087703).

10:15 a.m.- "Novel Grafting of Peptides onto Plant-Based Peptides for Modulation of CD2-CD58 Protein-Protein Interaction: Implications in Autoimmune Diseases"

Rushikesh Sable[1], Thomas Durek[2], David Craik[2], Sandeep Pallerla[1], Seetharama Jois[1]
[1]Department of Basic Pharmaceutical Sciences, School of Pharmacy, University of Louisiana at Monroe LA
[2]Institute for Molecular Bioscience, The University of Queensland, St. Lucia, Australia

Background: The importance of CD2 and CD58 molecules is vital in inflammatory and autoimmune diseases such as rheumatoid arthritis (RA). The objective of this project was to design and characterize stable small peptide like molecules which will inhibit CD2-CD58 protein-protein interaction. For the same, we adopted cyclotide grafting technique. Cyclotides are plant-derived peptides or mini-proteins that have multicyclic structure with disulfide bonds, are resistant to thermal, chemical, and enzymatic degradation, and are orally bioavailable.
Methods: All peptides were designed using YASARA molecular modeling software and synthesized by F-moc based solid synthesis method. A lymphocyte-epithelial cell adhesion assay and E-rosseting assay were used to evaluate protein-protein interaction inhibition activity. For the determination of serum stability peptide was incubated in human serum for 72 hours and, thermal stability of the peptide was evaluated by assessment the change in ellipticity (Circular Dichroism) data after gradual increase in temperature of a solution containing peptide from 25 to 85 ^{o}C. Flow cytometry and SPR techniques were used to confirm the binding specificity of designed molecules. AutoDock software was used to show virtual binding of these designed molecules to the CD58 and CD48 proteins.
Results: Among five different grafted cyclotides, SFTI 1-1 showed promising cell adhesion inhibitory activity (IC_{50} ~ 51 nM). Flow cytometry and SPR assay results also reflected the binding specificity of SFTI1-1 towards CD58 protein. It also demonstrated good serum and thermal stability. Observations from docking studies showed that SFTI1-1 (grafted peptide) binds to the CD58 and CD48 proteins with relatively low docking energy and the peptide binds to adhesion domain of the proteins.
Conclusion: A novel grafted molecule SFTI1-1 helped to modulate the immune response by targeting the adhesion interaction between CD2 and CD58 which can be developed as a potential therapeutic agent against autoimmune diseases.
Acknowledgment: Funding for this research was from the National Institute of General Medical Sciences of the National Institutes of Health under grant number 8P20GM103424 via LBRN Summer Research Program 2015.

Neuroscience, Azalea C

Friday, November 13, 9 a.m.- 10:30 p.m.

9:00 a.m.- "The Effects of Early-Life Risperidone Administration on Dopamine Release and Uptake During Adulthood"

Mark E. Bardgett[1], Cliff Brown[1], George Quintero[2], Greg A. Gerhardt[2]
[1]Northern Kentucky University, Lexington, KY
[2]Highland Heights, KY, University of Kentucky, Lexington, KY

Background: The use of antipsychotic drugs in pediatric populations has increased significantly over the past two decades despite an absence of research assessing their possible long-term effects on brain development. We have found that young rats administered the antipsychotic drug, risperidone, are more active, demonstrate deficits in impulse control, and show enhanced behavioral responses to drugs of abuse. Since many of these behaviors are associated with forebrain dopamine function, a preliminary study was performed using high-speed chronoamperometry to assess dopamine release and uptake in the striatum of adult rats administered risperidone early in life.
Methods: Male Long-Evans rats received daily injections of 3.0 mg/kg of risperidone or vehicle between postnatal days 14-28. High-speed chronoamperometry was used to record potassium-stimulated dopamine release and uptake of locally infused dopamine at nine different dorsal-ventral locations within the dorsal and ventral striatum of isoflurane-anesthetized rats. Recordings were performed when the rats were adults (between postnatal days 75-135).
Results: Potassium-stimulated dopamine release was similar in adult rats administered risperidone or vehicle early in life. Dopamine release was significantly higher in the dorsal striatum compared to the ventral striatum. Dopamine reuptake appeared to be impaired in the adult rats administered risperidone early in life. Local infusion of dopamine produced a higher peak amplitude in the risperidone-treated rats and the time for the dopamine signal to decay by 80% was longer in this group.
Conclusion: Early-life risperidone administration impairs some aspects of forebrain dopamine neurotransmission. Such alterations in dopamine function may underlie some of the behavioral changes reported in adult rats administered risperidone early in life. These data raise concerns about the long-term effects of antipsychotic drug use in children and indicate the need for more basic research that addresses this issue.
Acknowledgment: This work was funded by NIH Grant 1R15MH094955 (to M.E.B.) and by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM103436.

9:15 a.m.- Highlighted Speaker: "KNDy Neurons and the Control of Mammalian Reproduction"

Michael Lehman
The University of Mississippi Medical Center, Jackson, MS

Background: In mammals, including humans, reproduction is governed by intricate neural and hormonal communication between the brain, pituitary gland and gonads. At the top of this hierarchy are gonadotropin-releasing hormone (GnRH) neurons that are responsible for the pulsatile secretion of luteinizing hormone (LH) from the anterior pituitary gland. Changes in the frequency of GnRH and LH pulses have a profound effect on the reproductive system, but identification of the "GnRH pulse generator" in the brain has remained a major unanswered question in neuroendocrinology for nearly fifty years.
Methods/Results: Using a combination of anatomical, physiological and pharmacological approaches, we have discovered a subset of neurons in the arcuate nucleus of the hypothalamus that appear to play a key role in the generation of GnRH pulses and their control by endogenous hormones and other signals. We have called these cells, KNDy neurons based on their unique co-expression of three neuropeptides (kisspeptin, neurokinin B, and dynorphin) each of which is critical for reproduction in humans and animals.
Conclusion: In this talk, we will briefly review current evidence from our lab and others that KNDy neurons are a core component of the GnRH pulse generator, as well as findings suggesting they play a major role in the regulation of reproduction by gonadal steroids and other stimuli. In addition, we will discuss recent evidence that KNDy neurons are altered in a common reproductive disease in which pulsatile GnRH secretion is altered, namely polycystic ovarian syndrome (PCOS).
Acknowledgment: This work was funded by NIH Grants R01 HD39916 and P01 HD44232 (Project II).

9:45 a.m.- "The Modulatory Effects of Caffeine on the Intrinsic Properties of Spinal Lateral Motoneurons"

Marla Rivera-Oliver[1,3], Yocasta Alvarez-Bagnarol[1,2], Manuel Diaz-Rios[2,3]
[1]Department of Biology, University of Puerto Rico, Rio Piedras Campus, San Juan, PR
[2]Department of Anatomy and Neurobiology, University of Puerto Rico, Medical Sciences Campus, San Juan, PR
[3]Institute of Neurobiology, University of Puerto Rico, Medical Sciences Campus, San Juan, PR

Background: Caffeine can produce similar behavioral effects as other classical psychostimulants, such as cocaine and amphetamines, mainly increasing motor activation, arousal, and reinforcing effects related to neural reward systems. Additionally, caffeine and other adenosine receptor antagonists and agonists have been shown to have anti-inflammatory and neuroprotective effects after a spinal cord injury in animal models and in human patients. Most of the studies assessing the effects of caffeine and/or adenosine receptor antagonists and agonists on locomotor behavior have been performed on freely behaving rodents using systemic administration of these drugs which can activate multiple neural pathways making extremely challenging to study specific mechanisms of action.
Methods: We proposed to elucidate the cellular mechanisms by which caffeine modulates the intrinsic membrane properties of spinal lateral motoneurons, which are an essential components of the lumbar neural network producing hindlimb locomotion in mammals through bath perfusion of caffeine, an adenosine receptor antagonist, onto upper lumbar mouse spinal cord slices combined with perforated patch clamp recordings.
Results: Recent data from our laboratory from extracellular recordings on spinal lumbar nerves in the presence of serotonin (5-HT), NMDA and dopamine (DA), which are known to elicit a fictive locomotor pattern, shows that caffeine modulates motor activity by enhancing the burst properties of motoneurons. Thus, we studied the neuromodulatory effects of caffeine and other adenosine receptor antagonists on the intrinsic properties of spinal lateral motoneurons using pharmacological blockade and perforated patch clamp recordings. The application of 5-HT and NMDA, in the presence of synaptic blockers of inhibitory transmission depolarized the membrane potential of most motoneurons reversibly. The addition of caffeine (50uM in the presence of 5-HT and NMDA, significantly depolarized the membrane potential (~10-15mV), hyperpolarized the action potential (AP) threshold and decreased the AP After-hyperpolarization (AHP) in 70% of the recorded lateral motoneurons. In 17% of the recorded lateral motoneurons caffeine showed an inhibitory effect by increasing the AGHP and no effect in the AP threshold. Additionally, in 13% of the recorded lateral motoneurons, the application of caffeine did not produce any significant effects on any of the parameters measured.
Conclusion: The excitatory effects produced by caffeine on most of the lateral motoneurons suggest that they could be primary target for the neuromodulatory effects of caffeine in the lumbar region of the spinal cord of mammals.
Acknowledgment: This work was funded by NIH RISE Grant (2R25GM061151-13), NIH NIGMS COBRE grant (1P20GM103642), and by an RCMI/UPR-MSC grant (G12RR03051).

10:00 a.m.- "Design and Synthesis of Fluorinated Anthocyanins as Neuroprotective Antioxidants"

David A. Colby, Robert A. Hazlitt, Changho Han, Que-Lynn Tran
The University of Mississippi, University, MS

Background: The anthocyanins are a class of naturally occurring antioxidants that has recently shown therapeutic potential as neuroprotective agents. On the other had, the anthocyanins are heavily metabolized, and this pharmacokinetic profile substantially limits their clinical translation. The goal of this project is to synthesize derivatives of anthocyanins that are resistant to metabolism through the incorporation of fluorine, which is a common strategy in drug design.
Methods: The candidate anthocyanin is metabolized by de-glycosylation and the resulting aglycone does not cross the blood brain barrier. We have designed an analogue in which the labile O-glycosyl bond is replaced with a stable CF_2-isostere.
Results: Synthetic studies have already provided access to a CF_2-sugar for incorporation into the anthocyanin framework. The complementary coupling fragment has been accessed through flavone. Additionally, a new synthetic method to unite these two pieces has been developed.
Conclusions: The design of CF_2-sugars on to complex organic molecule is a novel strategy to limit metabolism by de-glycosylation. New synthetic methods to produce these targets have been developed.
Acknowledgment: This work was funded by the NIA under grant number R21AG039718 and by a Pilot Grant through NIGMS under grant number P20GM104932.

10:15 a.m. "Alternative Splicing in Photoreceptor Neurons"

Dr. Visvanathan Ramamurtny, Peter Stoilov
Department of Biochemistry & Ophthalmology, Center for Neuroscience, West Virginia University, Morgantown, WV

Photoreceptor cells express unique isoforms of ubiquitously expressed genes. In particular, our RNA-seq analyses show an inclusion of smaller exons "microexons" in the photoreceptor cells. This is prevalent in genes that are crucial for ciliogenesis leading to our main idea that alternative splicing events in photoreceptors contribute to the generation and function of specialized elaborated cilium, the outer segment. In this talk, I will discuss some of our recent findings on the mechanism controlling alternative splicing in photoreceptors and the role of alternative splicing in shaping the function of photoreceptor cells.

Genomics, Azalea D

Friday, November 13, 9 a.m.- 10:30 p.m.

9:00 a.m.- Highlighted Speaker: "Leveraging Rat Genetic Models to Understand Human Diseases of the Kidney"

Ashley C. Johnson[1], Xuexiang Wang[1], Zhen Jia[1], Michael R. Garrett[1, 2, 3]
[1]Departments of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS
[2]Departments of Medicine, University of Mississippi Medical Center, Jackson, MS
Departments of Pediatrics (Genetics), University of Mississippi Medical Center, Jackson, MS

Background: Our lab utilizes complex genetic rodent models (i.e. contain multiple genes or genetic factors involved in disease processes) to develop a better understanding of different types of human kidney disease as well as to identify novel therapeutic targets. In particular, we have performed genetic and genomic analysis of two rodent models-- the Dahl salt-sensitive (S) rat, a model of chronic kidney disease (CKD) and the heterogeneous stock derived model of unilateral renal agenesis (HSRA), a model of congenital abnormalities of the kidney.
Methods: A host of genetic [e.g., linkage analysis, genome-wide association studies (GWAS), positional cloning, etc], molecular (e.g., cloning, genetically modified cells, etc) and genomic techniques (e.g., microarray, RNA sequencing, and whole genome sequencing) have been utilized to identify genetic variation involved in causing kidney disease.
Results: Using the Dahl S rat, genetic variation in *Arhgef11* (Rho guanine nucleotide exchange factor) has been identified that causes chronic activation of Rho-ROCK pathway in specific kidney cells (proximal tubule), thereby promoting onset and progression of kidney injury and reduced kidney function. Identification of this gene/pathway has provided a novel target to develop new therapies to treat CKD. For the HSRA model, bioinformatics analysis of GWAS, whole genome sequencing, and fetal kidney RNA sequencing have identified several novel genes [*Met* and/or *Wnt2* (RNO4), *Fam58b* and/or *Nlk* (RNO10), and *c-Kit* (RNO14)] highly likely to cause failure of one-kidney to develop as well as impact development in the remaining kidney.
Conclusion: The use of animal models, genetic and genomic techniques, and *in* vitro studies using genetically modified cells provide important understanding into naturally occurring genetic variation that leads to various form of kidney diseases in the rat, which is highly relevant for understanding human health.
Acknowledgment: This work was funded by grant support to M.R.G. through NIH/NHLBI HL094446, Robert M. Hearin Foundation, UMMC IRSP grant. The work performed through the UMMC Molecular and Genomics Facility was supported, in part, by funds by P20 GM103476 [MS-INBRE-(Elasri)]; P30 GM103328 [CPN-COBRE (Stockmeier)]; and P20 GM104357 [Cardio-Renal (Hall)].

9:30 a.m.- "Histone Deacetylases 1 and 2 Balance Nephron Progenitor Renewal and Differentiation During Kidney Organogenesis"

Hongbing Liu, Shaowei Chen, Xiao Yao, Zubaida Saifudeen, Samir El-Dahr
Department of Pediatrics and Tulane Hypertenison & Renal Center of Excellence, Tulane University School of Medicine, New Orleans, LA

Background: Congenital Anomalies of the Kidney and Urinary Tract (CAKUT) are a major cause of morbidity in children, constituting approximately 20~30% of all anomalies identified in the prenatal period. CAKUT plays a causative role in 30~50% of cases of end stage renal disease (ESRD) in children, and predisposes to the development of hypertension and other renal-cardiovascular diseases in patients that survive to adolescence and adulthood. The long-term goal of our study is to uncover the epigenetic mechanisms accounting for CAKUT. Here, we investigate the nephric lineage-specific functions of class I histone deacetylases (HDACs), HDAC1 and HDAC2, in kidney development. HDACs are an evolutionarily conserved group of enzymes that remove acetyl groups from histones as well as non-histone proteins.
Methods: A genetic model of conditional HDAC1/2 deletion in renal progenitor cells was used for the investigation of HDAC1 and 2 function in renal progenitor cells during kidney organogenesis. Mice bearing conditional null alleles of HDAC1 and HDAC2 were crossed to Six2-CreEGFP transgenic mice to delete HDAC1 and 2 genes, specifically in nephron progenitor cells (NPC, also known as cap mesenchyme cells).
Results: Our data revealed that mice with three or less deleted alleles for HDAC 1 and 2 live until adulthood with normal growth and development, whereas concurrent deletion of both HDAC1 and 2 resulted in early postnatal lethality. At birth, $NPC^{HDAC1,2-/-}$ mice exhibit bilateral renal hypoplasia, including small kidney size, decreased number of nephrons and formation of multiple cysts. Double deletion of HDAC 1 and HDAC2 in the NPC depletes the cap mesenchyme and blocks nephron formation at the renal vesicle stage, due to defective cell proliferation and repression of the HNF-Notch/Lhx1 pathways. We also found that $NPC^{HDAC1,2-/-}$ kidneys ectopic expression of Wnt4 which indicates that HDAC1/2 prevents premature differentiation of CM cells through inhibition of Wnt/ β-catenin target genes, including *Wnt4*.

Conclusion: This study shows that Histone Deacetylases 1 and 2 are required for gene expression and the balance of self-renewal and differentiation of renal progenitor cells.
Acknowledgment: Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number P30GM103337 and RO1-DK096373-03 The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

9:45 a.m.- "Unappreciated piRNA Biology of the Two-Spot Spider Mite"

Mosharrof Mondal, Alex S. Flynt
The University of Southern Mississippi, Hattiesburg, MS

piRNAs are a class of small non coding RNAs that are involved in epigenetic and post-transcriptional gene silencing of transposable elements in animal germ line cells. They are different from other classes of small RNAs mainly by their size (25-31nt), biogenesis pattern and biological function. Arachnids diverged during the pre-cambrian radiation, thus it is likely that they have unique, unappreciated biology. The two-spot spider mite *Tetranychus urticae*, which is a major agricultural pest, has indeed shown divergent piRNA biology. By manual curation of piRNA loci we have found outstanding level of piRNA expression across the entire genome in both adult and embryo stages animals. Canonical piRNA biogenesis through ping pong mechanism produces characteristic 10nt overlap between sense and anti sense piRNA whereas we have observed many different overlap patterns other than 10nt in *T. urticae.* Nonetheless, piwi protein, which is the main effector of piRNA function, has expanded in number in *T. urticae and in situ* hybridization has also demonstrated their expression in both of the developmental stages. piRNA biology is essentially playing broader role in spider mite's life cycle that can be utilized to control this devastating pest. Interestingly, we have observed that ingested exogenous RNAs are incorporated in *T. urticae's* piRNA pathway, which suggests that plant transgenes encoding RNAi triggers may be best designed targeting piRNA pathway of spider mite.

10:00 a.m.- "Nutrition Intervention to Profile Metabolic, Microbiome and Vascular Health in Young Adults at Risk for Disease: FRUVEDomic Pilot Study"

Oluremi Famodu, MS, RDN, Melissa D. Olfert, DrPH, RDN, Marianne Downs, PhD, Pamela Murray, MD, Joseph McFadden, PhD, Christopher Cuff, PhD, Mark Olfert, PhD
West Virginia University, Morgantown, WV

Objective: To investigate the effects of an eight-week nutritional intervention on the microbiome, metabolome and cardiovascular indicators "at risk" young adults (18-28 years) for metabolic syndrome (MetS).
Methods: Thirsty-seven subjects were recruited at West Virginia University and randomized into one of three intervention groups; 1) "Fruved" (50% fruit & vegetable); 2) "Fruved+LowCHO" (low refined carbohydrate); and 3) "Fruved+LowFat" (low fat). Venous blood and stool sample were collected pre and post intervention. Alterations in the fecal microbiome were assessed using high throughput sequencing of the V3-V4 regions of the 16S rRNA gene (Illumina MiSeq). Targeted and non-targeted metabolomic approaches are currently being used to investigate the influence of diet on ceramides and its potential downstream regulators and to obtain a global understanding on the metabolome.
Results: A wide range of microbiome species diversity was found across the study population, where none of the diets altered species diversity when comparing pre- and post- samples from each participant. Significant increase in Firmucutes and decrease in Bacteriodetes was observed in Fruved + LowCHO group, whereas decrease in Firmucutes and increase in Bacteriodetes observed in Fruved+ low FAT group. No changes were found in the Fruved only group. Amino acid, carbohydrate, fatty acid, and sphingolipid metabolism is being analyzed to make correlations with the microbiome.
Conclusions and Implications: Identifying markers among those "at risk" of metS will help quantify disease risk and generate personalized nutrition prescription.
Acknowledgment: Funding provided by the WV Clinical and Translational Science Institute (NIH/MIGMS Award Number U54GM104942)

10:15 a.m.- "DNA methylation in Healthy Aging of Twins"

Sangkyu Kim, Jennifer Wyckoff, S. Michal Jazwinski
Tulane University Health Sciences Center, New Orleans, LA

Background: One approach to human aging is to identify genetic and epigenetic factors associated with aging-related changes and study their cellular mechanisms. Human longevity is heritable with its heritability estimated between 0.15 and 0.35. This indicates that a greater portion of the variation in human aging depends on non-genetic factors. The significant contribution of non-genetic factors is evident in monozygotic (MZ) co-twins who show high degrees of discordance in aging-related changes.
Methods: We recruited MZ and dizygotic (DZ) twins who are 60 -100 years old. As a phenotypic indicator of healthy aging, we generated a frailty index, called FI_{23}, based on 23 health variables. FI_{23} is heritable. Using the Illumina Human Methylation450 BeadChip assay, we obtained genome-wide DNA methylation data for blood and buccal cell samples from each twin.
Results: Genome-wide DNA methylation profiles of leukocyte samples were very different from those of buccal cell samples, recapitulating the cell-type specificity of epigenetic markers. More variation in genome-wide DNA methylation was observed between DZ co-twins than between MZ co-twins. An age-dependent decrease in genome-wide DNA methylation was also observed in both cell types. Currently, we are examining differentially methylated CpG sites that are correlated with FI_{23}. We are also investigating differentially methylated regions that are found in discordant MZ co-twin pairs but not in concordant twin pairs
Conclusion: Our twin samples show genome-wide properties similar to those reported in other DNA methylation studies. The search for differentially methylated sites or regions will lead us to identification of regulatory elements where DNA methylation plays an important role in healthy aging of the elderly.
Acknowledgment: This work was funded by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM103629.

Bioinformatics, Poster Session A

Thursday, September 12, 11 a.m.-12:30 p.m.

A 1.1- "Superconvergence of Nonconforming Finite Element Approximation for the Second Order Elliptic Problems and Numerical Examples"

Anna Harris
University of Arkansas at Pine Bluff, Pine Bluff, AR

Finite element method (FEM) is based on the premise that an approximation to any complex engineering problem can be reached by subdividing the problem into smaller, more manageable elements. Using FEMs partial differential equations that describe the behavior of structures can be reduced to a set of linear equations that can easily be solved using the standard techniques of matrix algebra. The main objective of the superconvergence using various FEMs is to improve the accuracy of the existing approximation solution by applying certain post-processing techniques that are easy to implement. The method of local averaging has been a popular and useful technique in the study of superconvergence. The underlying assumption of the existing superconvergence technique is that the finite element mesh has some special properties such as uniformity, local point-symmetry, local translation-invariance, or orthogonality. Wang proposed
and analyzed a general post-processing method for conforming finite element solutions. The post-processing method is to improve the finite element solution via the standard L^2-projection method. The role of the L^2-projection method is to eliminate and to reduce any pollution caused by mesh irregularity. The goal and objective of this paper are a) to obtain mathematical theories for the superconvergence of the nonconforming finite element method (NCFEM), b) to perform
numerical experiments to support the theoretical results by writing computer programs, c) to verify the theoretical results for the superconvergence of the conforming finite element method (CFEM) with numerical experiments by L^2-projection methods, and d) to find medical application.

A 1.2- "Determining Potential Yeast Longevity Genes via PPI Networks and Microarray Data Clustering Analysis"

Bernard Chen[1], Roshan Doolabh[1], Fusheng Tang[2]
[1]Department of Computer Science, University of Central Arkansas, Conway, AR
[2]Department of Biology, University of Arkansas at Little Rock, Little Rock, AR

Background: Identification of genes involved in lifespan extension is a pre-requisite for studying aging and age-dependent diseases. So far, very few genes have been identified that relate to longevity. The process of analyzing each single gene one at a time can be a very long and expensive process. It is known that approximately 10% of 6000 yeast genes are lifespan related genes; however, less than 100 genes are identified as longevity genes. The interconnection of multiple genes and the time-dependent protein-protein interactions make researchers use systems biology as a first tool to predict genes potentially involved in aging.
Methods: In this study, we combined analyses of protein-protein interaction data and microarray data to predict longevity genes. A dataset of all 6000 yeast genes was utilized and a protein-protein interaction ratio was used to narrow the dataset. Next, a hierarchical clustering algorithm was created to group the resulting data.
Results: From these clusters, conclusion of 6 highly possible longevity genes was drawn based on the amount of longevity genes in each cluster. Based on our latest information, one of our predicted genes is identified as a longevity gene. Wet lab experiments are applied to our predicted genes for supporting the findings.
Conclusion: In this paper, we show an approach to find the candidate yeast longevity genes based on the PPI and Microarray data. We search potential longevity genes based on our proposed PPI_Ratio. Then we put those genes with known longevity genes in the same pool and cluster them with the Microarray experimental results. By using the concept of "Genes that are clustered with dominate longevity genes group have a higher potential to be longevity genes", we are able to further extract high potential longevity genes.

A 1.3- "microRNA-148/152 Family Members Concordantly Target Genes Important for Tumor Progression and Chemoresistance"

David Oliver[1], Hao "Emily" Ji[1], Samuel Lee[2], Ellen Gardiner[1], Mythreye Karthikeyan[3], Homayoun Valafar[4], Michael Shtutman[1]
[1]Drug Discovery and Biomedical Sciences, South Carolina College of Pharmacy, Columbia, SC
[2]Biology, University of South Carolina, Columbia, SC
[3]Chemistry and Biochemistry, University of South Carolina, Columbia, SC
[4]Computer Science and Engineering, University of South Carolina, Columbia, SC

Background: To date, over 1800 human precursor miRNAs have been identified (miRBase 21) targeting virtually every mRNA. The means by which miRNAs exert their effect on protein translation is usually through (i) inhibition of translation, (ii) mRNA de-adenylation, or (iii) direct mRNA degradation. Which mechanism of translational repression occurs depends on miRNA/mRNA specific interactions. In

addition, it is well known that each miRNA can target multiple mRNAs and conversely, many mRNAs are regulated by multiple miRNAs. The complexity and sensitivity of regulatory networks that these interactions can produce is astounding and require genomic scale experiments to tease out. The microRNA-148/152 family is a highly conserved miRNA family made up of three miRNAs - miR-148a, miR-148b, and miR-152. miR-148b and miR-152 are encoded in the COPZ1 and COPZ2 genes, respectively while miR-148a is transcribed independently. The sequence of these miRNAs differ in only three base pairs outside of the canonical seed region making target prediction difficult. In addition, the microRNA-148/152 family members are differentially regulated during cancer progression. Specifically, miR-148a and miR-148b are often maintained or upregulated while miR-152 becomes silenced in many cancers.
Methods: We generated two prostate cancer cell lines (PC-3 and DU145) which overexpressed each of the microRNA-148/152 family members and performed high-throughput sequencing using Illumina HiSeq platform. Top up and down regulated genes were validated by qPCR. Network analysis was performed using protein-protein interactions and signaling pathways curated by GeneMANIA in Cytoscape v3. In addition, within network clusters were identified using the MCODE function of ClusterMaker 2.0 in Cytoscape. Finally, a variation on the cross-linking immunoprecipitation of Argonaut was performed to identify direct miRNA targets in-vivo.
Results: Analysis of the RNA-seq data from 2 different prostate cancer cell lines over-expressing each of the three microRNA-148/152 members shows that the majority of regulated genes are concordantly regulated rather than differentially regulated as expected from cancer progression data. These results suggest that the miR-148/152 family may behave in a rheostat model to regulate target mRNAs. RNA-seq data suggested, that the microRNA-148/152 family regulate genes and pathways involved in cancer development and progression, such as EMT, EGFR signaling, and DNA repair. Furthermore, network analysis revealed that concordant regulation by the microRNA-148/152 family focused on extracellular matrix reorganization as well as cell-cell adhesion and cell-substrate adhesion. Interestingly, further network analysis revealed a cluster of genes which were involved in mitotic progression suggesting a potential mechanism for selective expression of miR-148b during cancer progression. Additional results obtained using the modified cross-linking immunoprecipitation will be discussed.
Conclusions: This study provides additional evidence regarding the role of the microRNA-148/152 family in cancer progression. Furthermore, this study greatly increases the current understanding of the global impact of the entire microRNA-148/152 family including its role in EMT, DNA damage, and mitotic progression.
Acknowledgements: This work was funded in part by AFPE Pre-Doctoral Fellowship (DO), NIGMS grant 1P20GM109091-01 (MS), NIH grant P30 GM103336 to the Center for Colon Cancer Research at the University of South Carolina (MS), and SC INBRE pilot award (MS)

A 1.4- "Genome-wide Distribution of G-quadruplex Forming Sequences and Their Role in Stability During Meiosis"

Julia H. Chariker, Donald M. Miller, Eric C. Rouchka
University of Louisville, Louisville, KY

Background: G-quadruplex structures are found throughout the human genome and are known to play a regulatory role in a variety of molecular processes. Structurally, they have many configurations and can form from one or more DNA strands. At the gene level, they regulate gene expression, protein synthesis, and alternative splicing.
Methods: Sequences capable of forming G-quadruplex structures were identified on the current assembly of the human genome (hg38). Chromosomal-level patterns of distribution were analyzed to evaluate a long-standing hypothesis about their role in maternal and paternal chromosome pairing during meiosis.
Results: Here we show significant correlations between G-quadruplex forming sequences on forward and reverse DNA strands, and unique high density banding patterns are demonstrated for individual chromosomes, providing a unique signature for correct pairing of maternal and paternal chromosomes. The highest density of G-quadruplex sequences occurs within four megabases of one end of most chromosomes, which is in line with studies of homologue pairing in other species.Conclusion: The G-quadruplex forming sequence distribution identified in this study supports a role for G-quadruplex structures in homologue pairing in humans and provides justification for further investigation in the laboratory.
Acknowledgment: This work was funded by the National Institute for General Medical Sciences (NIGMS), National Insititutes of Health (NIH) Grant P20GM103436 (Nigel Cooper, PI).

A 1.5- "Computational Investigations of Enantiospecificity of Mutated CYP2C9"

Logan Bond[1], Grover P. Miller[2], Martin D. Perry Jr.[1]
[1]Ouachita Baptist University, Arkadelphia, AR
[2]University of Arkansas for Medical Sciences, Little Rock, AR

Background: In previous research conducted in our lab, the enantiospecificity of CYP2C9, the most enantiospecific member of a family of enzymes known

as cytochrome P450's, was examined. The data obtained from these studies revealed that there were significant residues within CYP2C9 that contributed to the overall enantiospecificity of CYP2C9. In the current study, we mutated these significant residues and followed the same procedures as the previous research in order to compare our results to those previously obtained. The differences were then analyzed and graphed.
Methods: We used a molecular modeling suite, Sybyl-X, in order to simulate oxidative reactions between a crystalized structure of CYP2C9 and six NSAIDs. After simulation, we used a written program to analyze the topography data output of Sybyl-X. We then graphed the total energy per residue by calculating the energies using the Van der Waals and hydrogen bonding energy equations.
Results: Final analysis of the energies by residue revealed that a mutated CYP2C9 can have entirely different enantiospecificity after mutation of a single, significant residue. This suggests that in the un-mutated version of CYP2C9, these significant residues play an important role in CYP2C9's expressed enantiospecificity.
Conclusion: The results reveal that critical residues within CYP2C9 contribute to CYP2C9's expressed enantiospecificity in a significant way. In certain cases where CYP2C9 preferred one enantiomer over the other, one mutation caused CYP2C9 to change its preference toward the other enantiomer of the ligand. Also, this study shows that mutations of CYP2C9 cause no consistent pattern of change from ligand to ligand in terms of CYP2C9's preference for one form or another.
Acknowledgment: This project was supported by the Arkansas INBRE program, with grants from the National Center for Research Resources - NCRR (P20RR016460) and the National Institute of General Medical Sciences - NIGMS (P20 GM103429) from the National Institutes of Health.

A 1.6- "Lower Level of Sestrin2 in Aging Sensitizes Heart to Ischemia and Reperfusion Injury"

Nanhu Quan, Wanqing Sun, Lin Wang, Xingchi Chen, Ji Li
University of Mississippi Medical Center, Jackson, MS

Background: A novel stress-inducible protein, Sestrin2, declines in the heart with aging. AMP-activated Protein Kinase (AMPK) has emerged as a pertinent stress-activated kinase shown to have substantial cardioprotective capabilities against myocardial ischemia/reperfusion (I/R) injury. We recently identified the interaction between Sestrin2 and AMPK in the ischemic heart.Hypothesis: The ischemic AMPK activation modulated by Sestrin2-AMPK complex in the heart is impaired in aging that sensitizes heart to ischemic insults.
Methods: Young C57BL/6 mice (4-6 months) and aged C57BL/6 mice (24-26 months) were subjected to left anterior descending coronary artery occlusion for different time points of ischemia and I/R in order to detect the signaling activity in the left ventricle. The ex vivo working heart system was used for measuring substrate metabolism in the perfused hearts.
Results: The protein expression level of Sestrin2 in aged heart was significantly lower than that of Sestrin2 in young heart ($p<0.05$). Intriguingly, ischemic AMPK activation was blunted in the aged hearts as compared with young hearts ($p<0.05$), the AMPK downstream glucose uptake and the rate of glucose oxidation were significantly impaired in the aged hearts during I/R ($p<0.05$ versus young hearts). The myocardial infarction size was significant bigger in the aged hearts ($p<0.05$ versus young hearts). Respective left ventricle lysates were immunoprecipitated with Sestrin2 and the immunoblots revealed that Sestrin2 forms a complex with AMPK and AMPK upstream LKB1 during I/R, moreover, the binding affinity between Sestrin2 and AMPK is decreased in the aged hearts during I/R ($p<0.05$ versus young hearts). Furthermore, Sestrin2 knock out hearts demonstrate impaired ischemic AMPK activation and higher sensitivity to I/R-induced injury as compared to wild type hearts ($p<0.01$).
Conclusions: Sestrin2 is a stress-induced scaffold protein that mediates the activation of AMPK in the ischemic myocardium via an interaction with upstream LKB1. Moreover, lower level of Sestrin2 in aging leads to blunted ischemic AMPK activation and increased sensitivity to ischemic insults.
Acknowledgment: This work was supported by American Heart Association 14IRG18290014, American Diabetes Association Basic Sciences Grant 1-14-BS-131, NIH R21AG044820 and R01AG049835, National Natural Science Foundation of China 31171121, 81471394 and 81500264.

A 1.7- "A Framework to Feature Mining for Comorbidity Analysis: Age Related Macular Degeneration and Alzheimers Disease"

Pradeep Chowriappa[1], Sumeet Dua[1], Prerna Dua[2]
[1]Department of Computer Science, Louisiana Tech University, Ruston, LA
[2]Department of Health Informatics & Information Management, Ruston, LA

Background: The goal of this work is to discern potential metabolic connections between AD and AMD through shared genes, transcription factors (TF's), microRNA's (miRNAs), and protein-protein interactions (PPI's). Recent research indicates that the polymorphism of the complement factor H (CFH) gene (CFH Y402H) is believed to increase the risk for AMD and may predispose patients for comorbidity in AD. Furthermore, it is known that the CFH gene is significantly down-regulated in both AD and AMD,

which manifests in the form of drusen in AMD and senile plaques in AD. Additionally, a comparative genetic analysis of miRNAs in age related degeneration of both the human neocortex and retina has shown an increase in has-miR-146a, has-miR-125b, and has-miR-155. Preliminary transgenic models of Alzheimer's disease (Tg-AD) indicate the highest retinal abundance of Aβ42 peptides and highest deficits of CFH. We believe that there is a pathological connection in vision and/or visual processing disturbances in AMD and AD, and we hypothesize that the CFH mRNA is regulated by multiple miRNAs along the visual circuit. The objective of this work is therefore to effectively model and mine the regulatory pathways between both AD and AMD with the intent of discovering, evaluating, and gleaning valuable inferences on the potential links between AD and AMD.
Methods: The proposed computational framework consists of two modules. The first relies on the creation of an Interactome. We use a graph model to facilitate information representation without the loss of information. The creation of the interactome relies on the aggregation and integration of gene expression and miRNA expression data of both AD and AMD. The second module focuses on mining the created Interactome to identify genes-miRNA correlations and generate their functional significance. We use Bio2RFD in conjunction with the iProClass. Frequent pattern mining is used to find prominent patterns.
Results: We have been successful in the creation of the Interactome. Initial results of our frequent pattern mining of the interactome graph have indicated patterns of interest and require subsequent biological validation. We believe that - there exists sufficient patterns that support the claim that CFH mRNA regulates multiple miRNA's along the visual circuit.
Conclusion: The proposed study acts as a proof of principle in the creation of a unified framework that can support the inclusion of multiple data sources. We wish to extend this work to include the analysis of non-coding RNA sequences and discover correlation patterns between integenic sequences and gene expression patterns along the visual circuit of AMD.
Acknowledgement: Support from the National Institute of Health through the National Institute of General Medical Sciences Grant 8P20GN103424 is greatly acknowledged.

A 1.8- "The Effects of Residue Mutations on the Enantiospecificity of CYP2C9"
Trevor Meece[1], Grover P. Miller[2], Martin D. Perry, Jr.[1]
[1]Ouachita Baptist University, Arkadelphia, AR
[2]University of Arkansas for Medical Sciences, Little Rock, AR

Background: CYP2C9 is an enzyme that helps to detoxify many foreign molecules primarily in the liver. Previous research with CYP2C9 has shown that it has significant enantiospecificity in some of its residues, notably Leu366, Phe100, and Phe476. Understanding how important these residues are to the overall enantiospecificity of the enzyme and what properties that they have make them so important, will lead to a greater understanding of how the enzyme functions.
Methods: In order to obtain data on the residues in CYP2C9 cheaply and efficiently, we use a computer program called Sybyl-X. The data obtained from the program is theoretical and can be used to direct later studies in a biochemical lab. To determine the importance of the residues, we mutated them to amino acids with different properties and performed dynamics runs with NSAIDs similar to what was done in previous research in our lab.
Results: The residues that were deemed important to the enantiospecificity of CYP2C9 caused significant changes in the enantiospecificity of other residues after mutation. The mutated residues always showed differences from the non-mutated residues. In many cases, the mutation had such a significant effect that the enantiospecificity of the enzyme was changed.
Conclusion: The fact that the enantiospecificity of the residues were so greatly changed by the mutation of certain residues means that those residues are of particular importance to the overall enantiospecificity of CYP2C9. Mutations of certain residues show very specific effects. This data can be used to determine if these effects can be used synergistically with other mutations to create a desired effect on the enantiospecificity of CYP2C9 or even other properties.
Acknowledgment: This project was supported by the Arkansas INBRE program, with grants from the National Center for Research Resources - NCRR (P20RR016460) and the National Institute of General Medical Sciences - NIGMS (P20 GM103429) from the National Institutes of Health.

A 1.9- "Does the Gut Microbiome Mediate Associations of Menarche with Cardiovascular Disease Risk in Postmenopausal Women?"
Yan Wang, Robert Delongchamp, Mohammed El Faramawi, Mohammed Orloff, Jordana Bell, Tim Spector, Barbara Fuhrman
The University of Arkansas at Little Rock, Little Rock, AR

Early menarche is a known risk factor for cardiovascular disease (CVD). During childhood, the evolving gut microbiome may affect growth and development through its impact on energy balance and metabolism of endogenous hormones. In adulthood, it plays known roles in the development of CVD, as a modulator of the impact of diet on atherosclerosis and a cause of systemic inflammation. We hypothesize that the association of early menarche with CVD may reflect a shared cause, which is reduced gut microbial diversity. We have proposed to test this hypothesis in existing data from

the TwinsUK study, which includes 2747 adult female twins. They provided fecal samples for 454-pyrosequencing of microbial 16S rRNA, reported their age at menarche, and underwent measures of cardiovascular risk including blood pressure, lipids, waist circumference, fasting glucose and insulin. Microbial parameters will include measures of alpha diversity, beta diversity, and metabolic activity of the gut microbiome. We will use linear regression modeling to test whether microbiome measures are associated with menarche and then use mediation analysis to formally test whether the microbial measures mediate observed associations of menarche with cardiovascular risk markers.

A 1.10- "Discovery of Hidden Splice Junctions in Personal Reference Genomes"

Juw Won Park[1], Shayna Stein, Zhi-xiang Lu, Yi Xing
[1]The University of Louisville, Louisville, KY
[2]University of California, Los Angeles, CA

Background: It is very common that the alignment of RNA-seq reads to a genome reference produces a percentage of transcripts that are unmappable to the reference genome. The origin of those unmappable transcripts in the reference genome is often unknown. Unmappable transcripts could be from SNP (single nucleotide polymorphism) mutations in the individual which are hard to handle with the traditional RNA-seq alignment process.
Methods: By generating personal reference genomes that reflect SNPs in each individual, we developed a personal genome RNA-seq alignment method that detects the hidden splice junctions which exist in individuals but not in the reference genome.
Results: Our results show that there are personal splice junctions and many of them are from SNPs that create new canonical splicing sites GT-AG, AT-AC, or GC-AG. Further analyses on a cohort of 76 individuals suggest that many novel splice junctions have high frequency in a cohort.
Conclusion: This study shows that mapping RNA-seq reads to personal reference genome will find hidden splice junctions that are not annotated in the reference genome. Finding novel splice junctions is a good starting point towards further research on the effects of splice site mutations.

Cancer,
Poster Session A
Thursday, September 12, 11 a.m.-12:30 p.m.

A. 2.1- "MM-398 Accumulates in Metastatic Lesions and Prolongs Survival in an Experimental Model af Brain Metastases of Human Triple Negative Breast Cancer"

Afroz Shareef Mohammad, Chris E. Adkins, Emma Dolan, Emily Sechrest, Tori Terrell-hall, Paul Lockman
Department of Pharmaceutical Sciences, West Virginia University, Morgantown, WV

Background: 10-16% of women with advanced breast cancer will develop symptomatic brain metastases, the survival rate of which is less than 2 years. Due to the presence of the highly regulated Blood Brain Barrier (BBB), permeation of chemotherapy such as irinotecan is limited into these tumors, resulting in low survival rate. Conventional irinotecan has poor brain distribution in brain metastases; therefore, we hypothesized, MM-398, a liposomal irinotecan formulation will enhance irinotecan and its active metabolite SN38 exposure in brain metastases leading to increased cytotoxicity in brain tumors, which in turn increases the survival.
Methods: Female nude mice were intracardially injected with human brain seeking breast cancer cells (MDA-MB-231Br) and allowed it to metastasize. After confirming the metastases, the animals were administered with vehicle, irinotecan (50mg/kg), or MM-398 low and high doses (10mg/kg, 50mg/kg respectively) intravenously from day 21. Drug accumulation, tumor burden and survival were evaluated in the treated mice.
Results: MM-398 penetrates the blood-tumor barrier and accumulates in brain metastases. Treatment with MM-398 extended median survival time from 35 days in conventional irinotecan group to 51 days in MM-398 high group. Normalized BLI on day 43 showed significant reduction in tumor burden in both MM-398 groups when compared with both vehicle and conventional irinotecan group.
Conclusions: The liposomal formulation MM-398 penetrates and accumulates in the brain tumors and acts as depot for continuous and prolonged release of irinotecan and SN38. This elevated and prolonged tumor SN38 exposure after MM-398 administration appears responsible for increased survival in preclinical brain metastasis of breast cancer model.
Acknowledgement: This work was done in collaboration with Merrimack Pharmaceuticals.

A. 2.2- "Effects of Curly kale *Brassica oleracea var. sabellica* on Viability of Cultured Mouse Melanoma Cells"

Bilal Qizilbash
Qizilbash, LLC, Clinton, MS

The challenge with many cancers is not just killing the malignant cells, but doing so in a non-toxic manner. Plants are sources of many anti-cancer compounds, many of which have been developed into chemotherapies, *e.g.* taxanes from the bark of the Pacific yew tree and vinblastine from vincas. These chemotherapies are delivered in large doses and reduce tumor growth, but have severe side effects on normal tissue. Cruciferous vegetables such as broccoli, cabbage, and kale contain anti-cancer compounds which are being isolated and examined. Some of the compounds are anti-oxidants, while others have yet to be identified. Numerous studies have been conducted on curly kale, *B. oleracea sabellica,* to identify some of the compounds responsible for the health benefits of consuming the plant, in its raw or juiced form. Much of this research focused on sulforaphane, an isothiocyanate that is also found in foods such as broccoli, brussel sprouts, and cauliflower. Sulforaphanes, among other compounds, have been shown to decrease cell proliferation, reduce inflammation, and induce protective autophagy *in vitro*. There are no studies that have examined the effect of kale juice on cells. We hypothesize that the natural context of kale's bioactive compounds may provide significant anti-cancer effects. To test this hypothesis, kale juice was prepared and added to melanoma, epithelial, and fibroblast cells. Initially, four forms of juice were tested: juice made with a blender and three juices made with an electric juicer (juiced kale, juice that was filter-sterilized, and juice that was sonicated and then filter-sterilized). Serial dilutions were tested on B16F10 melanoma cells to determine the optimum dosage for inducing cell death. There was a dose-dependent decrease in cell growth and the lowest effective concentration was chosen for all subsequent experiments. The growth rate of cells treated with an equivalent amount of unfiltered lettuce juice was not different from the untreated cells. The sonicated and filter-sterilized extract also significantly reduced growth, but had different effects on melanoma and epithelial cells. When these experiments were repeated with non-cancerous cell lines, the juiced kale was found to be non-toxic to the epithelial cells and the fibroblasts at the dosage that kills melanoma cells. Future experiments will assess the safety and efficacy of kale juice for treating melanoma *in vivo.*

A 2.3- "Screening For Heterozygous Gene Mutations that Increase Loss of Heterozygosity in a Yeast System"

Cheyenne Hensley, Kellyn Hoffert, Erin Strome
Northern Kentucky University, Highland Heights, KY

Background: Loss of heterozygosity (LOH) is frequently observed in cancers. Using a yeast model system, we aim to discover heterozygous gene mutations that increase LOH events at the mating (MAT) locus. *Saccharomyces cerevisiae* is able to reproduce sexually in a haploid state as mating type a or α. After mating occurs, the diploid offspring are now heterozygous a/α for mating type and reproduction occurs asexually. LOH events will be identified based on diploid heterozygous *S. cerevisiae* deletion collection (SCDC) strains' ability to mate with haploid strains.
Methods: Two haploid strains were constructed: one of each mating type, each with the HIS3MX6 cassette knocked into the CAN1 gene, allowing strain growth on histidine deficient media. Strains from the SCDC are grown under nonselective conditions to allow LOH events to occur. Haploid strains are then introduced and mating can occur with diploids in which the MAT type locus is no longer heterozygous.
Results: Pilot studies have been run and protocols developed for timing of each step and recognition of LOH events. Systematic screening of the entire deletion collection has begun.
Conclusion: Human homologs to genes whose mutations result in high numbers of LOH events will be studied further for their contribution to cancer incidence.
Acknowledgement: Funding for this work was provided by a grant from Northern Kentucky University's Center for Integrative Natural Science and Mathematics (CINSAM).

A 2.4- "Folate-functionalized Polymeric Micelle for Combinatorial Therapy to Overcome Drug Resistant Breast Cancer"

Graham Temples[1], Wendy R. Cornett[2], Jeoung Soo Lee, PhD[1]
[1]Drug Design, Development and Delivery Laboratory, Department of Bioengineering, Clemson University, Clemson, SC
[2]Department of Surgery, USC Medical School, Greenville, SC

Background: Cancer continues to be one of the leading causes of death worldwide, with breast cancers accounting for nearly fourteen percent of all cancer related deaths in women.[1,2] A marker often overexpressed in breast cancers is folate receptor alpha (FA +), a protein with a limited distribution elsewhere in the body.[3] This potential expression differential was exploited as a means to selectively target breast cancer by conjugating a folate (FA) moiety to the surface of PgP (Poly (lactic-co-glycolic acid)-graft-polyethylenimine) micelle for targeted delivery of siRNA and chemotherapeutic agents.
Methods: The feasibility of PgP and FA-PgP as a nucleic acid carrier was evaluated using the Monster Green Fluorescent Protein phMGFP Vector (pGFP (Promega), 2 μg/well) in MCF-7, MDA-MB-435 [WT, ADR] (FA +) and MDA-MB-468 (FA -) cells in 10% serum-containing media. Additional transfections were performed in the presence of free FA in order to further characterize target specificity of FA-functionalized micelle.
Results: FA-PgP exhibited selectivity when comparing PgP transfection efficiencies in (FA +) and (FA -) breast cancer cell lines. Transfections with FA-PgP exhibited substantial decrease in efficiency compared to PgP in (FA-) cells, with less reductive effect noted in (FA +) cells. In the presence of free FA, transfection efficiency of both FA-PgP and mixed micelles were substantially decreased in (FA +) cell lines, but not in (FA -) cells.
Conclusions: Future work includes utilizing combinatorial therapy of therapeutically relevant siRNAs with chemotherapeutics such as Doxorubicin or Paclitaxel to overcome drug resistance in breast cancers.
Acknowledgement: Research reported in this publication was supported by NIGMS of the National Institutes of Health under award number 5P20GM103444-07.
References: 1. Sun et al, Nanoscale, 5:845-859 (2013) 2. Sayed et al, Ultrasonics, 53:979-91 (2013) 3. Hicks and Kulkarni, 129:263-273 (2008).

A 2.5- "Myxoma Virus Differentially Influences Human CD14$^+$ Myeloid Cells from Healthy Donors and Ovarian Cancer Patients"

Shana Chancellor[1], Jason Liem[1], Bernice Nounamo[1], Martin Cannon[1], Jia Liu[1,2]
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[2]Center for Microbial Pathogenesis and Host Inflammatory Response, College of Medicine, University of Arkansas for Medical Sciences (UAMS), Little Rock, AR

Background: Despite decades of treatment development, OC remains the most deadly gynecological malignancy among women in the US. The difficulty in OC treatment is largely due to the heterogeneity of the disease, but a common feature found among OC patients is the immunosuppressive tumor microenvironment. OC-associated CD14$^+$ myeloid cells play an important role in disease progression by regulating the immunosuppressive environment. One hallmark of this immunosuppressive function among these OC-associated CD14$^+$ cells is the non-canonical phosphorylation of STAT3 at the

Ser727 residue (PhosphoSer727). Myxoma virus (MYXV), a rabbit specific poxvirus, is a candidate of oncolytic therapy. M062R is a host range factor of MYXV that specifically antagonizes a human protein SAMD9. In this study, we examined the immunotherapeutic potential of WT and M062R-null virus in affecting OC-associated $CD14^+$ cells on cytokine expression and STAT3 PhosphoSer727 status.
Methods: Healthy donor $CD14^+$ and OC patient ascites-associated CD14+ cells were mock treated, or infected with either wildtype or an engineered MYXV for 16-18 hours. The supernatants from the infected were collected for Multiplex Immunoassay (eBioscience); while the cells were fixed, permeabilized, and stained with antibody recognizing STAT3 PhosphoSer727 for flow cytometry.
Results: MYXV infection in OC patient $CD14^+$ monocytes led to significant reduction in cytokines such as IL-10 and IL-6 but not IL-8 using the Multiplex Immunoassay. Infection with M062R-null MYXV stimulated cytokine secretion and antiviral gene expression in the healthy $CD14^+$ samples, while wildtype MYXV infection did not cause significant changes. In OC-associated $CD14^+$ patient monocytes, M062R-null infection ablated the STAT3 Ser727 phosphorylation.
Conclusions: This study shows that MYXV infection of ascites myeloid cells can reduce cytokines such as IL-10 and IL-6, both of which are important in maintenance of the immunosuppressive tumor microenvironment. In normal $CD14^+$ samples, while wildtype MYXV infection showed minimal response, M062R-null infection up-regulates cytokines and anti-viral gene expressions. M062R-null MYXV was able to ablate STAT3 Ser727 phosphorylation in patient $CD14^+$ monocytes, which can be linked directly to the activation of SAMD9 by M062R-null MYXV infection. Further studies will be conducted to determine the role of SAMD9 in the STAT3 signaling, as well as treatment benefit using M062R-null virus in a murine OC tumor model.
Acknowledgments: This work is supported in part by K22 AI099184, P20GM103625, and startup funds to JL at University of Arkansas for Medical Sciences (UAMS) College of Medicine Department of Microbiology and Immunology

A 2.6- "Silver Nanoclusters as Luminescence Probes"
Lauryn Ashford, William Gladney, Bidisha Sengupta Chemistry Department, Tougaloo College, Tougaloo, MS

Background: Water-soluble silver nanoclusters (Ag-NCs) were synthesized with single-stranded DNA as the scaffold matrix. The fluorescent Ag-NCs is formed in a slightly acidic aqueous solution where the C-rich DNA can form unusual i-motif structures, commonly found in human telomeres. Nicotine exposure via long-term smoking, nicotine replacement therapies or E-cigarettes- is associated with decreased renal function and accelerated progression of chronic kidney disease (CKD). Probing the modifications in biomacromolecules, induced by oxidative stress, is important.
Methods: Here we used DNA templated Ag-NC as luminescence probes to understand the structural alterations in DNA, caused by nicotine. We initiated studies with a 24 bases long single stranded DNA oligonucleotide in absence and presence of nicotine. Studies are performed using different concentrations of nicotine, DNA and silver ions. The influence of pH is investigated.
Results: The significant change in fluorescence properties of the nanoclusters in presence of nicotine implies the changes in DNA structure in presence of nicotine.
Conclusions: DNA template silver nanoclusters can be used as biological probes to understand oxidative stress in DNA molecules.
Acknowledgements: MS-INBRE grant number P20GM103476, MS-EpscoR grant # 0903787; HHMI grant # 52007562, at Tougaloo College.

B 2.7- DNA Templated Silver Nanoclusters as Fluorescence Probes for Oxidative Stress
Maria Muhammad, Corley Walters, Bidisha Sengupta Chemistry Department, Tougaloo College, Tougaloo, MS

Background: Nicotine exposure via long-term smoking, nicotine replacement therapies or E-cigarettes- is associated with decreased renal function and accelerated progression of chronic kidney disease (CKD). Probing the modifications in biomacromolecules, induced by oxidative stress, is important. Small silver clusters that form with short single stranded oligonucleotides are distinguished by their strong and unique spectroscopic properties. Here we used DNA templated silver nanoclusters as fluorescence probes to understand the structural alterations in DNA, caused by nicotine.
Methods: We initiated studies with a 28 bases long single stranded DNA oligonucleotide. This sequence form i-motif and is common at the telomeric regions of human chromosomes. The silver clusters are formed on the scaffold in absence and presence of nicotine. Studies are performed using different concentrations of nicotine, DNA and silver ions. The influence of pH is investigated.
Results and discussions: The remarkable fluorescence spectroscopic properties of silver nanoclusters is exploited, well, to understand the oxidative stress mechanism. DNA scaffolded silver nanoclusters can act as intrinsic probe to study

structural modifications of DNA in presence of nicotine.
Acknowledgements: MS-INBRE grant number P20GM103476, MS-EpscoR grant # 0903787; HHMI grant # 52007562, at Tougaloo College.

A 2.8- "Observations into the Role of the *MSH4-MSH5* Heterodimer in Yeast Genome Stability"

Olivia Davis, Miranda Combs, John Crum, Erin D. Strome
Northern Kentucky University, Highland Heights, KY

Background: Genome instability is a common phenotype of many cancers. Gene mutations that increase genome instability may act alone or in concert to alter lifetime cancer risk. The yeast model system *Saccharomyces cerevisiae* can be exploited for investigation of gene mutations that reduce chromosome fidelity, and thus whose homologs may be of interest in cancer progression. We investigated two candidate genes, *MSH4* and *MSH5*, to determine if mutations increase genome instability alone or in concert with checkpoint loss.
Methods: Strains heterozygously mutated for either *msh4* or *msh5* in both wildtype and *rad9*-deficient backgrounds were created to test haploinsufficient effects. Homozygously mutated strains are currently under construction. All strains are tested for both chromosome fragment and whole chromosome loss.
Results: Heterozygously mutating *msh5* significantly increases the rate of whole chromosome V loss in both strain backgrounds, whereas heterozygously mutating *msh4* results in a significant increase in chromosome loss only in a wildtype background.
Conclusion: This study shows that mutations in *msh4* and *msh5* significantly increase genome instability in *S. cerevisiae*. The increases in genome instability within the heterozygous strains demonstrate haploinsufficient effects of these genes, supporting investigation of their homologs as cancer susceptibility genes.
Acknowledgement: Research was supported by an Institutional Development Award (IDeA) under grant # P20GM1234 and student support was received through the FORCE grant # DUE-STEP-096928.

A 2.9- "Defining Mechanisms of Inhibin Action in Cancer"

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[1]Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC
[2]Department of Drug Discovery and Biomedical Sciences, South Carolina College of Pharmacy, Columbia, SC
[3]Davis Heart Lung Institute, College of Pharmacy, Ohio State University, Columbus, OH

Background: The transforming growth factor-β (TGF-β) superfamily members function to either suppress or promote cancer progression through mechanisms that remain to be fully defined. Inhibin is a TGF-β superfamily member that is elevated in the serum of patients with ovarian, prostate and lung cancers. However studies in Inhibin null mice indicate Inhibin as a suppressor of gonadal tumorigenesis. The Type III TGF-β receptor/ betaglycan is the only high affinity receptor molecule that has been shown to directly bind Inhibin, that does not induce signaling by itself, but is altered frequently in multiple cancer types both at the mRNA and protein level. The goal of these studies is to define the functional consequences of elevated Inhibin in cancers in the absence of betaglycan. Defining the specific role of Inhibin will allow us to therapeutically target Inhibin function effectively and prevent cancer progression and metastasis.
Methods: We used ovarian cancer cell lines SKOV 3 and OVCA 420 as models of high INHA producing cell lines. We determined the effect of Inhibin A (INHA) using either exogenous recombinant or using the conditioned media from ovarian cancer cell lines. shRNAs were used to reduce INHA expression where indicated. SMAD and non-SMAD pathways were examined in endothelial cells using a combination of western blotting and immunofluorescence. Endothelial cells employed include MEEC+/+ (Mouse embryonic endothelial cells) and HMvEC-1 (Human microvascular endothelial cells) and MEEC-/- (embryonic null for Endoglin). We examined angiogenesis in endothelial cells using Matrigel-based *in vitro* angiogenic assays.
Results: In our preliminary findings we find that Inhibin is largely expressed by epithelial cells and not by endothelial cells. Reducing Inhibin autocrine production in epithelial cells had a modest effect on cancer cell growth and *in vitro* invasion. However, loss of INHA reduced cell migration and colony formation on soft agar. Inhibin, due to its high affinity to betaglycan, has not been shown to induce any signaling in epithelial cells. Most strikingly however, we find that conditioned media from cells lines with high Inhibin production can induce SMAD1/5 activation in a paracrine fashion on endothelial cells. We find that Inhibin is an activator of endothelial cell signaling and angiogenesis specifically activating SMAD1 in a time and dose dependent manner and increasing endothelial tube formation *in vitro*. Inhibin can also activate non-SMAD pathways in endothelial cells including ERK1/2. The precise mechanism of action and effect of elevated INHA *in vivo* is currently under investigation.
Conclusion: Inhibin activity on endothelium is dependent on the TGF-β coreceptor Endoglin, as mice embryonic endothelial cells lacking Endoglin fail to respond to Inhibin induced signaling. Endoglin is abundantly found in endothelial cells and shares 71% sequence similarity and 63% sequence identity with betaglycan and is emerging as a marker for increased

angiogenesis in several cancers. Based on previous work and our preliminary data the *current working hypothesis is that Inhibin plays dichotomous roles in cancer acting as a tumor suppressor in the presence of betaglycan and loss of which results in switching Inhibin to a tumor promoter through paracrine effects on the endothelium to promote tumor angiogenesis and metastasis.*
Acknowledgment: This work was funded by NIH COBRE Grant P20GM109091-02. To KM (Target PI)

A 2.10- "The Effects of Cannabinoids on Tumor Vasculature in Ewing Sarcoma"
Rebekah Davis, RJ Quilao Jessie Little, Dr. Lori Hensley, Dr. Robert Griffin, Jessica Webber
Ouachita Baptist University, Arkadelphia, AR

Background: Ewing's sarcoma is the second most common pediatric bone cancer. With patients having a 5 year survival rate of 30%, alternative treatments must be developed. We studied the effects of various cannabinoids, particularly Ajulemic Acid (AJA) and Cannabidiol (CBD) on angiogenesis and tumor vasculature in models of Ewing's sarcoma.
Methods: We performed tumor insterstitial fluid pressure (TIFP) studies in mouse xenograft ES tumors, looking at the effects of cannabinoids on IFP over the course of 30 minutes. After 24 hours, we then homogenized the tumors, and performed an angiogenic array looking for significant differences in protein expression.
Results: The TIFP decreased significantly with CBD, showing a 41% decrease in tumor pressure. AJA also decreased TIFP by 27%, while the control did not affect the TIFP significantly. The tumor angiogenic array did not show substantial differences in the regulation of fifty-five angiogenic proteins.
Conclusion: Our study showed that AJA and CBD both have potential as anti-tumor therapeutics through their effects on tumor vasculature. A decreased TIFP is largely correlated with a more positive prognosis in cancer patients. Our previous studies also show that cannabinoids can inhibit angiogenesis. However, the mechanism is still largely unknown. Because there was no substantial difference in angiogenic protein expression, we are doing a follow-up study to see if cannabinoids are inducing apoptosis in endothelial cells, which would cause an inhibition of angiogenesis.
Acknowledgment: This work was funded by NCRR grant P20RR016460 and NIGMS grant P20 GM 103429 from the National Institutes of Health.

A 2.11- "Cannabinoids' Effects on the Vasculature of Ewing's Sarcoma Tumors"
R.J. Quilao[1], Rebekah Davis[1], Sydney Heslep[1], Jessie Little[1], Jessica Webber[2], Klressa Barnes[1], Robert Griffin[3], Lori Hensley[1]
[1]Ouachita Baptist University Department of Biology, Arkadelphia, AR
[2]University of Arkansas for Medical Sciences Department of Pathology, Little Rock, AR
[3]University of Arkansas for Medical Sciences Department of Radiation Oncology, Little Rock, AR

Background: Ewing's Sarcoma (ES) is the second most common pediatric bone cancer. Ajulemic acid (AJA) and cannabidiol (CBD) are two cannabinoid compounds with no known psychotropic effects. In this study, we conducted two experiments to investigate AJA and CBD's effects on vasculature and angiogenic potential of ES cells/tumors.
Methods: We conducted an angiogenic array assay on ES cell supernatants treated with 0μM, 5μM, and 10μM AJA. In a separate experiment, we measured the acute effects of AJA and CBD on tumor interstitial fluid pressure within xenograft ES tumors in nude mice. Change in TIFP was measured over thirty minutes using an SPR-671 Mikro-Tip catheter transducer.
Results: Our angiogenic array potentially identified TIMP1 as differentially regulated by AJA. We are currently further investigating this finding via ELISA assays. TIFP's in nude mice displayed an average drop of 27% and 41% with AJA and CBD, respectively. Sole administration of the vehicle produced no statistically significant change.
Conclusion: As elevated TIFP's are correlated with grim prognoses in cancer patients, AJA and CBD's drastic lowering of TIFP *in vivo* supports their validity as legitimate cancer treatments. With further studies, TIMP1's identification as a differentially regulated protein may help elucidate the mechanism by which cannabinoids affect ES tumor vasculature.
Acknowledgement: This project was supported by the Arkansas INBRE program, with grants from the National Center for Research Resources - NCRR (P20RR016460) and the National Institute of General Medical Sciences - NIGMS (P20 GM103429) from the National Institutes of Health.

A 2.12- "Association of Alcohol Consumption and Skin Allergy with Non-Melanoma Skin Cancer: Findings from the 2012 National Health Interview Survey"

Samuel Kakraba[1], Ke-Sheng Wang[2]
[1]UALR/UAMS Joint Graduate Program in Bioinformatics, University of Arkansas at Little Rock, Little Rock, AR
[2]Department of Biostatistics and Epidemiology, College of Public Health, East Tennessee State University, Johnson City, TN

Background: Little is known about the associations of alcohol consumption and skin allergy with non-melanoma skin cancer.
Objectives: In this study, we estimated the prevalence of non-melanoma skin cancer in the United States (US) adults and examined the associations of alcohol consumption, anxiety, smoking, skin allergy, obesity, and moderate activity with non-melanoma skin cancer. Gender, age group, education, and income were included as covariates.
Materials and Methods: This was a cross-sectional study (n= 33,865, 640 had non-melanoma skin cancer) from the 2012 National Health Interview Survey (NHIS) data. Weighted univariate and multiple logistic regression analyses were used to estimate the odds ratios (ORs) with 95% confidence intervals (CIs).
Results: The overall prevalence of non- melanoma skin cancer is 2.0% (2.0% for males and 2.1 % for females). The prevalence increased with age (1.2% and 5.6% for age groups 18-64 and 65+ years, respectively). Multiple logistic regression analyses showed that elder adults (65+ years) (OR=5.01, P<0.0001), having skin allergy (OR=1.61, P=0.0045), with no moderate activity (OR=1.32, P=0.0291), higher education status (OR=1.50, P=0.0003), current and former alcohol consumption (OR=1.62, P=0.0033 and OR=1.91, P=0.0021, respectively) and higher income (OR=2.09, P<0.0001) were all positively associated with non-melanoma skin cancer.
Conclusions: The prevalence of non-melanoma skin cancer was higher among US adults who were elderly (65+years), had skin allergy, with no moderate activity, higher education, and used alcohol status (formal or current). In view of the findings, effective strategies may be needed to manage the risk of developing non-melanoma skin cancer.

A 2.13- "Using Antibody and Aptamer Modified Gold Nanomaterials for Detection and Destruction of Prostate Cancer Cells"

Santanu Banerjee
Tougaloo College, Tougaloo, MS

Background: Prostate cancer is the most common malignancy among American men. Prostate cancer is also the second leading cause of cancer death among men. Currently available treatments are mostly ineffective in advanced stage cancers and cause severe side effects. As a result, new approaches to treat cancers that do not rely on traditional methods are essential. In this study, gold nanomaterials of different sizes and shapes with optical properties tunable in the near-infrared (NIR) window of biological tissues are utilized for the targeted sensing and hyperthermal destruction of cancer.
Methods: We use multimodal Gold Nanomaterials (GNP) for photothermal therapy of LNCap prostate cancer cells. We use nanotechnology based NIR light driven approach to selectively target and destroy low concentration cancer cells. For selective sensing, therapy and monitoring of therapy progress, we have conjugated gold nano-material by multiple Prostate Specific Membrane Antigen (PSMA) specific targets: 1) anti PSMA antibody and 2) Raman dye (Rh 6G) attached A9 RNA anti-PSMA aptamer. We use a 670 nm continuous wavelength diode-pumped solid state laser for Raman sensing of nanoparticle attached prostate cancer cells and for nano-therapy. We will use a continuous wavelength laser operating at 785 nm, as an excitation light source for photothermal therapy.
Results: Using Transmission Electron Microscopy (TEM) we show the PSMA and A9 Aptamer modified GNP Aggregation in presence of prostate cancer cells. We show a strong Surface Enhanced Raman enhancement on addition of 1000 LNCap cells/ml and the concentration dependence of Raman sensing to lower concentrations of a few hundred cells/ml. We also show the Surface Plasmon Resonance driven hyperthermal destruction of prostate cancer cells using 785 nm NIR laser.
Conclusion: This study shows that new approaches using modified GNP may be used for sensitive and selective sensing of LNCap prostate cancer cells. The prostate cancer cells may also be selectively destroyed using NIR lasers. The procedure is quite general and can be modified to treat other types of cancer and simultaneous detection and destruction of multiple cancer types.
Acknowledgment: This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of General Medical Sciences or the National Institutes of Health.

A 2.14- "Analysis of PARP1 Interactions with Cannabinoids"

Sydney Heslep, Lori L. Hensley, Martin D. Perry, Jr.
Ouachita Baptist University, Arkadelphia, AR

Background: Cannabinoids show promising results for the treatment of Ewing's Sarcoma (ES), a pediatric bone cancer with a low survival rate. Cannabinoids would be ideal drugs because they have been shown to relieve pain and inflammation, and induce apoptosis in ES cell lines without the controversial psychoactive effects. While there is a lot of promise with cannabinoids, the biochemical pathway is still widely unknown. PARP1, a protein expressed in ES cells that functions in DNA repair, is a promising target for the drug.

Methods: In order to test the interactions between the drugs and PARP1, a computational docking suite within SYBYL-X-2.1.1, is used to dock cannabinoids to the ligand-binding domain of PARP1. The results show binding between the protein and the drug. An ELISA is used to investigate the presence of cleaved PARP1 in cannabinoid-treated ES cells in order to elucidate the potential mechanism of cannabinoids for apoptosis.

Results: Surflex Docking shows similar hydrogen binding of PARP1 inhibitors and the glycine 202 residue of PARP1. ELISA methods show cleavage of ES cells with cannabidiol, hemp oil, and possibly ajulemic acid.

Conclusion: Cannabinoids have great potential for ES treatment because they have the ability to bind and cleave PARP1. When combined with chemotherapy, a drug that cleaves PARP1 would be a great candidate for Ewing's Sarcoma treatment to eliminate the ES cells and prevent their return.

Acknowledgment: This project was supported by the Arkansas INBRE program, with grants from the National Center for Research Resources - NCRR (P20RR016460) and the National Institute of General Medical Sciences - NIGMS (P20 GM103429) from the National Institutes of Health.

Cardiovascular Research, Poster Session A

Thursday, September 12, 11 a.m.-12:30 p.m.

A 3.1- "Myocardial Glycolytic Rate Regulates Exercise Capacity and Cardiac Hypertrophy"

Andrew Gibb, Aruni Bhatnagar, Bradford G. Hill
University of Louisville, Louisville, KY

Background: Regular exercise decreases all-cause mortality and improves quality of life, which are in part due to improvements in cardiovascular health. Frequent exercise enhances cardiac function by promoting cardiomyocyte growth and increasing perfusion, and is associated with increases in myocardial glucose and fatty acid utilization; however, it is unclear whether such changes in cardiac metabolism cause adaptations to exercise. In this study, we tested the hypothesis that exercise-induced increases in myocardial glycolysis are required for adaptations to exercise.
Methods: Adult, male FVB/NJ wild-type (WT) mice and transgenic (Tg) mice expressing a validated, cardiac-specific, dominant-negative form of phosphofructokinase 2 (kinase-deficient PFK2; kd-PFK2), which decreases myocardial glycolytic rate, were subjected to sedentary (SED) conditions or forced treadmill exercise (EXE) for 30 days. Exercise capacity and echocardiographic analyses were measured to assess whole-body and cardiac-specific adaptations to exercise. Indices of physiological cardiac growth and molecular adaptations were assessed by gravimetric, immunohistochemical, qPCR and immunoblotting techniques.
Results: In WT mice, exercise training increased running distance by 42% ($p<0.0001$) and work performed by 69% ($p<0.0001$). Exercise promoted cardiac growth (heart-to-tibia length: SED, 6.7±0.2 mg/mm vs. EXE, 7.7±0.4 mg/mm; $p<0.0001$), which was due to myocyte hypertrophy (myocyte cross-sectional area: SED, 461±43 mm^3 vs. EXE, 564±40 mm^3; $p<0.01$). Mitochondrial abundance and Akt phosphorylation were elevated significantly in the exercised heart. The kd-PFK2 Tg mice performed 27% less pre-training work (WT, 3.3±0.4 kg-m vs. kd-PFK2, 2.4±0.4 kg-m; $p<0.0001$) and 13% less post-training work (WT, 5.6±0.6 kg-m vs. kd-PFK2, 4.9±0.5 kg-m; $p=0.008$) compared with WT mice. Whereas WT mice showed an exercise-induced increase in stroke volume (SED, 27±3 µl vs. EXE, 32±5 µl; $p=0.004$), which was driven by increased end diastolic volume (SED, 38±3 µl vs. EXE, 45±5 µl; $p=0.001$), these indices of cardiac function were unchanged by exercise in kd-PFK2 Tg mice. In contrast to WT mice, the kd-PFK2 mice showed no increase in cardiac growth following 30 days of exercise training.
Conclusion: Diminished cardiac glycolytic rate appears to diminish cardiac and systemic adaptations to exercise.
Acknowledgment: This work was funded by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM103492.

A 3.2- "A Novel Maternally Restricted Pro-angiogenic Therapeutic for Preeclampsia"

Gene L Bidwell III, Heather Chapman, Fakhri Mahdi, Omar Logue, Eric M George
The University of Mississippi Medical Center, Jackson, MS

Background: Preeclampsia is a major obstetrical health concern, affecting 5-8% of all pregnancies. Hallmarked by hypertension and endothelial dysfunction the origin of the disease remains obscure, though it is generally accepted that placental insufficiency/ischemia is a central cause. In response, the placenta secretes pathogenic factors, in particular the anti-angiogenic protein sFlt-1. Currently, there is no effective therapy for the management of the preeclampsia patient. We have recently produced a novel synthetic peptide based on placental growth factor (PlGF) which is maternally restricted by fusion to the synthetic carrier elastin like polypeptide (ELP). Here, we describe its in vivo pharmacokinetics and biodistribution and demonstrate its ability to antagonize sFlt-1 activity.
Methods: Fluorescently labeled ELP-PLGF was administered i.v. and blood sampled serially to determine clearance kinetics. Long-term pharmacokinetics and biodistribution was performed after subcutaneous administration of labeled peptide. Measurements were made on serially drawn blood, and in the whole animal by in vivo imaging. ELP-PlGF activity was assayed by endothelial tube formation and proliferation by MTS assay.
Results: ELP-PlGF exhibited markedly more favorable pharmacokinetics than the normal half life of PlGF. Chronic administration found highest levels accumulating in placenta and kidney (two favorable targets for preeclampsia) and liver. A single subcutaneous administration at 100mg/kg resulted in sustained therapeutic plasma concentrations for over 10 days. In vitro, ELP-PLGF reversed the inhibition of both tube formation and proliferation by sFlt-1. Likewise the inhibition exhibited by culturing cells in serum from rats with placental ischemia was reversed by ELP-PlGF.
Conclusion: These data demonstrate that ELP-PlGF has favorable pharmacokinetic and biodistribution profiles. Furthermore, ELP-PlGF directly antagonizes sFlt-1 in culture, as well as the inhibitory effects of rats with placental ischemia-a model for human preeclampsia. Future studies to assess the in vivo effectiveness of ELP-PlGF in managing placental ischemia induced hypertension and endothelial dysfunction are currently in progress.
Acknowledgment: This work was supported by NIH

grants R0121527 (GLB), T32HL105324 (OCL), P01HL51971, P20GM104357 (EMG), and R00HL116774 (EMG).

A 3.3- Sildenafil Treatment is Protective Against Progression of Renal Injury in the Preeclamptic Dahl Salt Sensitive Rat

Ellen E. Gillis, Jennifer N. Mooney, Michael R. Garrett, Jennifer M. Sasser
Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS

Background: Preeclampsia, a hypertensive disorder of pregnancy, is characterized by transient glomerular damage and increased risk for kidney disease later in life. Recent studies in our lab have identified the Dahl salt sensitive (Dahl S) rat on a 0.3% NaCl diet as a spontaneous model of superimposed preeclampsia. In the current study, we hypothesized that sildenafil treatment would result in an improvement in renal function during the preeclamptic pregnancy exhibited by the Dahl S rat.
Methods: Female Dahl S rats (n=4-9) were mated, and after confirmation of pregnancy, rats were randomly divided into control and sildenafil treated groups. Sildenafil was administered to the treated group via food on gestational days 10-20 at a dose of 50 mg/kg/d. Rats were placed in metabolic cages on gestational day 19 for 24-hr urine collection, and blood and tissues were harvested on gestational day 20. Urinary protein excretion, nephrin excretion, and plasma and urinary creatinine concentrations were measured by Bradford assay, Exocell ELISA, and the picric acid method, respectively. Kidney sections were stained with Masson's trichrome, and glomeruli were measured (n=20 per rat) and analyzed using Nikon software.
Results: Sildenafil treatment significantly improved renal function, as observed in the increase in creatinine clearance and corresponding decrease in plasma creatinine (Table, *p<0.05 vs control). Treated rats exhibited less renal injury, with a significant decrease in proteinuria and nephrinuria during late pregnancy (Table). Histological analysis showed that the treated rats did not exhibit glomerulomegaly during pregnancy compared to the untreated controls (Table).
Conclusions: This study provides preclinical evidence that sildenafil prevents the progression of renal injury during preeclampsia.

A 3.4- "Cardiac SIRT1 Deficiency Causes an Impaired AMPK Signaling Response and Sensitizes Heart to Ischemia and Reperfusion Injury"

Lin Wang, Wanqing Sun, Nanhu Quan, Xingchi Chen, Courtney Cates, Ji Li
The University of Mississippi Medical Center, Jackson, MS

Background: Introduction: A "longevity" gene, sirtuin 1 (SIRT1) is a conserved NAD^+-dependent protein deacetylase that regulates life span, apoptosis, neuronal protection, cellular senescence. AMP-activated protein kinase (AMPK) is a conserved energy sensor that regulates cellular metabolism. Interestingly, AMPK and SIRT1 signaling pathways have common activators such as caloric restriction, oxidative stress and exercise. Hypothesis: Cardiac specific SIRT1 deficiency could impair ischemic AMPK activation and increase the sensitivity of heart to ischemic insults caused by ischemia and reperfusion.
Methods: Male $SIRT1^{flox/flox}$ mice and tamoxifen-inducible, cardiac-specific SIRT1 KO mice were subjected to I/R, which were induced by occluding the left anterior descending coronary (LAD) and subsequent releasing it. Echocardiography was used to monitor the heart function during I/R. The western blotting was used to characterize the signaling pathways.
Results: Adult cardiac-specific SIRT1 KO demonstrated significant cardiac hypertrophy, fibrosis and macrophage infiltration under normal physiological conditions versus the $SIRT1^{flox/flox}$ hearts by the immunohistochemical staining (all $p<0.05$). The cardiac functions measured by echocardiography and pressure-volume loop remained normal in SIRT1 KO mice as compared with $SIRT1^{flox/flox}$ mice under physiological conditions. The infarct size of SIRT1 KO hearts was 1.7-fold larger than that of $SIRT1^{flox/flox}$ hearts ($p<0.05$). The immunoblotting data demonstrated that SIRT1 KO hearts had impaired responsive activation of AMP-activated protein kinase (AMPK) signaling and augmented inflammatory NF-kappaB signaling during I/R versus the $SIRT1^{flox/flox}$ hearts. Furthermore, real-time qRT-PCR showed that cardiac SIRT1 KO resulted in up-regulation of pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin-1 β and growth differentiation factor15 (GDF15), as well as a heart failure marker ANP (atrial natriuretic peptide) versus $SIRT1^{flox/flox}$ hearts during I/R.
Conclusions: SIRT1 plays a role in regulation of AMPK signaling and inflammation induced by I/R. SIRT1 specific agonists may have therapeutic potential for treatment of ischemic heart disease.
Acknowledgment: This work was supported by American Heart Association 14IRG18290014, American Diabetes Association Basic Sciences Grant 1-14-BS-131, NIH R21AG044820 and R01AG049835, National Natural Science Foundation of China 31171121, 81471394 and 81500264.

A 3.5- "Subcellular Control of Calcium Influx in the Diabetic Vasculature"

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Background: Calcium influx in vascular smooth muscle via L-type voltage-dependent Ca^{2+} channels (LTCCs) initiates contraction and alters transcriptional activity. During acute hyperglycemia and diabetes, enhanced LTCC activity increases arterial myocyte $[Ca^{2+}]_I$ in a mechanism that involves cAMP-dependent protain kinase (PKA). Whereas increases in global cytosolic cAMP and PKA activity are classically associated with smooth muscle relaxation, we hypothesized that contractility during hyperglycemia relies on precise scaffolding of PKA in microdomains with plasmalemmal LTCCs by the scaffolding protein AKAP150.
Methods: Superresolution microscopy was used to examine the spatial relationship between LTCCs and PKA in wild type, AKAP150-null ($AKAP150^{-/-}$) and knock-in animals expressing mutant AKAP150 that is selectively unable to bind PKA (Δ36). In myocytes/arteries from these animals, we examined LTCC phosphorylation and activity, global intracellular $[Ca^{2+}]_I$, and arterial tone.
Results: We found that ablation of AKAP150 results in a loss of PKA catalytic subunits targeted (≤100 nm) to LTCC channels. Potentiation of LTCC-mediated current, elevation of arterial myocyte $[Ca^{2+}]_I$ and enhanced constriction associated with elevating extracellular D-glucose all required adenylyl cyclase and PKA activity and the presence of AKAP150. Elevated extracellular D-glucose was associated with an increase in phosphorylation at the putative PKA target residue serine 1928 of $Ca_V1.2$ in arterial myocytes from wild type, but not AKAP150-null mice. Moreover, loss of AKAP150-PKA interactions in Δ36 animals mimicked effects of global AKAP150 deletion.
Conclusion: Together, our results indicate an essential role for AKAP150-mediated compartmentalization of PKA activity in enhancement of vascular LTCC activity during hyperglycemia.
Acknowledgement: This work was funded by the American Heart Association 13POST12730001 and the National Institutes of Health R01 HL098200 and P20 GM103492 (to A. Bhatnagar).

A 3.6- "Protein Tyrosine Phosphatase 1B (PTP1B) Deficiency in Pro-Opiomelanocortin (POMC) Neurons Improves Glucose Tolerance but does Not Decrease Body Weight in Mice Fed a High Fat Diet"

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Background: Type 2 diabetes (T2D) accounts for 90-95% of all diabetes mellitus and is closely related to obesity. Treatment of T2D has been hampered by limited availability of effective antidiabetic drugs and the difficulty of achieving sustained weight loss. We previously showed that activation of leptin receptors in POMC neurons has a powerful antidiabetic effect in non-obese or type 1 diabetic rodents. However, obesity and T2D are associated with resistance to the antidiabetic effects of leptin as well as insulin. Although the mechanisms involved in leptin resistance are still unclear, one potential factor is protein tyrosine phosphatase 1B (PTP1B), a negative regulator of leptin and insulin receptor signaling.
Methods: In this study we tested the hypothesis that obese mice with selective deficiency of PTP1B signaling in POMC neurons ($PTP1B^{flox/flox}$/POMC-Cre; n=4) have improved glucose regulation and enhanced metabolic responses to hyperleptinemia compared to control obese mice ($PTP1B^{flox/flox}$; n=7) fed a high fat diet (HFD) from 6-22 weeks of age. A glucose tolerance test (GTT) was performed at 19 weeks of age and the mice were placed in specialized metabolic cages for continuous measurements of oxygen consumption (VO_2), motor activity and body weight. Blood samples were also analyzed at baseline, on the last day of leptin and after recovery for fasting glucose concentration.
Results: Compared with controls, $PTP1B^{flox/flox}$/POMC-Cre mice had improved glucose tolerance (AUC 8636±1539 vs. 5421±668 mg/dL x 120 min; *P*=0.04). The mice were also infused with leptin for 7 days (4 μg/kg/min) and changes in fasting blood glucose, VO_2, motor activity and body weight were measured. Compared with controls, $PTP1B^{flox/flox}$/POMC-Cre mice had decreased fasting plasma glucose after chronic leptin infusion (178±12 vs. 138±5 mg/dL; *P*=0.02), VO_2 was also significantly increased in $PTP1B^{flox/flox}$/POMC-Cre mice (64±5 vs. 89±9 ml/kg/min respectively; *P*=0.007) but this increase was not significant in controls (84±8 vs. 93±7 ml/kg/min, respectively). PTP1B/POMC-Cre mice also has significantly higher motor activity than $PTP1B^{flox/flox}$ (7499±1802 vs. 4644± 740.0 cm/day; *P*=0.001) but no difference in body weight reduction after leptin infusion (-2.5±0.3 vs. -2.7±0.7 g).
Conclusion: These results demonstrate that PTP1B deficiency in POMC neurons improves glucose regulation in obese mice fed a HFD. Furthermore, PTP1B deficient mice also demonstrated increased VO_2 and motor activity compared with controls during chronic hyperleptinemia. PTP1B could be an important drug target for treatment of T2D treatment via its effects to attenuate obesity-induced leptin resistance. |
Acknowledgments: This work was funded by NHLBI-PO1HL51971 and an Institutional Development Award (IDeA) from the NIGMS P20GM104357.

A 3.7- "Retinoic Acid and Sodium Butyrate Attenuate Renal Fibrosis and Inflammation in Guanylyl Cyclase-A/Natriuretic Peptide Receptor-A Gene-Targeted Mice"

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Background: Mice lacking functional guanylyl cyclase/natriuretic peptide receptor-A (GC-A/NPRA) gene (*Npr1*) exhibit hypertension, kidney disease, and heart failure. The objective of the present study was to elucidate the effect of all-trans retinoic acid (ATRA) and histone deacetylase inhibitor, sodium butyrate (NaBu) on attenuation of renal fibrosis and remodeling in *Npr1* gene-targeted mutant mice.
Methods: Mice (24-week old) were injected intraperitonealy with ATRA (0.5 mg/Kg/day), NaBu (0.5 mg/Kg/day), and ATRA-NaBu (1.0 mg/Kg/day) for 2-week.
Results: The results showed that *Npr1* gene-disrupted heterozygous (1-copy; +/-) mice exhibited renal fibrosis and augmented levels of proinflammatory cytokines compared with wild-type (2-copy; +/+) and gene-duplicated heterozygous (3-copy; ++/+) mice. Retinoic acid and NaBu treatment significantly lowered systolic blood pressure in 1-copy mice and increased plasma cGMP levels in *Npr1* gene dose-dependent manner with pronounced effects in 1-copy mice. Treatment with ATRA-NaBu combination synergistically attenuated renal fibrosis by 70%, immunoexpression of renal **α**-SMA by 75%, and plasma and renal levels of proinflammatory cytokines TNF-**α** and IL-6 in 1-copy mice compared with vehicle-treated control mice.
Conclusion: The present results provide direct evidence that ATRA and NaBu act as potent immunosuppressive and antifibrotic agents and repair the abnormal renal pathology in mice with reduced *Npr1* gene copy number, which will have important implications in prevention of hypertension-related renal pathophysiological conditions.
Acknowledgment: This work was funded by NHLBI Grants HL57531 and HL62147 (to K.N.P) and by an Institutional Development Award (IDeA) from the NIGMS under grant number P30GM103337.

A 3.8- "High Fat Diet Intake Differentially Affects Adipose Inflammation in Obesity-Prone and Obesity-Resistant Rats"

Stefany D. Primeaux[1,2], Flavia Souza-Smith[1], Jonquil Poret[1], Tony H. Tzeng[1], H. Douglas Braymer[2], Lisa M. Harrison-Bernard[1]
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Background: Excess visceral adiposity, rather than total adiposity, is associated with obesity-related increases in the risk for developing cardiovascular disease (CVD). As a consequence of expanding visceral adiposity, inflammation is prevalent and may link obesity and CVD. The current study tested the hypothesis that high fat diet (HFD) consumption leads to differential expression of inflammatory markers in visceral fat depots of obesity-prone, Osborne-Mendel (OM) and obesity-resistant, S5B/Pl (S5B) rats.
Methods: OM and S5B rats were fed either a HFD (60% fat; n=6) or low fat diet (LFD, 10% fat; n=6) for 7 weeks. As expected, body weight and % adiposity were increased by HFD intake to a greater extent in the obesity-prone OM rats. A multiplex rat cytokine/chemokine panel was used to assess the expression of inflammatory markers in epididymal fat and subcutaneous fat depots.
Results: Higher basal levels of Il-6, TNF-α, IL-1α, and IL-1β were detected in the epididymal fat depots of obesity-prone rats. Consumption of a HFD increased Il-6, TNF-α, IL-1α, and IL-1β in the epididymal fat of both obesity-prone and obesity-resistant rats. HFD intake increased TNF-α expression to a greater extent in obesity-prone rats. HFD consumption did not alter inflammatory marker expression in subcutaneous fat in either obesity-prone or obesity-resistant rats, though basal expression of IL-6, IL-1α, and IL-1β was higher in the subcutaneous fat of OM rats, compared to S5B rats.
Conclusion: An increase in pro-inflammatory cytokine expression in the visceral adipose depot, but not in the subcutaneous fat, following HFD intake supports the role of visceral fat in the deleterious effects of dietary fat. Higher basal levels of pro-inflammatory cytokines in the adipose tissue of obesity-prone rats suggests that these rats are at greater risk for developing comorbidities associated with obesity, including CVD.
Acknowledgment: This work was funded by COBRE 1P30GM106392-01A1.

A 3.9- "Diabetes Alters the Molecular Mechanisms Underlying Plaque Rupture"

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Background: Intimal thickening, a component of both the initiation of atherosclerotic lesion formation and subsequent plaque development, is increased in diabetic subjects. Our prior data suggest this increase is driven by changes in the levels of non-coding RNA that impact mRNA activity. We hypothesize that diabetes induces alterations in RNA levels in the vasculature that promote acceleration of intimal thickening through decreases in apoptosis and increases in proliferation and migration.
Methods: We obtained carotid plaque tissue from patients undergoing carotid endarterectomy (CEA) either before (Asymptomatic) or within 5 days (Urgent) of a cerebrovascular event (i.e. a plaque rupture) in diabetic and non-diabetic patients. We used RNA-Seq methodology to compare the changes in mRNA to determine if diabetes altered the molecular mechanisms underlying plaque rupture.
Results: We identified 464 RNA transcripts that were differentially expressed between the asymptomatic (n=5) and urgent (n=7) non-diabetic patients. Of these 263 were altered in the same direction when comparing asymptomatic (n=6) and urgent (n=6) diabetic patients. Surprisingly 201 of these transcripts were altered in the opposite direction. Differentially regulated transcripts suggested increased inflammation and decreased re-modeling following rupture in the diabetic group.
Conclusion: These data suggest that the molecular mechanisms underlying plaque rupture are altered by diabetes mellitus. Future studies aimed reducing the increased risk of myocardial infarction and stroke in the diabetic population may require consideration of these changes.
Acknowledgment: This work was funded by NIGMS Grant U54GM104940 and by an Institutional Development Award (IDeA) from the NIGMS under grant number P30GM103337.

A 3.10- "Role of Vimentin in Pluripotent Stem Cell Differentiation to the Endothelial Phenotype"

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Background: Pluripotent stem cells (PSCs), with the capacity to differentiate to endothelial cells, are an attractive cell source for vascular tissue engineering and regenerative medicine therapies. Vimentin, a cytoskeletal intermediate filament, is often used as a marker of endothelial cells and has been implicated in vascular function where mice lacking vimentin have vascular abnormalities. Vimentin expression has been observed to be low in PSCs and increases with differentiation, but its role in differentiation is still unclear.
Methods: Vimentin knockout (VIM-/-) and wild type (WT) mouse PSCs were spontaneously differentiated as embryoid bodies (EBs). EB size was quantified using Image J analysis of phase images. Gene and protein expression for mesodermal (FLK1) and endothelial (TIE2, PECAM1, VE-CAD) markers was evaluated using quantitative PCR and immunocytochemistry, respectively.
Results: Unlike WT PSCs, VIM-/- PSCs did not spontaneously aggregate to form EBs, but instead required forced physical aggregation using microwell systems. Upon subsequent suspension culture, VIM-/- EBs had delayed growth kinetics as evaluate by size and a disrupted outer epithelial layer compared to WT controls. We also found that the lack of vimentin resulted in lower gene and protein expression of FLK1, TIE2, PECAM1, and VE-CAD.
Conclusion: This study shows that vimentin may be critical in PSC differentiation to an endothelial phenotype. As a result, the intermediate filament vimentin may be not only a structural cytoskeletal protein but a regulator of differentiation. This increased understanding of the cytoskeleton will help develop protocols for the efficient biomanufacturing of endothelial cells.
Acknowledgment: This work was funded by a COBRE grant from NIH NIGMS (#P20 GM103629).

A 3.11- "The Cardiac Deficiency of Pyruvate Dehydrogenase Complex Alters Cardiac Glucose Oxidation and Sensitizes Heart to Ischemic Insults"

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Background**:** Pyruvate Dehydrogenase Complex (PDHc), plays a key role in aerobic energy metabolism, occupies a central crossroad of glycolysis, and the tricarboxylic acid cycle. Our preliminary data showed that knock down PDHc in the heart causes higher mortality and significant hypertrophy.The glucose metabolism modulated by PDHc in the heart is critical for cardiac functions. The objective is to characterize the signaling mechanisms by which PDHc modulates glucose metabolism under physiological and pathological conditions.
Methods: Wild type or cardiac PDHc deficiency C57BL/6 mice were subjected to left anterior descending coronary artery (LAD) occlusion for ischemia and reperfusion (I/R) in order to detect the signaling activity in the left ventricle. The ex vivo working heart system was used for measuring substrate metabolism in the perfused hearts.
Results: The cardiac specific PDHc deficiency significantly increased myocardial infarction after

ischemia and reperfusion (I/R) ($p<0.01$ versus WT groups). Interestingly, the immunohistochemistry data showed that cardiac PDHc deficiency increased macrophage infiltration ($p<0.01$ vs. WT groups). Moreover, the staining of wheat germ agglutinin (WGA) and Masson trichrome revealed a decrease in hypertrophy and fibrosis in the PDHc knock down hearts ($p<0.05$ versus WT hearts). Furthermore, in a working heart perfusion system, we measured the substrate metabolism of hearts. During ischemia and reperfusion, glucose oxidation rate demonstrated significantly impaired in the cardiac PDHc deficiency hearts as compared to WT hearts, while the PDHc activator, dichloroacetate (DCA), can augment the glucose oxidation rate in WT hearts during ischemia and reperfusion. The AMP-activated protein kinase (AMPK) signaling can lead to an increased glucose metabolism under ischemic stress in the heart. Intriguingly. The immunoblotting data demonstrated that cardiac PDHc deficiency attenuates ischemic AMPK activation while DCA treatment enhances the ischemic AMPK activation.

Conclusions: The present results strongly suggest that cardiac PDHc deficiency cause an impaired ischemic AMPK signaling response that could sensitize heart to ischemic injury induced by ischemia and reperfusion.

Acknowledgment: This work was supported by American Heart Association 14IRG18290014, American Diabetes Association Basic Sciences Grant 1-14-BS-131, NIH R21AG044820 and R01AG049835, National Natural Science Foundation of China 31171121, 81471394 and 81500264.

Cell Signaling,
Poster Session A
Thursday, September 12, 11 a.m.-12:30 p.m.

A 4.1- "Creation of a NPC1L1 Knockout Using CRISPR Technology in Danio rerio"
Christian Pritchard, Benjamin Duncan, James Walters
Bluefield State College, Bluefield, WV

Background: Atherogenic dyslipidemia affect millions of people around the world. We investigated the uptake of cholesterol into the larval zebrafish intestines. NPC1L1 is the main cholesterol transport protein of intestinal enterocytes and NPC1L1 variants may impact cholesterol absorption. We are establishing an *npc1l1-/-* line in zebrafish using CRISPR methodologies. We will ultimately investigate how a putative low-absorption variant (**Δ**R110H) of *npc1l1* affects dietary cholesterol uptake in zebrafish. CRISPR/Cas9 constructs will be used to target specific sites within the genome of *Danio rerio* and knockout the gene.
Methods: The CRISPR targeting plasmid is assembled in a one-step digestion/ligation reaction and will produce Cas9 mRNA. Two plasmids containing the Cas9 protein and an *npc1l1* gRNA target sequence were injected into single cell embryos.
Results: After injection, the gRNA and newly made Cas9 protein should combine to form the Cas9 duplex. We will screen for frame shift mutations resulting in early stop codons within the *npc1l1* coding sequence.
Conclusion: The CRISPR method of producing frame shift mutations is an established and efficient method for creating knock-outs in zebrafish and can be utilized to establish a line of *npc1l1-/-* (null background) zebrafish. Future experiments will expose *npc1L1-/-* and TL (wild-type) strains to compare how cholesterol absorption is affected by low and high fat diets.
Acknowledgements: funded by NIH Grant P20GM 103434 to the West Virginia IDeA Network for Biomedical Research Excellence

General Biomedical Sciences, Poster Session A

Thursday, September 12, 11 a.m.-12:30 p.m.

A 5.1- "Regulation of Air Sac Primordium Development by a Cathepsin-L in *Drosophila melanogaster"*

Ajay Srivastava, Qian Dong, Breanna Brenneman, Christopher Fields
Department of Biology and Biotechnology Center, Western Kentucky University, Bowling Green, KY

Background: In *Drosophila*, the adult thoracic Air Sacs supply oxygen to the flight muscles. These Air Sacs develop from a group of larval cells that form the Air Sac Primordium (ASP). The ASP is located superficially over the larval wing imaginal disc and in response to Fibroblast Growth Factor (FGF) signal, migrates and invades into the wing imaginal disc to occupy a more deep seated position. The regulation of invasive behavior during ASP development is not well understood. In this talk data will be presented that implicate a Cathepsin-L in ASP development and invasive cellular behavior.
Methods: Utilizing immunohistochemistry and confocal microscopy we assessed the expression of Cathepsin-L in larval wing imaginal discs. The knockdown of Cathepsin-L in ASP was achieved by UAS-Gal4 driven RNAi and the overexpression of Cathepsin-L was performed using the UAS-Gal4 system of gene expression in *Drosophila*.
Results: We found that a single Cathepsin-L is upregulated in the ASP and downregulation of this gene results in aberrant ASP development, loss of Filopodia from ASP and suppression of invasive behavior. Furthermore, we demonstrate that overexpression of Cathepsin-L results in degradation of the Basement Membrane (BM).
Conclusion: Our data demonstrate that a Cathepsin-L is involved in the development of ASP by regulating the invasive cellular behavior and by degrading the BM. These data are particularly significant given that Cathepsin-L is also expressed during early human lung development and invasive cellular behavior and degradation of the BM are critical events during tumor metastasis.
Acknowledgment: Research in my laboratory at WKU is supported by the WKU Department of Biology startup funds, WKU Research Foundation RCAP-I grant # 11-8032 and by a KBRIN-AREA grant funded through a parent grant from the National Institute of General Medical Sciences of the National Institutes of Health under award number 5P20GM103436-13. This study was also supported in part by a WKU graduate school grant to QD.

A 5.2- "Dual Function Sers Active Nanoparticle Platform For Melamine Sensing"

Anant K. Singh, Yolanda Jones
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In recent years, there were two reported outbreaks of food borne illness associated with melamine. Melamine alone is of low toxicity; however it is able to form an insoluble complex with cyanuric acid, which can lead to kidney toxicity. The presence of melamine and its related compounds in milk, feed, and other foods has resulted in the need for reliable methods for the detection and accurate identification of this class of contaminants. Surface-enhanced Raman spectroscopy (SERS) has lagged behind other analytical techniques utilized in routine chemical analyses despite the information-rich spectra produced. This can be attributed in part to the difficulties in finding effective substrates that provide high sensitivity and highly reproducible SERS responses. Here in we have developed a chemically stable highly efficient SERS oxide plasmonic gold nano particle hybrid. Experimental data with melamine show that the SERS enhancement factor for hybrid platform is about two orders of magnitude higher than only nanoparticle. Our results demonstrate that in hybrid SERS platform, grapheme oxide will enhance the SERS signal via chemical enhancement. We show that hybrid SERS platform can be used for highly selective and ultra sensitive detection of melamine in parts per quadrillion level. Our results provide a good approach for developing ultra sensitive SERS platform for toxin chemical detection and analysis.
Acknowledgement: This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476

A 5.3- "Metabolic Dysfunction Due to a High Concentration of Dietary Fish Oil"

Alessandro Subauste, Simran Batth, Omonuwa Adah, Robert L. Hester, Angela Subauste
The University of Mississippi Medical Center, Jackson, MS

Background: Dietary supplementation with fish oil has multiple, well established health benefits. While fish oil supplementation reduce the risk of cardiovascular disease by approximately 10% it has not been shown to have a consistent effect on incident diabetes. Indeed some studies have shown an increase in incident diabetes. In this study we determined the metabolic effect of a diet high in fish oil in a mouse model of diet induced obesity.
Methods: C57Bl6 mice were fed a high-fat diet (HFD:60% fat) ad libitum from 6 weeks of age. At 12 weeks the HFD mice were either left on HFD or

switched to an isocaloric HFD-containing 30% fish oil (FO). The animals were fed for an additional 10 weeks during which mice from each diet were exercise trained for the last 8 weeks. At the end of the study mice underwent a glucose tolerant test and determination of running capacity.
Results: The body weights in exercise trained HFD groups was significantly less as compared to the FO diet exercised mice (35.3 g vs 39.8 g $p<0.05$). FO fed mice run a shorter distance to exhaustion and attained lower speeds (38.1 vs 29.75 m/min $P<0.05$) when compared to the HFD mice. After a glucose challenge FO mice had a significantly higher blood glucose when compared to HFD.
Conclusion: A diet with 30% of fish oil worsens glucose tolerance, decreases running capacity and dampens the weight loss effect associated with exercise.
Acknowledgement: This work was funded by the Department of Medicine, UMMC.

A 5.4- "Differential Effects of Sildenafil and Tadalafil in Human Penile Smooth Muscle Cells: New Insights for Old Mechanisms"

BM Rezk[1], AA Moustafa[2], P Sangkum[2], ZY Abd Elmageed[2], S Sikka[2], Abdel AB Mageed[2], WJG Hellstrom[2]
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[2]Department of Urology, Tulane University, School of Medicine, New Orleans, LA

Background and Objective: Normal penile smooth muscle structure and function are necessary for the initiation and maintenance of erection. Improvement in the relaxation of the cavernosal smooth muscle via phosphodiesterase 5 inhibitors (PDE5i) is attributed to the inhibition of PDE5 enzyme resulting in accumulating of cGMP and reduction of cytosolic calcium. This study investigates the additional mechanism(s) of the effects of sildenafil and tadalafil on human penile smooth muscle cells.
Materials and Methods: Primary human corpora cavernosa smooth muscle cells (HCCSMC) were isolated from penile tissues. HCCSM cells (passages: 3-7) were seeded in petri dishes (1 x 106/ml) for 24 hr. Then, the cells were treated with 100 μM of sildenafil or 100 μM tadalafil for 4 h and 24 h. Results: HCCSMC showed an elevation of mRNA expression of nNOS with 100 μM of sildenafil (19.4 ± 7.4, $p = 0.035$) compared to tadalafil (8.5 ± 7.4, $p = 0.19$). However, the mRNA expression of endothelial eNOS was slightly downregulated with sildenafil (0.64 ± 0.22, $p = 0.17$) while, tadalafil induced insignificant increase in the mRNA expression of eNOS (2.8 ± 1.6, $p = 0.19$). Although both sildenafil and tadalafil are phosphodiesterase 5 inhibitors, the PDE5 mRNA expression increased after treatment with sildenafil (2.15 ± 0.53, $p = 0.20$) but decreased with tadalafil (0.37 ± 0.2, $p = 0.12$). On the other hand, levels of cGMP were significantly elevated with sildenafil (1.82 ± 0.23 pM/mg protein compared to control 1.28 ± 0.15 pM /mg protein with p value of 0.04). However, tadalafil showed no effect on the cGMP levels compared to control. PKG mRNA expression levels were increased 6.88 ± 3.41 fold with sildenafil ($p = 0.08$), and 2.3 ± 1.07 fold with tadalafil ($p = 0.2$).
Conclusion: These findings suggest differential effects of sildenafil and tadalafil on HCCSMC. Sildenafil elevates mRNA levels of nNOS and PKG and cGMP protein levels. However, tadalafil enhances eNOS mRNA expression while, downregulating PDE5 mRNA. These need further investigation possibly using in vivo approaches.
Significance: Erectile dysfunction (ED) is a common medical disorder that has a negative impact on the quality of life men. The results of this study highlight for the first time the differential effects of sildenafil and tadalafil on HCCSMC. The effect of sildenafil is primarily through elevation in nNOS, cGMP and PKG. However, tadalafil enhances SMC relaxation via elevation of mRNA expression of eNOS and downregulation of mRNA of PDE5.

A 5.5- "Silver Clusters Inhibit the Biofilm Formed by *Bacillus Thuringiensis*"

Bidisha Sengupta[1], Christa Corley[1], Keith Cobb, Jr.[1], Sudarson Sinha[2], Elrica Brown[3], Paresh Ray[2], Bianca Garner[3]
[1]Chemistry Department, Tougaloo College, Tougaloo, MS
[2]Chemistry Department, Jackson State University, Jackson, MS
[3]Biology Department, Tougaloo College, Tougaloo, MS

Background: Biofilms are catenation of bacteria on a surface, which are caged by the extracellularly secreted proteins, carbohydrates, and/or DNA. The phenotypes of these multicellular aggregates are distinct from those of planktonic cells. The biofilm formation is resistant to anti-microbial agents, which give rise to chronic bacterial infections and death in human beings. The present study is designed to explore the effect of silver nanolcusters on the biofilm formation by the bacteria *Bacillus Thuringiensis*.
Methods:The silver clusters was made on the single stranded DNA oligonucleotide CCCACCCACCCTCCCA without and with a 5' aptamer attached, in distilled water using $AgNO_3$ and $NaBH_4$ as the reducing agent. UV/Vis absorption and fluorescence emission studies characterized the absorption and emission properties. Bacterial growth and biofilm (using crystal violet stain) assay were performed in 96 well plates at 600 nm and 540 nm, respectively. Gram staining confirmed the purity of the samples. Transmission electron microscopy (TEM) was performed on each sample.

Results: Silver nanoparticles formed on the cell membranes of the bacterial samples grown in presence of $AgNO_3$, but no biofilm was observed in samples treated with Ag+ or silver nanoclusters. MTT assay, TEM and light microscopic studies confirmed that as little as 2 μM of Ag^+ in the form of nanoclusters can prevent biofilm formation.
Conclusions: The present study proved the efficiency of silver as a potent anti-biofilming agent and opens a new door to the future perspectives for its possible usage in therapeutics. Further studies are underway.
Acknowledgements: MS-INBRE grant number P20GM103476, MS-EpscoR grant # 0903787; HHMI grant # 52007562, at Tougaloo College.

A 5.6- "Antibacterial Activity of the Novel C5-Curcumin-2-Hexadecynoic Acid Conjugate"

David J. Sanabria-Ríos, Yaritza Rivera-Torres, Joshua Rosario, Ricardo Gutierrez, Yeireliz Torres-García, Nashbly Montano, Gabriela Ortíz-Soto, Eddy Ríos-Olivares, José W. Rodríguez, Néstor M. Carballeira
The Inter American University of Puerto Rico, Metropolitan Campus, San Juan, PR

Background: Infectious diseases caused by multi-drug resistant bacteria (MDRB) add substantial costs to the nation's health care system since these infections require prolonged and expensive treatments, extended hospital stays, additional physician visits, and healthcare use, which results in increased rate of disability and death. Despite the fact that several compounds are being evaluated as antibacterial agents, it is urgent to develop new antibiotics. Among the compounds that are being evaluated, curcumin (Curc) has demonstrated to have both anticancer and antibacterial properties. Although preclinical and clinical studies have shown that Curc is not toxic against normal human cells, several pharmacokinetics disadvantages, such as poor bioavailability, fast metabolism and the requirement of repetitive oral doses has been reported, which limits its pharmacological applications. In this study, the first synthesis of C5-Curcumin-2-hexadecynoic Acid was C5-Curc-2-HDA) conjugate was successfully performed aimed at improving the antibacterial activity of C5-Curc.
Methods: The C5-Curc-2-HDA conjugate was obtained in 13 % overall yield through a three-step synthesis and tested for antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) strains using antibacterial susceptibility tests. In addition, mechanistic studies involving *S. aureus* DNA gyrase inhibitory tests were performed to further understand the mode of action of C5-Curc-2-HDA.
Results: Experimental results revealed that C5-Curc-2-HDA conjugate was active against eight MRSA strains at MICs that range between 31.3 and 62.5 μg/mL, demonstrating that the chemical connection of 2-HDA to C5-Curc enhances the antibacterial activity. In addition, it was investigated that conjugate C5-Curc-2-HDA conjugate can affect the replication process of *S. aureus*, since this compound inhibited the supercoiling activity of the *S. aureus* DNA gyrase at minimum inhibitory concentrations (MIC) of 250 μg/mL (IC_{50} = 100.2 ± 13.9 μg/mL). Moreover, it was observed that the presence of 2-HDA in C5-Curc-2-HDA conjugate improves the anti-topoisomerase activity of C5-Curc towards *S. aureus* DNA gyrase, which is in agreement with results obtained from antibacterial susceptibility tests involving MRSA strains.
Conclusions: This study clearly demonstrated that the chemical connection of 2-HDA to C5-Curc in C5-Curc-2-HDA conjugate improves both the antibacterial activity of C5-Curc against MRSA strains and its inhibitory effect in inhibiting the supercoiling activity of DNA gyrase, an important enzyme involved in the DNA replication process in bacteria.
Acknowledgement: This work was funded by the National Center for Research Resources and the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 5 P20 GM 103475-13. This project also was supported in part by the PRCTRC Grant through the Grant Numbers U54 RR026139 and 8U54MD 007587-03, and National Institute on Minority Health and Health Disparities of the National Institute of Health through the Grant Number 8G12MD007583-28.

A 5.7- "Expanding the Repertoire of Amino Acid and Nucleic Acid Analogues for Use in the Synthesis of Novel Peptides"

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Background: *Aminoacyl-tRNA synthetases (aaRSs) are a critical family of translation factors. These enzymes are responsible for linking amino acids (AAs) with the cognate tRNA to produce aminoacyl-tRNAs (aa-tRNAs). The purified recombinant elements (PURE) system is a cell free translation system capable of utilizing the plasticity of AARS substrate recognition to produce peptides containing nonstandard AAs. To date, approximately 75 unnatural AA substrates have been incorporated into peptides using the PURE system. Building on this platform, we have expanded our list of substrates to include additional AA substrates such as phosphatidylserine (PS) and nucleic acid substrates such as peptide nucleic acids (PNAs).*
Methods: *PS was examined as an AA substrate for seryl-tRNA synthetase (SerRS). A hybrid nucleic acid duplex composed of a peptide nucleic acid (PNA) and RNA was developed to mimic the acceptor stem of $tRNA^{Ala}$. This hybrid duplex was examined as a substrate for alanyl-tRNA synthetase (AlaRS).* Both

substrates were examined using an α^{32}P-radiolabled assay. This assay monitored the formation and decomposition of the aminoacyl adenylate (aa-AMP) high energy intermediate.
Results: Preliminary results suggest that both PS and the RNA/PNA hybrid are viable substrates for SerRS and AlaRS, respectively. In the presence of SerRS the assay clearly showed formation of PS-AMP and subsequent decomposition upon $tRNA^{Ser}$ addition. Similarly, charging of the PNA/RNA hybrid duplex was evidenced by the decomposition of Ala-AMP in the presence of AlaRS.
Conclusion: PS and the PNA/RNA hybrid duplex have been identified as possible substrates for incorporation into the PURE system.
Acknowledgment: This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

A 5.8- "Impaired Glucose Metabolism in the TREML-1 null mice: A link to Obesity"
Elizabeth Castro-Rivera, A. Valance Washington
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Background: The triggering receptor expressed in myeloid cells (TREM) like transcript-1 (TLT-1) is a putative type-1 receptor found inside platelets alpha-granules of both mice and humans that is released to the surface upon platelet activation. TLT-1 has both membrane bound and soluble forms (sTLT-1), with the latter been found to enhance platelet activation as well as platelet-endothelial cell interactions. Because of its location and release mechanism, TLT-1 is not found in the plasma of healthy individuals. Therefore, we hypothesize that increased levels of sTLT-1 in plasma during the progression of any specific disease implicates TREML-1 may be playing a role. Recent work from our laboratory has associated TREML-1 as a potential target for intervention during atherosclerosis, diabetes mellitus (DM) and obesity. Using our *treml1*$^{-/-}$ mice model, previously shown to have a bleeding predisposition in response to an inflammatory challenge during experiments involving sepsis patients, we crossed it onto the *apoe*$^{-/-}$ background to yield the *apoe*$^{-/-}$/*treml1*$^{-/-}$(DKO) mice. These DKO mice demonstrate to have higher cholesterol and fat percentage, than *apoe*$^{-/-}$ controls all when on a high fat diet. A previous Single Chain Polymorphism, shows that a causative SNP (rs34254490) is associated with DM, this supports the idea that TREML-1 is important in glucose regulation.
Methods: We performed both intraperitoneal (IP) glucose and insulin tolerance test (GTT or ITT) on fasting 6-9 week old *treml1*$^{-/-}$, *treml1*$^{+/-}$, *apoe*$^{-/-}$, DKO and wt mice. Serum insulin levels where measured with ELISA from fasting 6-14 week old *treml1*$^{-/-}$, *treml1*$^{+/-}$, and wt mice.
Results: Evaluation of basal plasma glucose shows a significant difference in glucose levels that correlates with gene dosage. Although the *treml1*$^{-/-}$ mice present a decreased in glucose levels, the DKO mice show a more prominent decrease in comparison to *treml1*$^{-/-}$, *treml1*$^{+/-}$, and *wt* mice. An IP-GTT revealed significant differences in plasma glucose clearance, further analysis of total area under curve (AUC) revealed a significant increase in *wt* control mice relative to DKO, *treml1*$^{-/-}$ and *treml1*$^{+/-}$ mice. Evaluation of serum insulin via an ELISA revealed no significant differences between *treml1*$^{-/-}$, *treml1*$^{+/-}$, and *wt* mice. Interestingly, an IP-ITT revealed *treml1*$^{-/-}$ and *treml1*$^{+/-}$ mice present an apparent insulin hypersensitivity that's represented by the steady glucose decline 15 minutes post injection, whereas *apoe*$^{-/-}$ and DKO mice express a steady glucose decline seconds after injection; all with no signs of ameliorating the effects of insulin after 2 hours.
Conclusion: DKO and *treml1*$^{-/-}$ mice show clear inverse relationships between glucose levels and insulin function, and suggest that insulin hypersensitivity is responsible for the rapid processing and the lower levels of blood glucose seen in these mice. Suggesting possible problems in glycogen breakdown and/or adrenocorticotropic hormone regulation of gluconeogenesis. These data support a model where glucose, once taken in by cells, is moved toward storage leading to lipid spill over and the obese phenotype observed in our mice.
Acknowledgment: Support for this project was obtained through grants 2P20GM103475, G12RR-03035, 8U54MD007587-03, and 1R01HL090933-01A2.

A 5.9- "FEA of Orthopedic Implants towards the Development of Optical Strain Sensors for Fracture Fixation"
Hunter Pelham, Melissa M. Rogalski, Nakul Ravikumar, John D. DesJardins, Jeffrey N. Anker
Clemson University, Clemson, SC

Background: Orthopedic implants provide alignment, stability, and load support to fractured bones during bone healing. Our goal is to create an optical strain measuring device integrated within orthopedic screws to monitor the load sharing between the implant and the bone to track bone healing. We use optical fringes to translate strain into color changes that can be detected through tissue with a spectrometer.
Methods: We use finite element analysis to evaluate strain along the length of orthopedic screws used in fracture fixation of the tibia under various loading conditions. We have also performed tensile testing of orthopedic screws with integrated optical sensors.
Results: FEA results along the length of the orthopedic screws show a dominance of axial strain compared to radial strain in almost all loading conditions. Results from tensile testing of optical

strain measuring screws show an 83% color change at a maximum load of 1500 N with high repeatability.
Conclusion: The current design of our optical strain measuring orthopedic screw is appropriate due to the dominance of axial strain on the screws. The high amount of repeatability in our strain measuring system is also required in any measurement tool. Additional mechanical testing for other loading conditions is required.
Acknowledgment: This work was supported by *NIGMS* of the National Institutes of Health under award number 5P20GM103444-07.

A 5.10- *"Spatial Control of Shoc2-Mediated ERK1/2 Signaling Requires Remodeling Activity of the Atpase PSMC5"*

Eun Ryoung Jang, HyeIn Jang, Ping Shi, Gabriel Popa, Myoungkun Jeoung, Emilia Galperin
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Background: Shoc2 is the scaffold protein that accelerates the ERK1/2 signaling pathway in response to growth factors. Initially identified in C. elegans as SUR-8/SOC2, Shoc2 is a critical positive regulator of the ERK1/2 signaling pathway that integrates the Ras and RAF-1 components of the ERK1/2 pathway into a multi-protein complex. Mutations in Shoc2 result in Noonan-like RASopathy, a developmental disorder with a wide spectrum of symptoms. The amplitude of the ERK1/2 signals transduced through the complex is fine-tuned by the HUWE1-mediated ubiquitination of Shoc2 and its signaling partner RAF-1. However, the mechanistic basis of how ubiquitination of the Shoc2 scaffold and RAF-1 is controlled is unknown.
Methods: Biochemical approaches (Yeast two-hybrid screening assays, sucrose gradient subcellular fractionation, immunoprecipitation, western blot analysis, immunofluorescence staining, and etc.) together with the cell-based assays (Cell growth and trans-well assays) were utilized in this study.
Results: We demonstrate that PSMC5 triggers translocation of Shoc2 to endosomes and then displaces the E3-ligase HUWE1 from the scaffolding complex to attenuate ubiquitination of Shoc2 and RAF-1. We show that Noonan-like Rasopathy mutation that changes the subcellular distribution of Shoc2 lead to alterations in Shoc2 ubiquitination due to the loss of accessibility to PSMC5.
Conclusion: Our results demonstrate that PSMC5 is a novel critical player involved in regulating ERK1/2 signal transmission through the remodeling of Shoc2 scaffold complex in a spatially-defined manner. This study makes a significant advance in our understanding of how the ERK1/2 signaling pathway is governed by the critical scaffold Shoc2. Furthermore, this study describes a novel mechanism of how scaffolds can regulate specificity and dynamics of cellular networks through remodeling mechanisms.
Acknowledgment: This work was funded by National Institute of General Medical Sciences (P20GM103486), the National Cancer Institute (R00CA126161 to EG), the National Institute of General Medical Sciences (P20GM103486), the American Cancer Society (RSG-14-172-01-CSM to EG) and from American Heart Association (15PRE25090207 to HJ).

A 5.11- "Transposable Element LINE-1 in Response to Ionizing Radiation"

Isabelle R. Miousse, Lijian Shao, Rupak Pathak, Jianhui Chan, Etienne Nzabarushimana, Sara Prior, Alan Tackett, Martin Hauer-Jensen, Daohong Zhou, Igor Koturbash
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Background: Ionizing radiation (IR), aside from its genotoxic potential, can also target the cellular epigenome, DNA methylation, in particular. Accumulating evidence indicates that alterations in global DNA methylation caused by IR are primarily associated with the methylation of repetitive elements. LINE-1 (L1) is the most abundant repetitive element that comprises 17-23% of mammalian genomes. Earlier considered as "junk DNA," L1 is now a well-accepted driving force of evolution and a critical regulator of the expression of genetic information. Recent advances in computational biology allowed classifying L1 elements according to their unique 5'-UTR sequences and evolutionary age.
Methods: We used the mouse model (C57BL/6J male mice) to investigate the mechanisms of LINE-1 response to exposure to ionizing radiation, and modulation of radiation response upon administration of methionine-deficient and methionine-supplemented diets.
Results: We report that L1 methylation status is not uniform between the families and is dependent on L1's evolutionary age. Furthermore, IR selectively alters methylation of primarily evolutionary young, rather than old, L1 elements. At the same time, we show that DNA methylation, however, is not a universal mechanism of regulation of L1 expression and loss of DNA methylation is not a necessary pre-requisite of L1 reactivation. Finally, we show that methionine/choline dietary modifications may alter the methylation and expression status of L1 and its response to IR.
Conclusion: Altogether these findings suggest that the methylation and expression status of certain L1 families may be used as a biomarker of radiation exposure and dietary modifications may have significant potential to modulate the cellular response to IR via targeting L1 elements.
Acknowledgement: This work was funded by NIH NIGMS Grant 1P20GM109005.

A 5.12- "Characterizing Modulators of the L1 Endonuclease"

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Background: Long interspersed element-1 (L1) is the only active autonomous retroelement in the human genome. Studies characterizing L1 function show that the L1 endonuclease activity is associated with the formation of DNA double strand breaks (DSBs). While L1 mutagenic insertion events have been associated with diseases such as breast and colon cancer as well as muscular dystrophy, the full effect of L1 endonuclease upon stability of the genome is undetermined. Data suggests that the damage from L1 endonuclease activity may be greater that previously considered seeing as though only a small fraction of L1-induced DSBs results in a retrotransposition event.
Methods: We have utilized an in vitro fluorescence based assay to identify novel small molecule inhibitors of the L1 endonuclease. Short term and long term cellular viability assays were also used to determine the toxicity of the drugs to cells. Standard retrotransposition assays were performed to determine if the drugs prevent LINE1 retrotransposition.
Results: We have identified three small molecules that inhibit L1 endonuclease activity in our fluorescence-based assay. The drugs also prevent L1 retrotransposition but do not appear toxic under the conditions examined.
Conclusion: This study shows that novel small molecule inhibitors have been identified that can be further modified to create potent and selective inhibitors of the L1 endonuclease.
Acknowledgment: This work was funded by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM103424 and from the Louisiana Cancer Research Consortium, the NIH-RCMI grant #8G12MD007595-05.

A 5.13- "NHR-80 is Required for Oxygen-Deprivation Tolerance in Aging *Caenorhabditis elegans"*

James Mercado, Jo M. Goy
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Background: The number of aged people (>65 years) in America recently passed 13% providing incentive to identify physiological processes that regulate healthspan. We previously showed age to be a factor in *C. elegans* tolerance of Oxygen-Deprivation (OD) stress. Wild type *C. elegans* exposed to OD show an increase survival rate at adult day 3 and 5 compared to day 1, indicating age affects OD tolerance. Lipid metabolism is a regulator of healthspan. A screen for genes required for OD tolerance in *C. elegans* identified the lipid desaturase genes *fat-5/6/7*.
Methods: To investigate the interaction between age and lipid metabolism we asked if lipid desaturases are required for aging worms to tolerate OD stress. The nuclear hormone receptor *nhr-80* regulates expression of *fat-5/6/7* and is required for normal lifespan. Loss of *nhr-80* results in elevation of saturated fatty acids. To determine the role of lipid desaturation in OD tolerance aging *nhr-80(tm1011)* were exposed to 72h of OD stress.
Results: Following a 24h normoxic recovery animals were scored as unimpaired (move normally) or impaired (abnormal movement) or dead (no movement if touched with a pick). At day one *nhr-80(tm1011)* survival rates were not different from expected for wild type (N2). However, at adult day three and five *nhr-80* survival was less than expected for wild type (day 3: 10% and 57% and day 5: 33% and 74%, respectively). We noted that *nhr-80* mutants survived OD stress better as they aged, perhaps due to reproductive senescence or fat desaturation demand.
Conclusion: Our data suggest that fatty-acid desaturation is necessary for aging *C. elegans* to tolerate oxygen-deprivation stress. To further investigate the role of NHR-80 in OD stress tolerance we will compare survival rates in *glp-1(e2141)* mutants known to be OD tolerant with *(glp-1(e2141);nhr-80(tm1011)* double mutants. We expect that loss of NHR-80 will suppress OD tolerance in *glp-1(e2141)* mutants.
Acknowledgement: This work was funded by a McNair Scholar's Program Fellowship, Harding University (to JM), a Beth Baird Scholarship Award, University of North Texas (to JMG) and the Department of Biology, Harding University.

A 5.14- "NaDC1 Knockout: Effects on Blood Pressure and Urine pH"

Federico Jose Teran[1,2], Weitao Huang[1], L. Lee Hamm[1,2,3], Kathleen S. Hering-Smith[1,2].
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Background: NaDC1 reabsorbs filtered citrate (Cit); thus its regulation is important in preventing calcium nephrolithiasis. Importantly NaDC1 reabsorbs other Krebs cycle intermediates such as succinate (Suc) and αKG. Recently Suc and αKG have been found to be important in paracrine signaling as their luminal presence stimulates distal nephron G protein coupled receptors GPCR91 and GPCR99 respectively. Luminal Suc via GPCR91 has been found to stimulate

renin release; while αKG via GPCR99 stimulates bicarbonate secretion by type B intercalated cells. The purpose was to determine whether knockout of NaDC1 produces hemodynamic or urine pH changes that might be anticipated by activation of these receptors.
Methods: Adult NaDC1 KO, heterozygous (Het), and wild type mice (WT), under normal or acid diet for 72 hr, were studied using standard clearance techniques. After anesthesia direct measurements of hemodynamic parameters were determined in real time. Urine, blood and tissue were collected for measurement of Suc, Cit, αKG, and pH.
Results: NaDC1 KO produced 2, 4, and 10 fold increases in urinary Suc, Cit, and αKG, respectively. There was substantial residual reabsorption of Cit and Suc in KO mice, indicating other transporters involved in reabsorption of these substrates. Despite the increase in urinary αKG there was no significant increase in urine pH (normal or acid diet); in fact on normal diet urine pH was lower in KO mice (5.41 ± 0.04 vs 5.90 ± 0.13 WT; $p < 0.001$). The lower urine pH in KO mice on normal diet may be a response to loss of potential bicarbonate in the form of increased Krebs cycle intermediate excretion. There was no change in the ability of KO animals to excrete ammonia. In regard to potential hemodynamic effects mediated by NaDC1 KO and increased urinary Suc, no change in MAP was determined comparing WT, Het or KO either on normal or acid diet.
Conclusions: In sum, NaDC1 is responsible for significant reabsorption of filtered αKG and Suc, but knockout of NaDC1 does not impair acid-base homeostasis or BP changes on normal or acid diets.
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A 5.15- "Spectral Rulers for Non-invasively Monitoring Load-Sharing Changes between Orthopedic Fixation Devices and Healing Bone"
Melissa M. Rogalski, Nakul Ravikumar, Hunter Pelham, Donald Benza, Joshua Lake, John D. DesJardins, Jeffrey N. Anker
Clemson University, Clemson, SC

Background: *In vivo* strain measurements are critical for assessing load-sharing between implanted fixation devices and healing bone fracture. We have developed luminescent spectral rulers to non-invasively evaluate load-sharing by measuring micron scale displacements through tissue.
Methods: The spectral rulers measure relative motion between two substrates: an "encoder" patterned with alternating luminescent lines and a transparent "analyzer mask" patterned with opaque lines that overlay and mask a portion of the encoder. Moving the encoder with respect to the analyzer generates a color change that is detected by spectrometer. To monitor strain through tissue we fabricated 2 sensors, an X-ray excited optical luminescent (XEOL) sensor and a fluorescent sensor. The XEOL sensor contains an X-ray scintillator film (Gd_2O_2S:Eu) overlaid with a dye patterned encoder. A portion of the Gd_2O_2S:Eu luminescence spectrum is absorbed by the dye in a position-dependent manner. The fluorescent sensors are excited with a 633 nm laser and are patterned with spectrally distinct CdSeS/ZnS core shell quantum dots.
Results: The spectral rulers have measured displacements in the range of ~15 microns to 2 mm through 6 mm thick chicken breast tissue.
Conclusion: The sensors provide a non-invasive method for measuring micron scale displacements through tissue.
Acknowledgment: This work was supported by *NIGMS* of the National Institutes of Health under award number 5P20GM103444-07.

A 5.16- "Sds-Page Buffer Can Be Reused For Multiple Times, But Not Towbin Transfer Buffer In Western Blotting"
Oluwabukola Omotola, Rajiv P. Heda, Jamie Avery-Holder, Ghanshyam D. Heda
Mississippi University for Women, Columbus, MS

Background: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting are two of the most commonly used biochemical methods for protein analysis. Proteins are electrophoretically separated based on their molecular mass by SDS-PAGE, and then electro-transferred to a solid membrane surface for subsequent protein-specific analysis by western blotting. Both of these procedures use a salt-based buffer with later procedure consisting of 10-20% methanol as an additive. Methanol serves in removing SDS from the protein bands as well as facilitate their binding to membrane, however, known for its toxicity. Reusing of Towbin Transfer Buffer (TTB) may help reduce this toxic waste. Previous reports present a contradictory view in favor [Pattegrew et al, 2009] and against [Y. Dorri et al, 2010] reusing of TTB, and hence is a subject of debate.
Methods: In this report, we present a detailed analysis supported by scanning data and their statistical analysis of not only reusing TTB but also SDS-PAGE buffer. Both buffers were saved at the end of each run and were reused for subsequent SDS-PAGE and/or western blotting.
Results: Our results suggest that SDS-PAGE buffer can be reused at-least for 5 times without compromising the electrophoretic separation of a

broad range proteins markers in the range of 10-260 kDa. Successive multiple use of TTB on the other hand, however, diminished the signal of a high molecular weight plasma membrane protein called CFTR in western blotting.
Conclusions: SDS-PAGE buffer can be used at-least for 5 times without any loss in terms of quality and separation of proteins. Reuse of TTB on the other hand reduced the signal of CFTR protein in western blotting procedure.
Acknowledgements: This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM103476 and Faculty Research Award to GDH.

A 5.17- "Nuclease Stability of Intramolecular Four Way Junctions and DNA-PNA Four Way Junctions"

Paul J. Stoulig III[1], Tamara Bell[2], Sana Solanja[1], Doug Iverson[1], Anthony Bell Jr.[1]
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[2]Alcorn State University, Lorman, MS

Background: High Mobility Group B1 (HMGB1) is an abundant multifunction protein that is responsible for critical nuclear and extracellular functions. In the nucleus, HMGB1 binds preferentially to bent and cruciform DNA such as four way junctions (4WJs) to control transcription, chromatin remodeling, and DNA recombination and repair. Alternatively, HMGB1 has been found to function in the extracellular matrix (ECM) where it functions as a redox sensitive cytokine that signals proinflammatory responses. This response occurs in a variety of diseases and conditions such as cancer, lupus, rheumatoid arthritis, and sepsis. Hence, HMGB1 garners a great deal of attention as a disease biomarker and therapeutic target.
Methods: Our long-term goal is to investigate intramolecular (IM) 4WJs and hybrid 4WJs composed of DNA and PNA (peptide nucleic acids) as high affinity ligands against HMGB1. Our initial investigations have focused on the nuclease stability of these 4WJs against nucleases: Deoxyribonuclease I (DNase I), Exonuclease III (ExoIII), Lambda Exonuclease (Lambda Exo) nuclease digestion. DNase I is an endonuclease that cleaves DNA to produce oligonucleotides with 5'-phosphorylated and 3'-hydroxylated ends. ExoIII is the coordinated removal mononucleotides from 3'-hydroxyl termini of DNA. Lambda Exo catalyzes the removal of 5' mononucleotides from duplex DNA, working in the 5' to 3' direction.
Results: Preliminary data shows that IM 4WJs and the blunt-ended PNA junction (bPNA1,3:4WJ) composed of multiple PNA strands are more resistant to DNase I and Exo III vs the intermolecular DNA control, J1. Conclusion: Future studies will focus on the serum stability of hybrid PNA-DNA and IM DNA 4WJs.

A 5.18- "Stimulating Effect of Advanced Glycation End Products on Angiotensinogen Expression in Renal Proximal Tubule Cells"

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Department of Physiology and Hypertension and Renal Center of Excellence, Tulane University School of Medicine, New Orleans, LA

Background: Elevated plasma and tissue concentrations of advanced glycation end products (AGEs) are seen in hyperglycemic individuals and are implicated in renal dysfunction in diabetes mellitus (DM). Furthermore, intrarenal renin-angiotensin system, including proximal tubular angiotensinogen (AGT) augmentation, is also a key risk factor in the development of diabetic nephropathy. There is evidence that AGEs augment AGT expression in podocytes. However, stimulating effect of AGEs on AGT expression in proximal tubular cells (PTC) has not been established although these cells are the primary source of intrarenal AGT. Therefore, this study was performed to determine if AGEs increase expression of AGT in PTC.
Methods: To show elevation of intrarenal AGT and AGE levels in DM, urinary AGT and AGE levels in streptozotocin (200mg/kg)-induced DM mice were determined by ELISAs. The stimulating effect of AGEs on AGT expression was tested by using cultured rat PTC receiving 0-200 μg/ml AGE-BSA treatments for 24 hours. AGT mRNA, intracellular AGT protein, and secreted AGT levels were measured by real-time RT-PCR, western blot analysis, and ELISA, respectively.
Results: Urinary AGT and AGE levels were concomitantly greater in DM mice compared to control mice (AGT: 21.6±5.5 ng/day vs. 190.1±57.8 ng/day, AGE: 139.1±21.6 ng/day vs. 332.8±102.7 ng/day). Direct treatment of PTC with AGE-BSA increased levels of AGT mRNA (3.43±0.11-fold compared to control), intracellular AGT protein (3.60±0.38-fold), and secreted AGT (2.11±0.18-fold). Non-glycated BSA serving as a negative control did not alter AGT levels. Expression of AGE receptor in cultured PTC was demonstrated by western blot analysis and immunocytochemistry. Adding recombinant soluble AGE receptor, which competes with AGE receptor on plasma membrane, to culture medium resulted in attenuation of the AGE-induced AGT augmentation, suggestion that AGE-BSA stimulates AGT expression via activation of AGE receptor. Enhanced phosphorylation of ERK1/2, but not p38 MAP kinase, was observed in AGE-BSA-treated PTC.
Conclusion: The results indicate that AGEs directly increase AGT expression levels in PTC. In the system, ERK1/2 may play a role as a signal transducer. The findings suggest that elevated AGEs

can contribute to intrarenal AGT augmentation as well as hyperglycemia-induced AGT upregulation in DM and consequent development of diabetic nephropathy.
Acknowledgment: This work was supported by grant from the National Institute of General Medical Sciences IDeA Program (COBRE, P30GM103337). Joseph M. Garagliano was recipient of the Bourgeous Medical Student Research award.

A 5.19- "Role of Conserved Components of Germ Cell Organelles in Germline Development in *Drosophila"*

Samuel J. Tindell, Jimiao Zheng, Nhan Huynh, Ming Gao, Tanyaradzwa Katanda, Bishal Shrestha, Hieu D. L. Vo, Alexey L. Arkov
Murray State University, Murray, KY

Background: Germ cells eventually give rise to egg and sperm and therefore, they are responsible for the next generations. In many organisms, germ cells contain electron-dense organelles referred to as germ granules. Germ granules consist of RNA and proteins required for germline development. We have characterized novel interacting components of the granules.
Methods: Using chemical crosslinking approaches, we identified germ granule components which associate with scaffold Tudor protein required for germline development.
Results: Unexpectedly, we found that glycolytic enzyme Pyruvate Kinase directly associates with Tudor in germ granules. In addition, we showed that glycolytic enzymes play a specific role in the protection of germline DNA from mutations caused by mobile genetic elements (transposons). Interestingly, we demonstrated that Pyruvate Kinase contains a specific methylated arginine residue recognized by Tudor. Similar to Pyruvate Kinase, Tudor interacts with another component of germ granules, Piwi protein Aubergine, and we are currently investigating the structure and function of this multi-component complex in vitro.
Conclusion: Based on our characterization of novel interacting components of germ granules in germline development we are reconstituting distinct molecular complexes in vitro to determine their specific role in the granules.
Acknowledgment: This work was funded by NSF CAREER grant award MCB-1054962 and by a KBRIN Investigator Development Award (IDeA) from the NIH National Institute of General Medical Sciences under grant number 8P20GM103436-14

A 5.20- "Investigation of Phospholipids as Novel Reagents to Isolate Labile Protein-Nucleic Acid Complexes"

Vaniecia Wilson, Jalisa Keys, Alexis L.N. Sanders, Andrea Phang, Douglas Iverson, Anthony J. Bell Jr. , Ph.D.
The University of Southern Mississippi, Hattiesburg, MS

Background: High Mobility Group B1 (HMGB1) is an abundant multifunctional or "moonlighting" protein that controls critical nuclear and extracellular reactions. In the nucleus, HMGB1 mediates transcription by "loosening" or remodeling chromatin. During chromatin remodeling, HMGB1 binds and bends DNA to expose transcription factor binding (TF) sites that are recognized by TFs such as nuclear factor-kB. Hence, a great deal of effort has been put forth to define HMGB1-DNA binding mechanisms.
Methods: Electrophoretic mobility shift assays (EMSAs) have been used extensively to measure the binding affinity (K_D) of HMGB1 and its subunits (HMGB1a and HMGB1b) toward bent DNA substrates such as four-way junctions (4WJs).
Results: These studies show that the HMGB1a and HMGB1b bind 4WJs with a binding stoichiometry of four to one (4:1). A 4:1 complex consists of four protein monomers bound to one junction that migrate in EMSAs as a single species. The intermediate binding species (3:1, 2:1 and 1:1) are labile complexes that are not detected by EMSA or analytical ultracentrifugation. Here, we use competition EMSAs with a known HMGB1b extracellular substrate phosphatidylserine (PS) to isolate HMGB1b:J1 intermediate binding species. Preliminary data shows that PS can be used to detect 3:1 and 2:1 complexes.
Conclusion: Future studies will focus on optimizing the PS conditions to determine the ideal ratio of each component (HMGB1b, 4WJ and PS) to more accurately characterize HMGB1b:J1 binding mechanisms.
Acknowledgment: This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

A 5.21- "Total Synthesis of δ-Tocotrienol"

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Background: Vitamin E is a group of lipid-soluble compounds including four tocopherols (α, β, γ, δ) and four tocotrienols (α, β, γ, δ). The tocotrienols have attracted increased attention recently as they have been shown to have some biological effects not seen

with the tocopherols. It is very difficult and costly to isolate tocotrienols in pure forms from natural sources. A number of total syntheses of tocotrienols and tocopherols, including chiral resolution approaches and enantioselective approaches, have been reported. However, none of the reported synthetic routes are efficient/economical for generating substantial amount of products. Therefore, the purpose of our research is to develop an efficient and scalable synthetic route for the synthesis of tocotrienols.
Methods: We used δ-tocotrienol as a synthetic target to develop an efficient route that should be applicable to the synthesis of other tocotrienol homologues. The chromanol ring of δ-tocotrienol was assembled via Heck reaction between 2-bromo-6-methylhydroquinone and a chiral synthon derived from (-)-linalool, followed by pyran ring formation. The farnesyl side chain was assembled through Horner–Wadsworth–Emmons (HWE) olefination.
Results: δ-Tocotrienol was synthesized in high overall yield. Spectral data of the synthetic compound were in agreement with the isolated δ-tocotrienol from annatto oil. The synthesis of the key intermediate was highly efficient; however, further optimizations were needed for some of the remaining steps. Overall, the synthetic route was economical and should be applicable in large scale synthesis.
Conclusion: We developed an efficient and scalable synthetic route for δ-tocotrienol. The synthetic route is applicable to other three tocotrienols and Vitamin E derivatives such as (*S*)-γ-CEHC. In addition, advanced intermediates from this synthetic route could be used for the synthesis of tocotrienol analogues.
Acknowledgment: This work was supported by National Institute of General Medical Sciences of the NIH under grant number P20 GM109005.

Genomics,
Poster Session A
Thursday, September 12, 11 a.m.-12:30 p.m.

A 6.1- "Identification of Branch Points in Mirtrons"
Britton Strickland, Jaaved Mohammed, Matthew DeCruz, Mosharrof Hossain, Alex Flynt
The University of Southern Mississippi, Hattiesburg, MS

Abstract: Small RNA molecules called microRNAs are important regulators of gene expression in multi-cellular organisms. MicroRNAs are a heterogeneous group of gene products that can be generated by many pathways. Mirtrons are a class of microRNAs produced by splicing. During RNA splicing, introns are cut from the pre-mRNA and form a loop-like "lariat" structure, which is created when the 5' end of the intron is attached to a site in the intron called the branch point. Location of branch points in mirtrons have yet to be studied or identified, and the goal of this project is to uncover the contribution of branch point location to the processing of mirtrons into functional gene regulators. This project approaches this issue from two directions: using computational identification of branch points in genome wide datasets and a polymerase chain reaction (PCR) based branch point mapping strategy. We have identified the branchpoint of miR-1017 through computational analysis of specialized genome-wide RNA-seq datasets. Using the PCR based strategy, we have experimentally isolated, mutated, and verified the branchpoint location of mir-1017, and northern blotting further explained our findings. We will expand this analysis to other species of mirtrons with the aim of identifying similarities in the different lariat RNAs and their function in gene regulation.

A 6.2- "The UMMC Molecular and Genomics Core Facility: A Resource for Genomic Technology throughout Mississippi and Beyond"
Ashley C. Johnson[1], Zhen Jia[1], Michael R. Garrett[1, 2]
[1]Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS
[2]Director, UMMC Molecular and Genomics Core, University of Mississippi Medical Center, Jackson, MS

Background: The University of Mississippi Medical Center (UMMC) Molecular and Genomics Core Facility (MGCF) provides centralized access to molecular and genomics expertise and services for the University and external researchers. In recent years, it has become evident that a scientific approach using high-throughput genomic technologies, such as microarray and next-generation sequencing (NGS) can provide an enormous capacity to understand the complex interaction of biological systems associated with living organisms, human health, and disease.
Methods: The MGCF is currently supported through three extramural funding mechanisms (2 COBRE and Mississippi-INBRE) which provides highly competitive prices to researchers. The mission of the MGCF is to serve as a nucleus to develop research and educational programs to increase the competitiveness and enhance biomedical discovery of researchers. The MGCF is equipped with several genomics platforms, including Affymetrix 3000 7G and GeneAtlas Instruments for whole genome expression profiling and an Illumina MiSeq and NextSeq500 for small RNA sequencing (miRNA), ChIP-Seq, amplicon sequencing, mammalian level RNA sequencing, and whole genome sequencing.
Results: The MGCF provides the following services to generate high quality genomic data for researchers: (1) sample preparation, quality control, and storage; (2) sequencing and genotyping; (3) microarray, NGS, and validation via quantitative real-time PCR; (4) lentiviral methodologies (Center for Psychiatric Neuroscience-COBRE); and (5) preliminary bioinformatics analysis.
Conclusion: The MGCF provides cutting-edge genomic technologies and genomics expertise to academic institutions throughout the State of Mississippi to enhance scientific discovery.
Acknowledgment: The MGCF is supported by P20 GM103476 [MS-INBRE-(Elasri)]; P30 GM103328 [CPN-COBRE (Stockmeier)]; P20 GM104357 [Cardio-Renal (Hall)]; and UMMC (Office of Research).

Infectious Disease/Immunology, Poster Session A

Thursday, September 12, 11 a.m.-12:30 p.m.

A 7.1- "Knowledge and Awareness of HIV Facts, Infection Routes and Myths among the General Population in Jackson, MS and the Surrounding Metro-statistical Area"

Russell Baldwin[1], Amber Haymer[2], Dr. William K. Johnson[3]
[1]Belhaven University, Jackson, MS
[2]Mississippi Valley State University, Itta Bena, MS
[3]My Brother's Keeper, Inc., Jackson, MS

Background: The purpose of our research project is to attempt to find out if a lack of HIV knowledge and awareness is a factor which contributes to the high prevalence of HIV infections in Mississippi. Studies similar to ours have been conducted in Africa which demonstrate that a population's overall knowledge of HIV helps to reduce the rate of new infections per year, but we could not find any research for any study of a similar kind that has been conducted in Mississippi.
Methods: We decided the best way to conduct research of peoples knowledge of HIV was to survey them pertaining to their knowledge and awareness of HIV. We concluded that because of our short research window (8 weeks) we would do best to disseminate the surveys via hand to people of Jackson and the surrounding metro statistical area (MSA).
Results: Our results showed that the population overall was very knowledgeable of HIV, with a mean score of 77.21% on the knowledge portion of the survey. However, when we compared this percentage to males under 24 with the rest of the population, the results showed a discernable gap in knowledge. Males under the age of 24 scored a mean of 70.0% while the rest of the population scored a mean score of 82.3%. This difference of 12.3% could highlight one of the contributing factors behind Mississippians having the highest rate of new infections of males age 13-24.
Conclusion: This study observed that the sum score mean for our surveys was 4.63 out of 6 maximum points, which is 77.2% correct. This demonstrated that the average resident of Jackson and the surrounding MSA was fairly knowledgeable about HIV transmission. The question most frequently answered incorrectly pertained to the percent of HIV positive individuals who were unaware of their HIV status.
Acknowledgment: This work was funded by NIAID Grant 1R15AI099922 (to M.O.E.) and by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM103476.

A 7.2- *"Staphylococcus aureus* Superantigen-induced T Regulatory Cells in Mouse Splenocytes"

Barbara L. F. Kaplan, Yianelly Rodríguez Ruiz, Nogi Park, Eun-Ju Yang, Jeffrey Eells, Keun-Seok Seo
Mississippi State University, Mississippi State, MS

Background: Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) with unknown etiology. It affects over 2 million people worldwide causing variable clinical signs and symptoms depending on the affected area in the CNS, which may include motor, sensory, autonomic as well as cognitive disabilities. Experimental autoimmune encephalomyelitis (EAE) is the most common animal model used to study MS and causes paralysis and spinal cord inflammation. Regulatory cells have been shown to control EAE and MS, including $CD4^+CD25^+FoxP3^+$ cells (Tregs), as well as $CD8^+$ T suppressor cells (Ts). One potential therapeutic strategy to limit the severity of the disease is to increase Tregs. Staphylococcal enterotoxin C1 (SEC1) is a pyrogenic toxin produced by *Staphylococcus aureus*, and is a superantigen (SAg), which can induce Tregs at low concentrations.
Methods: The goal of this work was to characterize the degree to which Tregs and Ts could be induced in response to SEC1 in mouse splenocytes. First, we evaluated cellular proliferation in mouse splenocytes in response to SEC1 and found SEC1 induced proliferation at high concentration. Next we performed flow cytometry for CD25 and FoxP3 expression on $CD4^+$ and $CD8^+$ T cells using various SEC1 concentrations and time points.
Results: SEC1 induced modest CD25 and FoxP3 expression in $CD4^+$ cells but was more effective at lower concentrations. Although there was little FoxP3 expression induced in $CD8^+$ cells, the CD25 expression was high in response to SEC1.
Conclusion: The results suggest that treatment of splenocytes with SEC1 could provide a source of Tregs and Ts that could help control EAE.
Acknowledgment: This work was funded by a T35 training grant for Summer Research Experience for veterinary students (for YRR) and by a Center of Biomedical Research Excellence grant from NIGMS under grant number 5P20GM103646.

A 7.3- "Role of Polyamine Transport in Pneumococcal Pneumonia"

Aswathy N. Rai, John Stokes, Justin A. Thornton, Edwin Swiatlo, Imran Sunesara, Bindu Nanduri.
Mississippi State University, Mississippi State, MS
University of Mississippi Medical Center, Jackson MS

Background: *Streptococcus pneumoniae* (pneumococcus) is an encapsulated gram-positive bacterium that normally resides as a commensal in the nasopharynx of humans. Pneumococcus causes a

wide range of infections when the host immune system is compromised; pneumococcal pneumonia is the most common manifestation. Polyamines are ubiquitous small cationic molecules that are important for growth and virulence of human pathogens, including pneumococcus. Polyamines such as spermidine, putrescine and cadaverine are synthesized in bacteria using precursor amino acids. In addition, polyamine specific transport systems allow the uptake of polyamines from the extracellular environment. In pneumococci polyamine transport of putrescine/spermidine is carried out by a single transport system, potABCD.
Methods: We generated an isogenic deletion of polyamine transport operon in *S. pneumoniae* TIGR4 Δ*potABCD*. To identify specific host innate immune mechanisms that contribute to enhanced resistance in mice challenged with polyamine deficient pneumococci, C57BL/6 mice (n=5) were challenged with 1 x 10^7 CFU of wild type TIGR4, Δ*potABCD* by intranasal inoculation. Mice were euthanized 4h, 12h and 24h post infection (p.i.) and lungs were harvested for enumerating bacterial CFU, evaluation of immune cell infiltration, and cytokines/chemokines analysis.
Results: Our results indicated that deletion of polyamine transport led to attenuation in a mouse model of pneumococcal pneumonia. There was a significant difference in the *in vivo* bacterial burden with WT and Δ*potABCD* at all the time points measured. The mutant was cleared by the host immune system by 24h. Consistent with this observation we found significant differences in the infiltration of neutrophils in the lung tissue of mice challenged with Δ*potABCD.* Concentration of G-CSF, GM-CSF, IL-5, and MIP-1a were higher with the polyamine transport deficient strain.
Conclusion: Taken together, results of this study demonstrate that polyamine transport in the pneumococcus is essential to evade early innate immune responses.
Acknowledgment: This work was supported by grant # P20GM103646 (Center for Biomedical Research Excellence in Pathogen Host Interactions) from the National Institute for General Medical Sciences.

A 7.4- "Vaccination with a Live-Attenuated HSV-1 Vaccine VC2 Promotes Robust Immune Response and Complete Protection in Mice, Guinea Pigs, and Rhesus Macaques in Models of Ocular HSV-1 and Genital HSV-2 Infections"

Brent A. Stanfield[1], Rhonda Cardin[2], Bapi Pahar[3], Vladimir N. Chouljenko[1], Ronald Veazey[3], Konstantin G. Kousoulas[1]
[1]Department of Pathobiological Sciences and Division of Biotechnology and Molecular Medicine, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA
[2]Division of Infectious Diseases, Cincinnati Children's Hospital Medical Center, Cincinnati, OH
[3]Tulane National Primate Research Center Department of Comparative Pathology, Covington, LA

Background: We have previously published utilizing the mouse model for genital herpes infection that vaccination with the VC2 vaccine strain promotes significant immunity to vaginal challenge with virulent HSV-1 or HSV-2. Here we demonstrate the unique immunological phenotype demonstrated by vaccination with the HSV-1 VC2 vaccine strain in mouse, guinea pig, and primate models.
Methods: Mice received multiple injections of either VC2 or the parental virus F strain and challenged either ocularly with HSV-1 Mckrae strain or vaginally with HSV-2 G strain. Guinea pigs received multiple vaccinations with VC2 or an HSV-2 gD subunit vaccine adjuvanted with MLP/Alum. Animals were vaccinated in 3 week intervals. 21 days following final vaccination animals were challenged vaginally with virulent HSV-2. Gross clinical evaluations and vaginal swab samples were collected over the observation period. Macaques received multiple vaccinations with the VC2 vaccine strain. One week post vaccination vaginal biopsies were collected and used to analyzed changes in percentages of cells demonstrating markers of T cell proliferation.
Results: Vaccination with VC2 protected mice from any appreciable ocular disease following challenge with HSV-1 Mckrae strain. Vaccination with VC2 produced an altered and more robust IgG response post vaginal challenge with HSV-2 G strain. Mice vaccinated with VC2 demonstrated a higher anti-HSV-2 IgG titer and early responses of anti-HSV-2 IgG_1 and IgG_3 when compared to vaccination with F. Mice vaccinated with VC2 produced a IgG_{2a} specific response to challenge with HSV-2 G strain mice vaccinated with F strain produced a significant IgG_{2b} response. The VC2 vaccine produces a superior response compared to current HSV-2 gD subunit vaccines. Analysis of the swab samples demonstrated that VC2 largely prevented challenged animals from shedding infectious HSV-2. Rhesus Macaques vaccinated with VC2 demonstrated a dose dependent increase of proliferating CD4 and CD8 positive T cells in the vaginal mucosa.
Conclusions: Collectively these data suggest the vaccine vector VC2 promotes a unique immune response capable of preventing both HSV-1 and HSV-2 disease. More work needs to be done to identify specific immunological mechanisms required for these observed outcomes.

A 7.5- "Acyclovir Cannot Block HSV-1 DNA Accumulation in the Brain during Acute Viral Corneal Keratitis in ApoE e4/e4 Mice"

Christian Clement[1], Florence A. Clement[3], Alberto E. Musto[2], Timothy P. Foster[2], Partha S. Bhattacharjee[4], Harris E. McFerrin[4], Walter J. Lukiw[2], James M. Hill[2]
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[4]Xavier University of Louisiana, New Orleans, LA

Background: Herpes simplex virus type-1 (HSV-1) DNA in ganglia and brain can remain inactive for the entire life of the human host. However, under certain conditions the virus reactivates to cause encephalitis and severe corneal keratitis. HSV-1 has been associated with viral encephalopathy and with the amyloid beta 42 (Ab42) peptide-enriched lesions that characterize Alzheimer's disease (AD) neuropathology. Mice carrying the human apolipoprotein E (APOE) ε4 allele gene (ApoE e4/e4 mice) latent with HSV-1 would undergo frequent spontaneous virus reactivation irrespective of the virus reactivation phenotype. These findings were based on the use of high phenotypic reactivator (HPR), 17*Syn*$^+$ and low phenotypic reactivator (LPR), 17ΔPst(LAT$^-$). During this time, HSV-1 causes corneal lesions and ocular shedding of infectious virus causing stromal damage. Additionally, viral DNA accumulates in the left hippocampus and left cortex. This study investigates the effectiveness of ACV treatment in viral-induced brain inflammation, neuronal damage and corneal damage.
Methods: As an intervention, cohorts of ApoE e4/e4 mice were separately dosed by spiking their water supply with acyclovir (ACV) at 4 mg/ml (super-dose = 8 mg/ml) and oral gavage of 100 µl of solution containing 28 mg/ml ACV (super-dose = 56 mg/ml) equivalent to 140 µg/g (super-dose = 280 µg/g) respectively during the acute infection stage. The acute anti-herpetic treatment was to inhibit characteristic viral DNA deposition in the left brain and reverse or reduce the corneal damage.
Results: Antiviral treatment had no significant effect in attenuating HSV-1 DNA deposition in the left brain, the neuropathology, nor the severe ocular disease and mortality in the ApoE e4/e4 mice. Corneal lesions and ocular shedding of infectious HSV-1 occurred with no significant difference for both the ACV treated and the control (non ACV treated) transgenic mice. Additionally, viral DNA accumulated in the left hippocampus and left cortex about equally for both the ACV treated and the control (non ACV treated) transgenic mice.
Conclusion: The results suggest incremental deposition of viral DNA in the left hippocampus and left cortex from intense spontaneous neuronal HSV-1 reactivation/replication and severe corneal damage in the human APOE ε4 genetic background to be unaffected by ACV even at high doses. Therefore, the viral DNA accumulation in the left brain of the ApoE e4/e4 mice could be dependent on the human APOE ε4 genetic status irrespective of the phenotypic nature of the virus. Alternatively, we suspect the human APOE ε4 gene could render the latency silencing to be less effective, allowing higher frequency of HSV-1 reactivation invoking pathological and viral effects through HSV-1 challenge to human brain cells by simple virus reactivation/replication, viral DNA accumulation and ocular shedding of infectious virus. These observations need further investigations using a combination of *in vitro*- primary neuronal cell cultures and *in vivo*-new ocular mouse HSV infection model.
Acknowledgment: This work was funded by NEI grants R01-EY006311 (JMH), R01-EY14289 (JMH), R21-EY019144 647 (PSB); NIA AG18031 (WJL) and AG038834 (WJL), AG23085 (JMH); P20RR016456 grant from the National Center for Research Resources (HEM); Transitional Research Initiative grants (WJL); unrestricted grant to the LSU Eye Center from RPB, New York, NY; Alzheimer Association Investigator-Initiated Research Grant IIRG-09-131729 (WJL); the Louisiana Cancer Research Consortium; the Louisiana Lions Eye Foundation, New Orleans Louisiana Vaccine Center sponsored by the Louisiana Board of Regents; LBRN Faculty Support 2015 (CC).

A 7.6- "Puma - A Critical Component of Innate Immunity Against Extracellular Pathogens"

Dan E. Kennedy II, Katie E. Heath, Katie Hill, Keun Seo, Tim Morgan, Jim Cooley, Justin A. Thornton
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Background: Cells of the innate immune system are the first line of defense against bacterial pathogens. Essential to the resolution of infection and inflammation is the effective recruitment and organized death of these important cells. Organized cell death (apoptosis) is triggered by a variety of stressors following infection and serves to clear immune cells from infected tissues after they have performed their function and to subsequently down-regulate inflammation. We previously identified that the pro-apoptotic Bcl-2 family member Puma is essential for surviving *Streptococcus pneumoniae* infection in a mouse pneumonia model. We hypothesized that reactive oxygen species (ROS) produced following phagocytosis induces DNA damage and subsequent Puma expression and that this process is essential for apoptosis and down-regulation of inflammation. We also sought to determine if Puma's contribution to bacterial clearance extended beyond pneumococcal infection by utilizing a *Staphylococcus aureus* skin infection model.

Methods: Phagocytosis assays were performed by exposing HL-60 human granulocytic cells to opsonized *S. pneumoniae in vitro* at a 1:1 bacteria to cell ratio for 1h followed by plating aliquots on blood agar. DNA damage was quantitated by exposing cells to bacteria in the presence or absence of inhibitors and performing comet assay. Comet tail lengths were measured using ImageJ software (NIH). Production of ROS was quantitated by BioTek fluorescent plate reader following exposure of HL-60 cells to bacteria in the presence of 123 dihydrorhodamine. Expression of puma was assessed by qRT-PCR on cDNA synthesized from RNA isolated from HL-60 cells exposed to pneumococcus for 1h and 3h. Apoptosis of HL-60 cells and murine neutrophils was assessed following bacterial exposure by AnnexinV/PI staining and flow cytometry. To determine the effect of Puma on *S. aureus* clearance, 10-12 week old mice were challenged intradermally with LAC strain for up to 7 days. Imaging of dermonecrosis, plate counts from tissues, and quantitation of immune cells was performed at 4d and 7d post-infection.
Results: ROS production by HL-60 cells in the pneumococcal challenge model was found to independent of phagocytosis. However, DNA damage was increased when phagocytosis occurred. Interestingly, Puma induction was significantly greater in the absence of phagocytosis (7-fold vs 3-fold). Annexin V staining indicated increased apoptosis in the absence of phagocytosis in both HL-60 cells and bone marrow neutrophils. Puma-/- mice challenged with *S. aureus* demonstrated more severe dermonecrosis at both 4d and 7d post-infection and had greater numbers of *S. aureus* remaining in the tissue. Dramatic differences in the populations of immune cells were seen between Puma+/+ and Puma-/- mice following challenge.
Conclusion: While Puma appears to be important for cell survival following bacterial challenge, changes in its expression does not appear to be dependent on phagocytosis-induced ROS production or DNA damage. While DNA damage may contribute to Puma's role in innate immunity, other factors are likely involved including possibly ER stress and inflammatory signaling. Challenge of mice with S. aureus demonstrated differential recruitment or clearance of macrophages and neutrophils in Puma-/- mice. Together, these results indicate that during the innate immune response to bacterial infection, Puma regulates cell fates following cellular stress thereby preventing prolonged or ineffective resolution of inflammation.
Acknowledgment: This work was funded by an Institutional Development Award (IDeA) from the NIGMS COBRE grant number (P20GM103646).

A 7.7- "Analyzing the Expression of *M46*, A Mold Phase- Specific Gene, In The Pathogenic Dimorphic Fungus *Histoplasma capsulatum*"

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Background: *Histoplasma capsulatum (Hc)* is a dimorphic fungus, that is the causative agent for the respiratory infection histoplasmosis. The fungus is found in the environment as a multi-cellular saprophytic mold, and converts to a uni-cellular parasitic yeast in the lungs. The yeast is highly studied because it is the morphotype that is pathogenic. Studies on *Hc* mold is highly overlooked. This research focuses on characterizing the mold specific *M46* gene.
Methods: Northern blot analysis with four commonly used *Hc* strains was conducted to investigate the expression of *M46.* The M46 ORF was sequenced to determine if *M46* exist in all strains. The *M46* promoter from all four strains was sequenced, to determine if the promoter may be a reason for lack of *M46* expression in the latter strains.
Results: The Northern blot analysis has shown, that *M46* is up-regulated in strains G186AS and Downs, mold, but is down regulated in strains G184AS and G217B mold. The M46 ORF sequenced was conserved in all four strains. Sequencing of the *M46* promoter from all four strains revealed a 12 bp deletion and 10 bp insertion upstream from the TATA Box sequence of *M46* non -expressing strain G217B. The promoter sequence of *M46* in *M46* expressing strain G186AS is identical to the promoter sequence of *M46* in non-expressing strain G184AS.
Conclusion: The reason for lack of expression of *M46* in strains G217B and G184AS mold is unknown. The ORF is not a reason for lack of expression of *M46.* The deletion and insertion that is present in the *M46* promoter region of *M46* non-expressing strain G217B, could be a reason for lack of expression. The reason for lack of expression in strain G184AS could be due to a trans-acting element.
Acknowledgement: This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institute of Health under grant number P20GM103476.

A 7.8- "The Role of FTL_0129 in the Invasion of Erythrocytes by *Francisella tularensis"*

Devin Sindeldecker[1], Deanna Schmitt[1], Tricia Gilson[1], Alek Florjanczyk[1], Caleb Martin[1], Edward Beaumont[1], Jennifer Hickman[1], Donald Primerano[2], James Denvir[2], Joseph Horzempa[1]
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Background: *Francisella tularensis* is a bacterium that can infect humans with fewer than ten bacteria and is the causative agent of tularemia. If untreated, tularemia causes mortality in approximately 60% of those infected. Due to the ability of this bacterium to be aerosolized and the high mortality rate, *F. tularensis* has the potential to be used as a bioterrorism agent. During infection, *F. tularensis* invades erythrocytes, a phenomenon that enhances subsequent colonization of ticks following acquisition of a blood meal. Gaining more information regarding the pathogenesis and transmission of this organism will help us to develop new vaccines and therapeutics. We hypothesized that transcription of *F. tularensis* genes important for erythrocyte invasion would be induced in the presence of erythrocytes.
Methods: Here, we conducted mRNA profiling studies to determine *F. tularensis* genes expressed in response to human erythrocytes to identify genes that may be involved in red blood cell invasion. Subsequently, a mutation was made in the most highly upregulated non-essential gene. The resulting mutant was tested for erythrocyte invasion in vitro.
Results: An RNAseq analysis indicated that ~7% of *F. tularensis* genes were upregulated when exposed to erythrocytes. FTL_0129 was the most highly induced non-essential gene when *F. tularensis* came into contact with an erythrocyte. FTL_0129 was deleted, and the resulting mutant was tested for erythrocyte invasion using a gentamicin protection assay. Significantly lower levels of the mutant strain of *Francisella tularensis* survived the gentamicin protection assay as compared to wild-type *F. tularensis* LVS.
Conclusions: FTL_0129 is required for erythrocyte invasion.
Acknowledgement: This work was supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence.

A 7.9- "Activated caspase-1 in the Colon and Small Bowel as a Marker for Barrier Dysfunction in Inflammatory Bowel Disease"

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Background: Activated caspase-1 is a surrogate marker for barrier dysfunction in inflammatory bowel disease (IBD). We investigated the effect of age and sex on the expression of activated caspase-1 in the colon and small bowel of the IL10 knock-out (KO) mouse model of IBD. We also examined the role of intestinal microbiota on the activation of caspase-1 using germ-free and specific pathogen free (SPF) conditions.
Methods: A time-course study was performed where colon and small bowel mucosa were collected from male and female C57BL/6 WT and IL-10 KO mice, aged 5, 6 and 7 months, and levels of activated caspase-1 were measured by Western blot and correlated to histology scores. Similarly, activated caspase-1 levels in the small bowels from germ-free and SPF C57BL/6 wild-type and IL-10 KO mice were compared by Western blot.
Results: Increased levels of activated caspase-1 were observed in the colons and small bowels of both male and female IL10 KO mice in all age groups compared to WT mice, with female mice expressing higher levels in the small bowel early on in disease. SPF IL-10 KO expressed significantly higher levels of activated caspase 1 compared to wild-type mice. In contrast, levels of activated caspase-1 expression in germ-free IL-10 KO mice were similar to wild-type levels.
Conclusions: Our results suggest that ongoing barrier dysfunction is measured by higher levels of activated caspase-1 in the colons and the small bowels in the IL10 KO mouse model. Our results also suggest that in the absence of intestinal microbiota activated caspase-1 levels revert to wild-type levels, supporting the role of microbes in the development of barrier dysfunction.
Acknowledgements: This work was funded by grant P20 GM103625 from the Center for Microbial Pathogenesis and Host Inflammatory Responses.

A 7.10- "Th1/Th2 Imbalance by "Gain-Of-Function" Mutations Cause Fatal Theiler's Virus Infection in Mice"

Fumitaka Sato, Ph.D.[1], Eiichiro Kawai, M.D.[1], Nicholas E. Martinez, Ph.D.[1], Seiichi Omura, Ph.D.[1], Elaine Cliburn Stewart[1], Satoru Takahashi, M.D., Ph.D.[2], Keigyou Yoh, M.D., Ph.D.[2], Ikuo Tsunoda, M.D., Ph.D.[1]
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Background: Theiler's murine encephalomyelitis virus (TMEV) is a non-enveloped, positive-sense, single-stranded RNA virus that belongs to the family *Picornaviridae*. TMEV infection in susceptible SJL/J mice is used as a viral model of multiple sclerosis, since TMEV can induce an inflammatory demyelinating disease in the central nervous system (CNS), which is mediated by both viral persistence

and immune-mediated tissue damage (immunopathology). On the other hand, resistant C57BL/6 mice can clear TMEV with no persistent disease. Since T helper (Th) cell subsets can contribute to both viral clearance and immunopathology, the difference in Th responses may explain the difference in susceptibility among mouse strains. Recently, Th1–biased "gain-of-function" mutations in humans have been reported to make patients susceptible to infections with microbes, including viruses. We have established novel transgenic (Tg) mice on the C57BL/6 mouse background, Th1-biased T-bet transgenic Tg and Th2-biased Gata3-Tg mice, whose T cells overexpress T-bet and Gata3, transcription factors that are required for Th1 and Th2 cell differentiation, respectively. Thus, using the two Tg mouse strains, we tested whether "gain-of-function" Th subset mutations could alter the susceptibility to TMEV infection. Methods: Wild-type, T-bet-Tg, and Gata3-Tg mice were infected intracerebrally with 2 x 10^5 plaque forming units (PFU) of TMEV. We semi-quantified viral RNA in the brain by real-time PCR. We also measured anti-viral antibody titers and the levels of a Th1 cytokine, interferon (IFN)-γ, and a Th2 cytokine, interleukin (IL)-4, production by enzyme-linked immunosorbent assays (ELISA). Histology of the CNS and general organs were examined by Luxol fast blue staining, and hematoxylin and eosin (H&E) staining, respectively. Results: Similar to wild-type C57BL/6 mice, Gata3-Tg mice cleared TMEV and remained resistant to TMEV-IDD, although infected Gata3-Tg mice had lower IFN-γ and higher IL-4 production with increased anti-viral IgG1 antibody responses. Unexpectedly, infected T-bet-Tg mice developed encephalitic signs and paralysis with weight loss, and died 2 to 3 weeks after infection. T-bet-Tg mice had also higher viral titers and severe neuronal loss in the brain, particularly in the hippocampus and substantia nigra, while neither demyelination nor increased T cell infiltration was observed. Infected T-bet-Tg mice had lower IL-4 production and decreased anti-viral IgG1 antibody responses with atrophy of the thymus and spleen, while IFN-γ production remained high. These results were in contrast to our previous findings in Th17-biased RORγt-Tg mice, which became susceptible to TMEV-IDD with viral persistence, severe immunopathology, and higher IL-17 production. Conclusions: Our novel Tg mice could provide translational information about how Th-related "gain-of-function" mutations *in hosts* could alter anti-viral responses and/or immunopathology in neurotropic viral infections. Acknowledgments: This work was supported by an IDeA from the NIGMS of the NIH (5P30GM110703) and the fellowships (F. Sato and S. Omura) from the Malcolm Feist Cardiovascular Research Endowment, Louisiana State University Health Sciences Center-Shreveport.

A 7.11- "Characterization of Uninfected and *Rickettsia parkeri*-infected *Amblyomma maculatum* Tissues"

Gail M. Moraru, John V. Stokes, Andrea S. Varela-Stokes
College of Veterinary Medicine, Mississippi State University, Mississippi State, MS

Background: *Rickettsia* dynamics within vectors is not well understood. *Rickettsia parkeri* is a human pathogen using *Amblyomma maculatum* as its primary vector. We are using this model system to better understand rickettsial infection dynamics inside the tick vector. Here we attempt to characterize select tick tissues (midgut, salivary gland) and detect rickettsiae in tissues of experimentally infected ticks.
Methods: We first approached this using flow cytometry by generating a single-cell suspension of tissues (midgut, salivary gland) from uninfected ticks and analyzed plots based on forward and side scatter. Subsequently, we experimentally infected adult *A. maculatum* with *R. parkeri* expressing GFPuv by capillary feeding and later dissected the ticks at 4, 7, and 15 days post feeding (DPF). These tissues were also analyzed by flow cytometry for direct detection of expressed GFPuv. In order to refine our approach, we began pursuing production of monoclonal antibodies, first by using unique peptides identified by proteomic analysis of proteins extracted from salivary glands and midguts.
Results: Using flow cytometry, salivary glands were found to be the most promising tissue in terms of cell population discrimination, while distinct populations of cells could not be observed in suspensions of midgut cells. While we were able to detect *R. parkeri* GFPuv in Vero cell culture using flow cytometry, we were not able to do so in tissues of fed ticks, although the same ticks tested positive for rickettsial DNA by PCR assay. Using proteomic analysis of differentially expressed proteins, we selected two peptides based on significantly higher expression in either salivary gland or midgut. Monoclonal antibody production for tissues is underway, and antibodies will be screened for their utility in immunoblotting, immunocytochemistry, and flow cytometry.
Conclusion: With the pursuit of these studies and additional production of *Rickettsia* species-specific monoclonal antibodies, we anticipate being able to better understand dynamics of rickettsiae in specific tissues within infected ticks. Further, these tools will allow us to evaluate interspecies interactions among rickettsiae within co-infected ticks.
Acknowledgments: This work was supported by grant # P20GM103646 (Center for Biomedical Research Excellence in Pathogen Host Interactions) from the National Institute for General Medical Sciences.

A 7.12- "Role of 5'and 3' UTR Region to Regulate msaB Production in *Staphylococcus aureus"*

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Background: *Staphylococcus aureus* causes a wide range of acute and chronic infections including severe invasive biofilm-associated infections in humans. They possess a wide variety of virulence factors, the expression of which is carefully coordinated by a variety of regulators. *msaABCR* operon is one of those regulators that regulates virulence factors, biofilm development and antibiotic resistance. The *msaABCR* operon is composed of one protein coding gene, msaB, and three non-coding gene, *msaA, msaC and msaR*. Transcription of *msaABCR* generates several sub-transcripts including one that translates into the MsaB protein. However, the regulation mechanism of this operon and the role of the sub-transcripts is not yet understood. In this study, we investigate the role of 5' end and 3' end of the *msaABCR* operon in the regulation of production of MsaB. We also investigate mRNA stability and its role in biofilm development and virulence.
Method: We constructed a series of truncated *msaABCR* operon constructs (TC-1 to TC-12) from both the 5' end and the 3' end to study the role of 5' end and 3' end in transcript stability, MsaB production, proteases production, and biofilm development. We also performed the mutagenesis experiments to study the interaction between 5' end and 3' end of the *msaABCR* operon transcript and its role in mRNA stability and MsaB production.
Results: Full *msaABCR* operon transcript complemented the deletion mutant with regards to pigmentation, proteases production, biofilm development, and MsaB production. A series of 5' end and 3' end truncated complements showed different level of complementation and msaB production, which suggests its role in regulation of *msaABCR* operon. Two constructs, TC-5 and TC-9, complement the *msaABCR* deletion mutant and result in overexpression of MsaB. The constructs TC-1, TC-2, TC-3 and TC-4 did not complement the *msaABCR* deletion mutant and did not produce MsaB. Interestingly, TC-3 and TC-4 complemented biofilm formation suggesting a role for the 3' end in biofilm formation that does not require MsaB. These results also suggest that the 5' end and the 3' end of the transcript interact and play a role in the production of MsaB and biofilm development.
Conclusion: This study defines the regulatory functions of the 5' and 3' ends of the *msaABCR* transcript in the production of MsaB and Biofilm development. This study will allow us to eventually identify the environmental and/or host stimuli that control the functions of the *msaABCR* operon.

A 7.13- "Assessing Differential Gene Regulation Triggered by Environmental Temperature in *Borrelia turicatae"*

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Background: *Borrelia turicatae*, a tick-borne bacterial pathogen, encounters distinct environments during different stages of its enzootic cycle. The mechanism by which *B. turicatae* adapts to these different environments is undefined, but we have identified proteins that are differentially regulated in response to temperature.
Methods: To identify differentially regulated proteins, we performed proteomic comparisons of *B. turicatae* grown at 37˚C and 23˚C. To study the regulation of *bta121,* one of the identified temperature-responsive genes, we used a *bta121* knockout mutant, *bta121* genetic complementation, and *bta121* luciferase reporter constructs. BTA121 production was evaluated by western blotting and *bta121* transcription was measured by luciferase assays.
Results: Proteomic comparisons identified 102 proteins preferentially produced at 37˚C and 121 proteins maximally produced at 23˚C. *bta121* was successfully disrupted; however upon complementation with a construct expressing *bta121* from a short region upstream of the start codon, temperature-dependent gene regulation was not restored. Luciferase assays with the *bta121* transcriptional reporter confirmed these results.
Conclusion: Proteomic data indicates that *B. turicatae* undergoes significant regulatory changes in response to environmental temperature alone. Data obtained from the *bta121* knockout and its complement suggests the presence of an operator that represses the expression of *bta121* at elevated temperature. Luciferase assays performed using the upstream region of *bta121* confirm the presence of potential regulatory element(s), offering new insight into transcriptional regulation in *B. turicatae.*
Acknowledgment: This work was supported by a pilot grant from the Centers of Biomedical Research Excellence (COBRE) award through NIGMS (P20-GM103625).

A 7.14- "The *msaABCR* Operon Activates Capsule Production in *Staphylococcus aureus"*

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Background: Capsule production in *Staphylococcus aureus* plays a major role in pathogenesis. Capsule allows *S. aureus* to evade phagocytosis during

movement from one localized site to another in the course of an infection. *S. aureus* has developed a complex regulatory network that is responsible for regulating capsule production. We have identified and defined the *msaABCR* operon that regulates several virulence factors. In this study we show that the *msaABCR* operon regulates capsule production by activating the *cap* operon.
Methods: To examine the role the *msaABCR* operon plays in the regulation of capsule, we deleted the *msaABCR* operon in two strains of *S. aureus* that produce two different capsule serotypes. We tested the effects of the mutation on various conditions including transcription of *cap* genes, total capsule polysaccharide (CP) production, and functional killing assays using human polymorphonuclear neutrophils or (PMNs). Additionally, we used chromatin immunoprecipitation or (ChIP) and electrophoretic mobility shift assay or (EMSA) to determine if the regulation observed is directly related to a component of the *msaABCR* operon.
Results: We observed in both strains tested that deletion of *msaABCR* significantly reduces the expression level of *cap* genes. Measurement of the total crude capsule production showed that the reduction in *cap* transcription reduced total CP production to undetectable levels in the deletion mutants. We also observed that the mutants from both strains are significantly more susceptible to elimination by host immune components including PMNs. Additionally, we found that a protein product of the *msaABCR* transcript, MsaB, binds upstream of the promoter region of *cap* as an activator of CP production in a growth phase-dependent manner specifically in the late and post-exponential growth phases.
Conclusions: In this study we show that the *msaABCR* operon plays an important role in the regulation of CP production within *S. aureus* at the transcriptional level that significantly alters total CP production. Furthermore, we show that the *msaABCR* operon directly regulates CP production in *S. aureus*. We found that the MsaB protein binds upstream of the promoter region of the *cap* operon as an activator of *cap* transcription and ultimately CP production. We are currently exploring this regulation observed by the *msaABCR* operon and specifically the MsaB protein in further detail.
Acknowledgment: This work was funded by NIAID Grant 1R15AI099922 (to M.O.E.) and by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM103476.

A 7.15- "The Dose-Dependent Effect of Superantigens on Development of Immunosuppressive CD8+CD25+FOXP3+ T Cells"
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Background: *Staphylococcus aureus* is a significant pathogen in humans and animals. *S. aureus* typically colonized in the host and did not elicit any clinical manifestations but causes severe invasive diseases such as endocarditis and pneumonia in immunocompromised hosts. Staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin produced by *S. aureus* are superantigens (SAgs) that cause toxic shock in an exposure to a high dose of SAgs. However, *S. aureus* typically colonized and secreted a low dose of SAgs, and the effect of chronic exposure to a low dose of SAgs has not been characterized yet.
Methods: We isolated hunman peripheral blood mononuclear cells (PBMCs) from healthy donors and stimulated with a high (1 μg/ml) and low dose (1 ng/ml) of Staphylococcal enterotoxin C1 (SEC1). After stimulation, CD8+CD25+ T cells were purified by magnetic bead selection. We characterized the phenotype of Treg by flow cytometry and assessed their suppressive activity by measuring tritiated thymidine incorporation of CD4+CD25- responder T cells.
Results: Both high and low dose stimulations induced phenotypic markers related to suppressor T cells (Ts) including CD25, FOXP3, CD28, CTLA-4, IL-10, TGF-β1, and IFN-γ. However, CD8+ T cells from high dose stimulation did not suppress the proliferation of responder cells (CD4+CD25-) to anti-CD3/CD28 bead but those from low dose stimulation did. The suppression was mediated by both a contact dependent manner, but to a lesser extent, and a soluble factor dependent manner.
Conclusion: These results suggest that a chronic exposure to a low dose of SAgs by asymptomatic colonization of *S. aureus* induced immunosuppressive Ts cells that cause immunocompromisation, potentially leading to an outbreak of severe invasive *S. aureus* infections.
Acknowledgment: This work was funded by National Institute of Health, Center for Biomedical Research Excellence in Pathogen-Host interactions (1P20GM103646-01A1).

A 7.16- "Evaluation of Sex Education among Mississippi Students"
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Background: The purpose of this study is to evaluate student's feedback of the sex education in schools compared to in-depth sex education seminar given by Delta Region AIDS Education Training Center. House Bill 999 stated that every school in the state of Mississippi had to adopt a method of sex education by

June 2012 (Abstinence-only or Abstinence-plus). Based on research, sex education is leisurely fulfilling its purpose. The health disparities rates are gradually decreasing in Mississippi.
Method: Delta Region Aids Education Training Center (DRAETC) conducted a sex education seminar at Tougaloo College. The target population was 16 -18 year olds. This was conducted during the summer during the Summer Bridge program at the college. DRAETC provided a curriculum fit for the target population. At the conclusion of the seminar an evaluation was provided (through survey monkey an online survey site), to analyze their feedback and compare demographic information to the information studied in our research. The evaluation addressed demographic information, prior sex education experience, knowledge level before and after seminar, effectiveness of sex education seminar, and if available resources are available to them.
Results: There were 45 students that participated in the sex education seminar at Tougaloo College. Our evaluation was sent after the seminar through survey monkey and 10 out 45 students answered the evaluation (22% of the student population). Eight were female (80%) and two were male (20%). We asked if the students had prior sex education to our sex education seminar. Six of the ten participants (60%) have had sex education prior to the sex education seminar. Of those six, three have had comprehensive sex education (50%), two have had abstinence only (33%), and 1 has had abstinence plus (33%). Seven of the participants stated that their parents have had sex discussions with them that included contraceptives, condom use, and how to get pregnant and an STD and HIV. Six participants (60%) stated they were neutral of their overall sexual health before the seminar. One stated (10%) they were not knowledgeable at all of their overall sexual health. Two stated (20%) they were knowledgeable of their overall sexual health. We asked the participants what their experience was after the sex education seminar and eight participants stated (80%) they were knowledgeable of the subject matter and one stated (10%) they were neutral. We asked the participants was our sex education seminar effective, seven (70%) stated yes and two (20%) stated that the seminar was neutral. One skipped the question.
Conclusion: Based on our results the sex education seminar was very effective compared to prior participant's sex education experience. This research is valuable to facilitators leading the curriculum to evaluate their teaching and curriculum and figure out ways to be more effective for students in Mississippi.

A 7.17- "High-Throughput Screening of CRISPR-Cas9 Library for Host Factors Essential for HSV-1 Replication"

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Background: Herpes simplex virus (HSV) is an important human pathogen, infecting up to 90% of the global population, classically causing oral genital mucocutaneous vesicular lesions (e.g., fever blisters, cold sores). Other more severe clinical manifestations of HSV infection include encephalitis, meningitis and keratitis. Additionally, coinfection with HSV-2 is a major contributor to HIV transmission. Therefore, a better understanding of HSV and host interaction has broad implications for global healthcare.
Methods: To systematically investigate how host factors (HFs) affect HSV-1 replication, we set out to perform genome-scale loss-of-function screening in human cells with Cas9-sgRNA system.
Results: Having transduced a lentivirus library containing 122,411 sgRNAs and targeting 19,050 human genes into Cas9-expression Hep2 cells and performed screens for resistance to HSV-1 infection, we selected 80 cell clones and confirmed these clones are resistant to HSV infection. Five resistant cell clones were randomly chosen for further study, and preliminary data showed that HSV-1 replication was decreased over 1000 fold compared to that of wild type cells even though the level of viral gene expression in all the tested cells was comparable to that in wild type cells. The sgRNA coding regions of some of the resistant cell lines were amplified and sequenced. Some host factors known to be critical to HSV replication were identified, but importantly, some genes previously unknown to be essential for HSV replication were found. We will further investigate the function of UNC50 in HSV-1 replication which was identified to be targeted in the R2 cell line.
Conclusion: Our preliminary data demonstrate that RNA-guided CRISPR Cas9 screens are a powerful tool for rapid exclusive identification of host genes important for HSV replication. Data from this proof-of-principle project would lead to new areas of research to our understanding of HSV-1 replication cycle and discovery of new targets of treatment of HSV-associated diseases.
Acknowledgement: This work was funded by NIH/NIAID grant 1R21AI107382-01A1 (K.Y.) and a pilot grant (K.Y.) by LSU-Tulane NIH Center for Biomedical Research Excellence (COBRE) under grant number 5 P30 GM110760-02

A 7.18- "The Characterization of The Mold Specific Genes, *Ms88* and *Ms95*, In the Dimorphic, Pathogenic Fungi, *Histoplasma capsulatum"*

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Background: *Histoplasma capsulatum (Hc)* is the causative agent for the upper respitory infection, histoplasmosis. The organism can exist as a multicellular mold at 25˚C, or as a unicellular yeast at 37˚C. The yeast is the phase that causes histoplasmosis. This study focuses on the investigation of the mold specific genes, *MS88* and *Ms95*. The *Ms88* gene is 1145 bp long and encodes for a protein that is 219 amino acids long. A NCBI BLAST (p) search showed that *MS88* was classified in the CFEM family, this suggests that the gene may play a role in pathogenesis. The mold specific, *Ms95* gene, was discovered from a cDNA subtractive library enriched for mold specific genes and is homologous to the *DDR48* gene that is responsible for DNA repair in the yeasts, *S. cerevisae* and *C. albicans.*
Methods: An NCBI BLAST (p) www.ncbi.nlm.nih.gov domain search was conducted on the *MS88* gene to characterize the sequence. An *Ms88* overexpression construct was made to determine if *Ms88* is involved in dimorphism. The construct was made by fusing the H2B promoter to the *MS88* open reading frame via fusion PCR. The construct will then be electroporated into an *Ms88* expressing strain of *Hc*. An *Ms95* knockout was created to determine if *Ms95* is involved in DNA-damage repair. The *Ms95* knockout will be exposed to a susceptibility assay that involved various concentrations of 4-nitroquinoline 1-oxide and paraquat dichloride. The samples will be measured daily for 6-days, over the average 12-day growth cycle. A yeast growth curve with the *Ms95* knockout and parental strain will be conducted by measuring the absorbance on a Bio-Rad microplate iMark reader, every 12 hours for 4 days.
Results and Conclusion: A NCBI BLAST (p) search found that *MS88* was classified in the CFEM family that suggests that the gene plays a role in pathogenesis. The *Ms88* gene could be involved in pathogenesis due to the NCBI BLAST (p) domain search. The *Ms95* growth curve assays and stress susceptibility assay are currently on-going.
Acknowledgement: This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

A 7.19- "Non-structural Protein σ1s Enhances Reovirus Spread in Endothelial Cells"

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Background: Mammalian orthoreovirus (reovirus) traffics via the blood to from initial sites of infection to target organs and tissues, including the central nervous system. Reovirus non-structural protein σ1s is required for hematogenous reovirus dissemination. The mechanism by which σ1s facilitates systemic dissemination is undefined.
Methods: Endothelial cells are critical targets for reovirus infection of the host that are required for viral entry to and exit from the blood. We compared replication and cell-to-cell spread of wild-type (WT) and σ1s-null reoviruses in SV40 immortalized endothelial cells (SVEC). We also used antibodies to block interferon α receptor-1 (IFNAR1) signaling in SVECs, as well as WT and IFNAR $^{-/-}$ murine embryonic fibroblasts (MEFs) to determine if σ1s confers reovirus resistance to the type 1 interferon (IFN-1) response.
Results: We found that WT and σ1s-null viruses produced comparable viral yields from SVECs during the first round of viral replication. However, multistep growth analysis revealed that the σ1s-null virus failed to amplify beyond the initial round of infection. WT and σ1s-null viruses initially infected an equivalent number of cells, but only the WT virus spread to additional cells in culture. The σ1s-null virus remained restricted to a few isolated cells. Comparable levels of WT and σ1s-null progeny were present in the supernatant of infected SVECs after a single round of replication. WT and σ1s-null progeny virus were equivalently infectious when applied to new SVECs. We found that IFNAR1 antibody blockade restored the capacity of the σ1s-null virus to spread in SVECs. Similar to SVECs, replication of the σ1s-null virus was restricted in WT MEFs. However, WT and σ1s-null viruses replicated to equivalent titers in IFNAR1$^{-/-}$ MEFs.
Conclusions: Our results indicate that σ1s is dispensable for the initial round of reovirus replication in SVECs. However, σ1s is required for virus spread to uninfected cells in culture which amplifies viral replication. These findings suggest that σ1s is required to overcome a restriction in secondarily infected cells that is not present during the first round of replication. Further, restricted spread of the σ1s-null virus is overcome by abrogating the IFN-1 response. This finding suggests a critical role for σ1s in overcoming the IFN-1 response to allow reovirus spread. This data also implicates the IFN-1 response as a key host restriction to viral hematogenous dissemination.
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A 7.20- "Autoantibody Profiling and Immunosuppressive Medications in Systemic Lupus Erythematosus and Incomplete Syndromes"

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Background: Systemic Lupus Erythematosus is an autoimmune disorder currently affecting 5-7% of the population; this is more likely an approximation due to the difficulty of diagnosis in a clinical setting. In some sub populations, for example females, this rate increases exponentially. In order to receive a "diagnosis", one must meet 4 or more of 11 ACR criteria. The purpose of this study is to investigate the clinical prevalence of autoantibodies of incomplete lupus-like syndromes and confirmed lupus individuals in means of developing a more specific diagnosis in future studies.
Methods: A protein microarray composed of 77 possible targets of autoantigens was used to profile autoantibodies from 33 patients blood sera; SLE(n=18), ILE(n=15). Median fluorescent values were obtained and subsequently used for analysis with the program R.
Results: Significant differences were observed, but actual MFI differences between patients were marginal. Due to rather small sample populations of the clusters, significant values become increasingly weak when representing a much larger population. Prednisone shows a better effectiveness at lowering MFI of disease related autoantibodies of confirmed SLE diagnosis.
Conclusions: Characterization of this disease in disparate organ systems makes diagnosis an arduous task, which often times goes miss diagnosed. Any insight into narrowing down the plethora of possible blood markers would provide the scientific community more sensitive tests than ANA alone. Patients who were diagnosed with SLE and on Prednisone had lower antibody MFI of lupus related autoantibodies compared to those prescribed Hydroxychloroquine.
Acknowledgments: Dr. James of the Oklahoma Medical Research Foundation funded this work, and all work was done at Harding University genetics lab.

A 7.21- "The Dose-Dependent Effect of staphylococcal superantigens on Development of Functional CD4+CD25+FOXP3+ Regulatory T Cells"

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Background: *Staphylococcus aureus* is one of the most significant human pathogens for hospital- and community-associated infections. *S. aureus* is often colonized skin and mucosal membranes in the host and causes broad spectrum of diseases from mild skin infection to toxic shock syndrome. Staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin are a group of prototypic pyrogenic toxin superantigens (SAgs) that cause toxic shock in an exposure to a high dose of SAgs. However, the amount of SAg produced in chronic staphylococcal infection was significantly lower and the effect of such chronic exposure has not been well elucidated yet.
Methods: Human peripheral blood mononuclear cells (PBMCs) was isolated from healthy donors and stimulated with various doses of Staphylococcal enterotoxin C1 (SEC1). After stimulation, CD4+CD25+ T cells were purified by magnetic bead selection. The phenotype of regulatory T cells (Tregs) was examined by flow cytometry and their suppressive function was assessed by measuring tritiated thymidine incorporation of CD4+CD25- responder T cells.
Results: Expression of FOXP3, a canonical Treg lineage marker, was induced by SEC1 stimulation in a dose dependent manner, only CD4+CD25+FOXP3+ T cells induced from a low dose of SEC1 stimulation suppressed the proliferation of CD4+CD25- responder T cells. The phenotype of functional Treg stimulated by a low dose of SEC1 was characterized as CD25+, FOXP3+, CTLA-4+, IL-10+, and TGF-β1+. The suppression was mediated mainly by a contact dependent manner while soluble factor mediated suppression was also observed in a lesser extent.
Conclusion: *S. aureus* maintains life-long association with its host by manipulating host immune system favor to its colonization. Our results demonstrate that *S. aureus* achieves this goal by constantly secreting a low dose of SAgs that induced immunosuppressive CD4+CD25+FOXP3+ Treg cells. These results suggest that SAgs are important vaccine targets to prevent severe invasive *S. aureus* infections.
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A 7.22- "Cigarette Smoke Mediated Regulation of Autophagy in RAW264.7 Macrophages"

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Background: Chronic obstructive pulmonary disease (COPD) has become a major global public health problem. Although the mechanisms responsible for the pathogenesis of COPD remain unclear, there is no doubt that cigarette smoke (CS) is a major risk factor. CS contains numerous oxidants, free radicals and chemicals, which can induce oxidative stress in lungs, resulting in cell death and senescence. Autophagy, once considered as a basic catabolic process to remove damaged organelles and proteins, has now evolved into a highly regulated process capable of specificity. There is paucity of information regarding its role in COPD. Autophagic process is mainly executed by a series of autophagy related proteins (ATGs) regulated by Fork head box class O3 transcription factor. LC3 is an autophagy marker which conjugates to the sequestering membrane and controls the elongation of phagophore. LC3-I conjugated to phosphatidylethanomine (PE) that requires ATG7. The lipidated, form of LC3 II is attached on to the autophagosome membrane. New insights into the molecular mechanisms associated with autophagy will help in improving therapeutic strategies for COPD patients.
Methods: We challenged RAW 264.7 murine macrophages with CS or DMSO (control) for 24h and determined the expression of LC3 by immunofluorescence imaging. We also determined the expression of ATG7 which is important for recruitment of LC3 (ATG8) to the autophagic complex; and LC3-I and the cleaved LC3-II levels in CS challenged and DMSO (control) treated cells by immunoblotting.
Results: We observed increase in the expression of ATG7 and cleavage of LC3-I to LC3-II in CS challenged murine macrophages. Our results also warrant for investigating the role of upstream regulators particularly at post-translational levels associated with the process during CS exposure.
Conclusion: Our findings suggest that autophagic flux may play critical role in disease pathogenesis of COPD.
Acknowledgements: This work was supported by Young Clinical Scientist Award (FAMRI-123253_YCSA_Faculty); NIH R15 (7 R15 ES023151 02) and Southern University Foundation Grant (COSC0016) to SB.

A 7.23- "The Role of the Erythrocyte Cytoskeleton Protein Spectrin During Invasion by *Francisella tularensis*"

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Background: *Francisella tularensis* is classified as a Category A bioterrorism agent by the Centers for Disease Control and Prevention. Inhaling as few as ten of these bacteria can cause acute, lethal pneumonia. Studies using mouse models of infection have shown that erythrocyte invasion is a novel feature of *F. tularensis* during infection; however, the mechanism by which this bacterium enters red blood cells is unknown. Cytoskeletal rearrangement of other host cells such as macrophages and hepatocytes is required for entry of *F. tularensis*.
Methods: In this study, we evaluated the role of the major erythrocyte cytoskeletal component, spectrin, in red blood cell invasion. To test this, human erythrocytes were treated with venom from the Blue-bellied black snake (*Pseudechis guttatus*), which disrupts the spectrin in the cytoskeleton. We also investigated the role of band 3 protein in red blood cell invasion using antigen-binding fragments of anti-band 3 antibodies.
Results: Treatment with this venom significantly reduced the number of intra-erythrocytic bacteria suggesting that spectrin is involved in erythrocyte invasion. Importantly, the concentrations of venom used in this study did not reduce the number of intact erythrocytes. The data collected from the investigation of band 3 protein in erythrocytes during invasion is inconclusive thus far, however preliminary results suggest that band 3 may not play a significant role during invasion by *F. tularensis*.
Conclusion: This is the first study showing the involvement of an erythrocyte protein during invasion by *F. tularensis.* The disruption of spectrin in erythrocytes significantly reduced the ability of *F. tularensis* to invade during red blood cell invasion. Further studies testing the role of band 3 protein in red blood cell invasion are ongoing.
Acknowledgement: This work was supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence and funding from the WV Research Challenge Fund [HEPC.dsr.14.13]

A 7.24- "Modualtion of anti-*Mycobacterium tuberculosis* Adaptive Immune Responses-Role of IDO"

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Background*: Mycobacterium tuberculosis* (*Mtb*) infection causes ~ 9 million new infections and ~ 1.5 million deaths globally, every year. The failure to control TB stems from the lack of complete understanding of the virulence and pathogenesis of this pathogen and is directly related to the inability of the experimental models of TB to accurately recapitulate human TB. The local architecture of the granuloma results in the close apposition of activated IFNg secreting lymphocytes with macrophages, necessary for the activation of the latter. IFNg stimulates macrophages to kill *Mtb* via a variety of mechanisms. Yet, some bacilli survive this process. While the granuloma may be a protective strategy to limit bacterial replication, it may also offer survival benefits to *Mtb*.
Methods: We have developed a robust macaque model of TB, which can be leveraged to study *Mtb* infection in *in-vivo* setting. *Mtb* infects antigen-presenting cells, which recruit and stimulate $CD4^+$ T cells, in turn activating *Mtb* infected macrophages and granuloma formation.
Resuts: Our preliminary data indicates that the expression of IDO1 is dramatically enhanced in classical granuloma lesions present in the lungs of rhesus macaques and is confined to the macrophage rich inner region of the lesion. IDO1 encodes for indoleamine 2,3, dioxygenase, a tryptophan catabolic enzyme. IDO1 is a powerful immunosuppressant of activated $CD4^+$ T cells.
Conclusion: Based on the available data and literature, we hypothesize that IDO1 is responsible for keeping activated $CD4^+$ T cells away from the center of the granuloma, where *Mtb* infected macrophages as well as extracellular *Mtb* are present. This specialized inner ring distribution of IDO1 expression indicates that active *Mtb* infection programs infected and bystander macrophages to prevent activated $CD4^+$ T cells from accessing the pathogen infected regions of the granuloma.
Acknowledgment: This work was funded by Tulane National Primate Research Center, Pilot Grant (A52274G1) and startup funds to SM from LSU COBRE (166-41-6174).

A 7.25- "Promoter Analysis of *M46* gene, in the Pathogenic Dimorphic Fungus *Histoplasma capsulatum"*

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Background: *Histoplasma capsulatum (Hc)* is a dimorphic fungus that is found in contaminated soils, in bird and bat excrements. In the soil, it exists as mold at 25 ˚C. Once the soil is disturbed, and the *Hc* spores are released and inhaled, it is converted into yeast at 37˚C. The conversion from mold to yeast is a requirement for pathogenesis. It is the yeast form, which causes the upper respiratory infection histoplasmosis. This study focuses on the mold-specific *M46* gene. Mold specific genes are genes that are only expressed in the mold phase and not in the yeast phase.
Methods: We would like determine what region of the promoter is required for *M46* expression by deleting different sections of the G186AS M46 promoter region, and fusing the promoter in frame to the Green Florescent protein (GFP). Florescence will cease once the region that is required for expression is deleted.
Results: For strains G186AS and Downs, *M46* is expressed in the mold but is not expressed in G184AS and G217B mold. Reason for lack of expression is unknown. Observation of the sequence of the *M46* promoter insertion revealed a 12 base pair deletion, located 500 base pairs upstream from the transcriptional +1 site. It also revealed a 10 base pair repeat insertion that is approximately 50 base pairs upstream from the transcriptional +1 site.
Conclusion: The variation of sequences may be the reason *M46* is not expressed in strain G217B. However, G186AS which does express *M46,* and strain G184AS which does not express *M46* have the same sequence. This suggests that *M46* in strain G184AS is not expressed due to a trans- regulating factor.
Acknowledgment: This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476

A 7.26- "Role of *msaABCR* operon in Biofilm Development of *Staphylococcus epidermidis"*

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Background: *Staphylococcus epidermidis* is an important nosocomial pathogen that causes infections primarily related to those of indwelling medical devices. Their ability to form biofilm and evade host

immune defense systems allow for this opportunistic pathogen their increased survivability and antibiotic resistance. A newly described operon, *msaABCR* operon has recently been linked to biofilm development, virulence and antibiotic resistance in *Staphylococcus aureus*. While *msaA, msaC*, and *msaR* are thought to be regulatory genes, msaB is the only putative protein transcribed from this operon. Bioinformatics analysis showed a good homology between the msaB in *S. aureus* and *S. epidermidis.* Therefore, we hypothesized that *msaABCR* operon will play a similar role in the regulation of biofilm development in *S. epidermidis* strains. The mechanism of biofilm development between the two species is different. *S. epidermidis* develops PNAG-mediated biofilm, whereas, *S. aureus* develops biofilm independent of PNAG. However, decreased transcription of *icaADBC* operon has been observed in the deletion mutant of *msaABCR* in *S. aureus*, so we hypothesized that it will play a similar role in *S. epidermidis* and affect its biofilm development.
Methods: Clustal Omega was used to determine the homology between *msaABCR* operon in *S. aureus* and *S. epidermidis*. Deletion construct (pJB38-*msaABCR*) for the deletion of *msaABCR* in *S. epidermidis* was made by amplifying the upstream and downstream flanking regions, as previously described by Sahukhal & Elasri, 2014. Effect of DispersinB, sodium metaperiodate, proteinase K, and DNase I on the mature biofilm produced by wild type *S. epidermidis* RP62A strains was studied to determine the major component of the biofilm matrix. We also studied the effect of different environmental factors like NaCl, glucose, and ethanol on the biofilm development by RP62A strains.
Results: Bioinformatics analysis showed 84% and 93% homology in *msaA* and *msaB* gene between *S. aureus* and *S. epidermidis* RP62A strains. Interestingly, *msaC* showed only 28% homology. The only protein translated from this operon, MsaB showed 97% homology between these two strains, but is found highly conserved within all *S. epidermidis* strains. The *msaABCR* deletion construct (pJB38-*msaABCR*) has been made, verified and moved to *S. aureus* RN4220 strains. The construct will be introduced to *S. epidermidis* RP62A strains to delete the *msaABCR* operon gene by allelic replacement method and study its effect on the biofilm development. The mature biofilm produced by the wild type strains RP62A, is more sensitive to Dispersin B and Sodium metaperiodate compared to proteinase K and DNase I, thus suggesting its biofilm development is more PNAG dependent. Environmental stressors like NaCl, glucose, and ethanol were found to induce biofilm development in the RP62A strain.
Conclusion: This study will allow us to define the role of a new global regulator, *msaABCR* operon, in *S. epidermidis* strain that has been associated with major phenotypes in *S. aureus* such as biofilm development and virulence. The findings may be therapeutically relevant to control the biofilm-associated infections in both *S. aureus* and *S. epidermidis* strains.
Acknowledgments: This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.[2]

Neuroscience,
Poster Session A
Thursday, September 12, 11 a.m.-12:30 p.m.

A 8.1- "Leptin Inhibits Presympathetic Neurons in the Rostral Ventrolateral Medulla"
Hong Gao[1], Heike Muenzberg-Gruening[2], Andrea Zsombok[1], Andrei V. Derbenev[1]
[1]Department of Physiology, Tulane University Health Sciences Center, New Orleans, LA
[2]Pennington Biomedical Research Center, LSU, Baton Rouge, LA

Background: Leptin is greatly involved in the regulation of food intake and dysfunction of leptin or its receptors is associated with obesity. Numerous evidence indicate that obesity is linked to increased prevalence of hypertension. The sympathetic nervous system (SNS) is involved in the regulation of arterial blood pressure and neurons in the rostral ventrolateral medulla (RVLM) are critical components of both the SNS and cardiovascular regulation.
Method: In this study we used whole-cell patch-clamp recordings from presympathetic kidney-related RVLM neurons identified with retrograde viral labeling and tested the hypothesis that leptin reduces neuronal excitability of RVLM neurons.
Results: Application of leptin (500 nM) caused a rapid membrane hyperpolarization (3.7 ± 1.4 mV change) in half of the recorded presympathetic RVLM neurons without affecting the rest of the recorded cells. The hyperpolarization was accompanied with an increase of input resistance from 287 ± 95 MΩ to 336 ± 97 MΩ. In addition, leptin decreased the frequency of spontaneous excitatory postsynaptic currents (sEPSC) by 35.6 ± 0.1% without changing sEPSC amplitude. In contrast, leptin did not alter the inhibitory neurotransmission.
Conclusion: Our data demonstrate that leptin reduces the excitability of a subset of presympathetic RVLM neurons via pre- and postsynaptic mechanisms and suggest a potential control of SNS activity.
Acknowledgment: This work was supported by Tulane University COBRE in Hypertension (NIH P30GM103337) and AZ (NIH R01DK099598).

A 8.2- "Control of Cortactin Levels by Wg/Wnt Regulates Activity Dependent Synaptic Plasticity"
Daniel Alicea, Carihann Dominicci, Marizabeth Perez, Carolina Maldonado, Bruno Marie
Institute of Neurobiology, University of Puerto Rico, Medical Sciences Campus, Rio Piedras, PR

Background and Objective: The secreted signaling molecule Wg/Wnt has recently been shown to be essential to activity-dependent synaptic plasticity. A challenge remains to understand how this potent signal is transduced into the necessary cellular changes underlying this plasticity. Here we focus on the actin regulator Cortactin (a major organizer of protrusion, membrane mobility and invasiveness) and define Cortactin's new role in synaptic plasticity.
Methods: We use genetics, confocal microscopy and electrophysiological recordings to assess the role of Cortactin in regulating activity-dependent synaptic plasticity both at the structural and functional levels.
Results: We show that Cortactin is present pre and post-synaptically at the Drosophila Neuromuscular junction (NMJ) and that pre-synaptic Cortactin regulates activity-dependent modifications in synaptic structure and function. In addition, we show that Cortactin level is increased at stimulated synaptic terminals and that this increase requires de novo transcription and depends on Wg/Wnt expression. Finally, we present evidence of a correlation between the abundance of Cortactin at the synapse and the magnitude of the activity-dependent synaptic plasticity.
Discussion and Conclusion: We argue that Cortactin is an important element that determines the gradient of activity-dependent synaptic plasticity under the control of the Wg/Wnt signal.
Funding: NIH-NIGMS 1P20GM103642

A 8.3- "The Postmortem Brain Core of the COBRE Center for Psychiatric Neuroscience"
Craig A. Stockmeier, Gouri Mahajan
The University of Mississippi Medical Center, Jackson, MS

A comprehensive understanding of the brain from the gross anatomical to the cellular and molecular level is critical to basic and clinical neuroscience. Mission of the Postmortem Brain Core: To provide high-quality: 1) human brain tissue collected at autopsy from normal control subjects and subjects with a psychiatric illness (e.g. major depressive disorder, schizophrenia, bipolar disorder, or an alcohol or substance use disorder); 2) brain tissue from monkeys treated for 9 months with fluoxetine (an antidepressant) or vehicle; and 3) assistance to users in the selection and use of tissues. Psychiatric information is collected by an informant-based, structured clinical questionnaire, evaluated by a clinical team and diagnoses are determined by DSM-V criteria. Services: The Core provides guidance, technical support and tissue for research to better understand the pathology of mental illnesses and assists faculty by enhancing competitiveness for extramural funding. Access to high-quality tissue is accompanied by a detailed psychiatric and medication history, and toxicology and neuropathology reports. Services include tissue processing, celloidin embedding and sectioning, frozen sectioning, collection of cell types or tissue via laser capture microdissection from cryostat sections, routine immunohistochemical and Nissl staining, and 3-D cell counting. Fee for Service includes access to

Core equipment and/or performance of services by an investigator with the help of core manager; assistance with design and interpretation; development of new techniques. Information and a fee schedule are at: http://www.umc.edu/cpn/. Acknowledgment: Supported by IDeA Award P30 GM103328 and the University of Mississippi Medical Center.

A 8.4- "Pharmacoresistant Epilepsy: Methods for Measuring Efficacy vs. Toxicity of Cannabidiol and Structural Analogues"

Dennis R. Carty, Stephen J. Cutler, Mahmoud A. ElSohly, Ikhlas A. Khan, Kristine L. Willett
Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS

Background: While cannabidiol (CBD) and cannabidiol oil (CBO) show anecdotal efficacy in reducing seizure frequency, little is known about the potential adverse side-effects of CBD and CBO on child physiology, brain development, adult disease, and/or F1 offspring. Therefore, the goal of this project is to compare the relative morphological, behavioral, reproductive and multigenerational phenotypes that result after a developmental exposure to CBD, CBO and ∆9-tetrahydrocannabinol (THC). Additionally, there is a critical need for new, non-scheduled drugs for treatment of pediatric epilepsy.
Methods: Our study design, using an adverse outcome pathway paradigm, will allow us to determine the unique effects of CBD, CBO and THC and will provide insight into the molecular pathways of both toxic and therapeutic action. We will leverage our experience with the zebrafish model and extensive natural product and chemical libraries available in our department to develop a high-throughput screen for epilepsy drug discovery.
Results: The dose-dependent adverse effects of THC, a known morphological, neurodevelopmental, and behavioral toxicant, will be compared to CBD and CBO in developing and adult zebrafish. Additionally, sperm integrity and reproduction success will be analyzed following exposures. Lastly, positive leads with anti-epileptic activity will be pipelined: A) back into zebrafish toxicity assays; and B) submitted to the *in vitro* and *in vivo* cores of our NIH-Center for Biomedical Research Excellence Natural Product Neuroscience.
Conclusion: This work is highly relevant because human exposure to THC is known to cause reproductive deficits including: pregnancy loss and sperm malformations and in children, brain development deficits and social disorders. However, the developmental toxicity of CBD or CBO is unknown.
Acknowledgment: This project is funded by Grant Number P20GM104932 from the National Institute of General Medical Sciences (NIGMS), a component of the National Institutes of Health (NIH).

A 8.5- "Low Intensity Pulsed Focused Ultrasound Alters Long-Term Excitability Properties of Cortical Cells in Rats"

Leonardo Rodríguez-Negrón[1], Rafael Vázquez-Torres[2], Bermary Santos-Vera[2], Carlos A. Jiménez-Rivera[2], Eduardo J. Juan-García[1]
[1] Department of Electrical & Computer Engineering, University of Puerto Rico at Mayagüez, Mayagüez, PR
[2] Department of Physiology, Medical Sciences Campus, University of Puerto Rico, San Juan, PR

Background: Although the neuromodulatory effects of Focused Ultrasound (FUS) have been a research topic since the 1950's, the effects of FUS in long-term (> 30 mins) cell excitability and electrophysiological changes has been of limited study. The excitability properties of Sprague-Dawley rat's pre-frontal cortex (PFC) cells were analyzed after low intensity insonification in order to study the feasibility of FUS to induce long-term excitability or inhibition.
Methods: A total of 9 Sprague-Dawley rats were anesthetized with an intraperitoneal injection of chloral hydrate, decapitated and the brains promptly removed. The PFC was dissected into coronal slices (220 um) and incubated for 45 minutes in an oxygenated artificial cerebrospinal fluid (ASF) at 32˚C. PFC slices were insonified with 5 ms pulses of 250 kHz FUS at a rate of 100 Hz (50 % duty cycle) with an intensity of 12.8 W/cm^2 (spatial peak-temporal average). After insonification, PFC pyramidal cells were identified by visual inspection. Whole-cell current clamp recordings were obtained for input currents that varied from -100 pA to 500 pA.
Results: The number of action potentials (AP), AP amplitude, AP duration, initial inter-spike interval, rheobase and fast after-hyperpolarization were compared between experimental (n = 11) and control (n = 14) groups. An increase in AP amplitude and depolarization rates values was observed in FUS-treated cells ($p < 0.05$) for input currents greater than 300pA. Furthermore, decreased adaptation frequency values were observed for input currents greater than 150 pA.
Conclusion: Our results indicate changes in cortical cell excitability properties that persist long after (between 30 and 60 minutes) insonification, suggesting long-lasting electrophysiological changes induced by FUS.
Acknowledgment: This work is supported by NIH-GM-08224 to CAJR and by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM103475-13.

A 8.6- "The Imaging Core Facility of the COBRE Center for Psychiatric Neuroscience"

Grazyna Rajkowska, Mohadetheh Moulana, Craig A. Stockmeier
The University of Mississippi Medical Center, Jackson, MS

Mission: A comprehensive understanding of the brain and its cellular systems at structural levels ranging from gross anatomy to the cellular level is critical to basic and clinical neuroscience. The Imaging Core is funded by a Phase III COBRE grant and provides a centralized base for microscopic equipment with advanced capabilities in the visualization, quantification and interpretation of neuronal systems at the cellular level.
Services provided**:** 1) Design of experiments, performance and interpretation of results, if needed, 2) Image analysis including object identification, 3-D object counting, 2-D gray level analysis, confocal laser scanning microscopy, fluorescent microscopy, and digital photomicroscopy; 3) Techniques offered include general histology, single-and double-label immunohistochemistry, immunofluorescence labeling and Western blotting.
Equipment: Nikon C1 confocal scanning microscope with 3-dimensional counting capabilities, fluorescent microscope with 3-dimensional counting capabilities, Nikon research microscopes with MBF Stereoinvestigator and Neurolucida systems for 3-dimensional cell counting and object tracing, stereomicroscopes, MCID Image analysis systems, cryostat.
Type of support provided**:** Fee for Service includes access to Core equipment and/or performance of the image analysis or tissue staining by an investigator with the help of core manager; assistance with design and interpretation; development of new techniques. While the COBRE is focused on discovery of cellular pathology in psychiatric and neurological disorders, the Core provides services for any investigator. For information and a fee schedule, see http://www.umc.edu/cpn/.
Acknowledgement: This work was funded by an Institutional Development Award (IDeA) from NIGMS under grant number GM103328.

A 8.7- "Astrocyte Pathology in the Ventral Prefrontal White Matter in Major Depressive Disorder"

G Rajkowska, B Legutko, CA Stockmeier, JJ Miguel-Hidalgo
The University of Mississippi Medical Center, Jackson, MS

Background: The functions of astrocytes in white matter (WM) are much less understood than their functions in gray matter. Our recent study of WM in the ventral prefrontal cortex (vPFC) revealed alterations in the expression of myelin-related genes in major depressive disorder (MDD). Since WM astrocytes play a role in maintaining myelin, we hypothesized that the morphometry of WM astrocytes will be altered in MDD in the same prefrontal WM region in which myelin-related genes are altered. Moreover, we also predicted that the expression of the glial glutamate transporter-2 (EAAT-2) would be altered in this region of white matter in MDD as this transporter is located on astrocyte processes and on axons myelinated by oligodendrocytes.
Methods: Sections of WM underlying vPFC were examined in 8 matched pairs of MDD and control subjects. Astrocytes were immunostained with an anti-GFAP antibody. The packing density of astrocyte cell bodies and their sizes were measured using StereoInvestigator. The area fraction of astrocyte immunoreactivity (cell bodies + processes) was estimated with Image J. Punches of tissue from adjacent vPFC sections were used to estimate the level of EAAT2 protein by Western blotting.
Results: Two morphological types of GFAP-immunoreactive astrocytes, small and large, were distinguished in the WM of vPFC. A marked reduction (95%) in the density of large GFAP-immunoreactive astrocytes was found in MDD. A less-pronounced but also significant reduction (52%) in the density of small astrocytes was noted in MDD. The mean area fraction of all GFAP-immunoreactive structures (cell bodies + processes) was significantly reduced by 28% in MDD. The average expression of EAAT2 protein was significantly reduced by 75% in the same MDD subjects as those used for astrocyte density measurements.
Conclusions: The observed decreases in the density and area fraction of WM astrocytes may be related to myelin pathology in depression as astrocytes play a role in myelin maintenance and development. Astrocyte pathology in WM is consistent with altered expression of myelin-related genes observed recently in the same WM region in MDD. Astrocyte pathology in WM in MDD is also consistent with decreased expression of EAAT2 protein in this disorder. Moreover, EAAT2 is localized to astrocyte processes and cell bodies. P30 GM103328.
Acknowledgement: This work was funded by an Institutional Development Award (IDeA) from NIGMS under grant number GM103328.

A 8.8- "Polymeric Micelle as a RhoA siRNA Carrier for Axonal Regeneration in Rat SCI Model"

So Jung Gwak[1], Christia Macks[1], Ken Webb[1], Michael Lynn[3], Mark Kindy[2], Jeoung Soo Lee[1]
[1]Department of Bioengineering, Clemson University, Clemson, SC
[2]Department of Neurology, USC Medical School, Greenville, SC
[3]Dept of Pharmaceutical Sciences, College of Pharmacy, University of South Florida, Tampa, FL

Background: Physical trauma or ischemia results in significant damage to the central nervous system (CNS). The regenerative capacity of the injured adult CNS is extremely limited, due to both extrinsic microenvironmental factors and intrinsic, age-related changes in neuronal biochemistry. Many studies have shown that diverse extracellular inhibitors of neuroplasticity including both myelin associated inhibitors and chondroitin sulfate proteoglycans may act through common intracellular signaling pathways. Neurite growth inhibition in response to MAIs and CSPGs has been shown to be associated with activation of RhoA and Rho kinase (ROCK) and can be overcome by Rho/ROCK inhibitors. The long term goal of our work is to develop neuron-specific multi-functional polymeric micelle nanotherapeutics for combinatorial delivery of multiple bioactive molecules targeting different barriers to plasticity and axonal regeneration. To achieve this goal, we designed amphiphilic copolymers (poly(lactide-co-glycolide)–g-polyethylenimine: PgP) as a drug and nucleic acid delivery carrier. Here, we show that PgP micelle/RhoA siRNA efficiently knockdowns RhoA gene expression in B35 cells in presence of 10% serum *in vitro* and in a rat compression spinal cord injury model *in vivo*.
Methods: Poly (lactide-co-glycolide)-graft-polyethylenimine (PgP) was synthesized and characterized by ^{1}H-NMR/GPC. PgP/RhoA siRNA polyplexes were prepared at various (N/P) ratios ranging from 5/1 to 30/1. B35 cells were transfected in media containing 10% serum and total RNA was isolated at 72hrs post-transfection. The efficiency of Rho A gene knockdown was measured by real-time PCR using the QuantiTect SyberGreen PCR kit (Qiagen) with RhoA gene-specific primers. Relative changes in gene expression levels will be analyzed by the delta-delta C_T method.
Laminectomy was performed on the back of Sprague Dawley rats and the T9 spinal cord region was exposed. PgP/RhoA siRNA polyplexes (20 μg RhoA siRNA) at various N/P ratio were prepared and injected at the T9 spinal cord region. At 7 days after polyplex injection, the rats were sacrificed and spinal cord sites were retrieved and total RNA was isolated and real-time PCR were performed as described above.
Results: In B35 cells, RhoA gene knockdown of approximately 40% was achieved at N/P ratio of 30/1. In the SCI model, RhoA expression was upregulated (2.5x) in untreated SCI animal groups relative to the sham animal group. In PgP/RhoA siRNA treated animal groups, RhoA gene expression was not significantly different from the sham animal group.
Discussion and Conclusion: These studies demonstrated that PgP is a promising therapeutic siRNA delivery carrier in B35 cells in *vitro* and in rat compression spinal cord injury model *in vivo*. Currently, we are evaluating the feasibility of PgP as a drug delivery carrier in rat spinal cord injury model.
Acknowledgements: Research reported in this publication was supported by NIGMS of the National Institutes of Health under award number 5P20GM103444-07 and South Carolina Spinal Cord Injury Fund under award number SCIRF # 2014 I-02.

A 8.9- "Measuring Mitochondrial Size and Movement in Primary Mouse Neurons"

John Z. Cavendish, Saumyendra Sarkar, Sujung Jun, James W. Simpkins
West Virginia University, Morgantown, WV

Background: Neuronal mitochondria are dynamic organelles that divide, fuse, and move along axons and dendrites and neurons rely heavily on oxidative phosphorylation in mitochondria for their survival and proper function. It is hypothesized that abnormal mitochondrial function contributes to the pathogenesis of neurodegenerative diseases including Parkinson's disease, Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS). The goal of the current study was to develop a method for measuring mitochondrial structure and dynamics in living neurons. We then investigated mitochondrial parameters in neurons from Alzheimer's transgenic mice.
Methods: We generated primary neuronal cultures from embryonic day 19 mouse embryos of triple transgenic (PS1M146V/APPSwe/tauP301L) Alzheimer's mice (3xTg-AD) and wild type controls (WT). We transfected neurons with mito-dsRed2 plasmid and imaged fluorescently-labeled mitochondria in live neurons using an epifluorescence microscope. We measured length, width, cross-sectional area, and perimeter of mitochondria in neurites of cultured neurons, and we measured mitochondrial movement in time-lapse images captured every 10-15 seconds over 45-60 minutes. To determine if our measurements detected differences in mitochondrial structure or movement, we treated wild-type neurons with rotenone, an inhibitor of complex I.
Results: We observed significant reduction in average area, perimeter, and length of mitochondria as well as elimination of mitochondrial movement after application of 1μM rotenone. We found no significant differences in mitochondrial size or movement between WT and 3xTg-AD neurons.

Conclusion: We have shown that inhibition of mitochondrial function rapidly changes their morphology and intracellular behavior in neurons. The lack of changes in embryonic 3xTg-AD neuronal mitochondria suggests that AD-induced mitochondrial dysfunction, if present, occurs at later disease stages.
Acknowledgments: This work was funded by NIA grant PO1 AG022550, NIA grant P01 AG027956, and NIH/NIGMS grant P20 GM109098. Imaging experiments and image analysis were performed in the West Virginia University Microscope Imaging Facility, which has been supported by the Mary Babb Randolph Cancer Center and NIH grants P20 RR016440, P30 GM103488 and P20 GM103434.

A 8.10- "ShRNA Based Suppression of Cx43 in Vivo and In Vitro to Determine Depression-Related Cellular and Behavioral Abnormalitie"

José Javier Miguel-Hidalgo, Katherine Hall, Zhen Jia, Natalie J. Booker
The University of Mississippi Medical Center, Jackson, MS

Background: Major depressive disorder (MDD) is associated with reductions in connexin 43 (Cx43) in prefrontal cortex (PFC). Cx43 is located in astrocytes and forms gap junctions. Gap junctions are crucial for essential processes such as myelination. It is still unknown whether reductions in Cx43 in PFC in depression result in changes in myelin plasticity. To determine the influence of Cx43 on myelination and on depression-related behaviors, we are applying an shRNA-based technique using non-replicant LCMV-pseudocoated lentiviruses as vectors to suppress expression of Cx43 in the rat PFC.
Methods: These experiments examine the ability of transduced Cx43 shRNA to be expressed and suppress Cx43 in cultured astrocyte-like C6 glioma cells and *in vivo* in the rat PFC. We used non-replicant lentiviruses with one of 4 different shRNA sequences and one for green fluorescent protein as reporter. The lentiviruses were prepared in HEK293 cells at different titers, and the viruses then used for in vitro and in vivo transductions.
Results: Transduction of C6 cell with viral titers of 10^6 to 10^8 particles/ml resulted in ample GFP expression, and in reduction of Cx43 mRNA and Cx43 immunoreactivity only when Cx43 shRNA was employed, but not with scrambled shRNA. *In vivo* transduction resulted in the labeling of small-sized cells, many immunoreactive for the astrocyte marker glial fibrillary acidic protein (GFAP). Three weeks after bilateral infusion of Cx43 shRNA in rats, ultrasonic vocalizations appear to be reduced.
Conclusion: Use of Cx43 ShRNA transduced with lentivirus appears effective in suppressing Cx43 RNA expression in C6 cells and preferentially in glial cells in the rat PFC. Infusion of Cx43 shRNA caused a behavioral change that was similar to that noted after chronic unpredictable stress, a rodent model of depression-like behavior.
Acknowledgment: This work received support from the Molecular & Genomics Core and the Animal Behavior Core of the Center for Psychiatric Neuroscience at the University of Mississippi Medical Center funded by an Institutional Development Award (IDeA) from the NIGMS under grant number P30GM103328.

A 8.11- "Peripheral Inflammation During Pregnancy Leads to Increased BBB Leakage in the Post-Partum Period"

Kedra Wallace, Teylor Bowles, Shauna-Kay Spencer, Cynthia Bean
The University of Mississippi Medical Center, Jackson, MS

Background: Hemolysis Elevated Liver enzyme Low Platelet (HELLP) syndrome is associated with hypertension and inflammation during pregnancy and and affects up to 1% of pregnant or early post-partum women. We have recently reported that plasma from women with HELLP syndrome increases leakage of Texas Red Dextran from rat cerebral vessels compared to normal pregnant (NP) women, indicative of increased blood brain barrier (BBB) permeability. In the current study we wanted to determine if infusion of Texas Red into rats with HELLP syndrome was associated with BBB leakage and whether or not this persisted beyond pregnancy.
Methods: On gestational day (GD) 12, mini-osmotic pumps infusing sFlt-1 and sEng are placed into rats to induce HELLP syndrome and were not removed until 12-24hrs post-delivery. On GD19 or post-partum day (PPD) 3, 10 or 20, Texas Red was infused via carotid catheters and allowed to circulate for 10 minutes before being flushed out of the circulation. The rats were decapitated, brain removed and scanned with IVIS Spectrum. Regions of interest were scanned to determine what areas emitted the most light due to Texas Red leakage. Blood was collected from an un-infused set of rats who were euthanized at the same time to determine peripheral inflammation and circulating $CD4^+$ T cells.
Results: HELLP syndrome during pregnancy is associated with a significant increase in Texas Red emission leakage in the posterior cortex ($p=0.0006$), brainstem ($p=0.006$) and cerebellum ($p=0.014$) of rats that persists into the post-partum period. This was accompanied by a significant increase in circulating CD4 cells in response to HELLP syndrome that also persisted until PPD 10 ($p=0.0009$). Hypertension also persisted until PPD20 in rats with a history of HELLP syndrome compared to NP rats ($p=0.002$).
Conclusion: These results suggest that hypertension and peripheral inflammation during pregnancy contributes to an increase in BBB permeability and systemic inflammation. As women with a history of

HELLP syndrome are reported to be at an increased risk of developing postpartum depression or post-traumatic stress disorder, additional studies are being carried out to determine if changes in BBB permeability are also associated with alterations in behavior.
Acknowledgment: This work was funded by an Institutional Development Award (IDeA) from the NIGMS under grant number P30GM103328.

A 8.12- "The Functional Roles of miR-1017"
Matthew de Cruz
The University of Southern Mississippi, Hattiesburg, MS

microRNAs (miRNAs) are recognized as regulators of most mRNAs within multi cellular animals. Despite the pervasive nature of these genes relatively few have been characterized, let alone non-canonical family members. Here we investigate the functional roles of miR-1017, a 3' tailed mirtron. miR-1017 is encoded within an intron of, nAchRalpha-96Ab. Utilizing reporters for nAchRalpha-96Ab expression showed the mir-1017 host transcript present in a restricted pattern in *Drosophila* larval brains and broad expression in adults. Numerous miR-1017 predicted targets serve neurological roles. Interestingly, using mir-1017 knock out (KO) mutants, we found derepression of many predicted targets including nAchRalpha-34E, Rrp45 and most interestingly, its host transcript nAchRalpha-96Ab. Utilizing reporters for nAchRalpha-96Ab, we will examine the development of the *Drosophila* brain within mir-1017 KO mutants. Based on our data miR-1017 may be an important player in regulating acetylcholine receptor activity. Increased receptor activity has been reported to cause reactive oxygen species, which leads to neurodegeneration. Therefore we will examine whether varying levels of miR-1017 may take a functional or protective role during neurodegeneration.

A 8.13- "Evaluation of Berberine as an Inhibitor of Human Monoamine Oxidase A and B: Potential Application in Treatment of Neurological Disorders"
Deependra Singh[1], Narayan D Chaurasiya[1], Stephen J. Cutler[2], Larry A Walker[1,2], Babu L. Tekwani[1,2].
[1]National Center for Natural Products Research, The University of Mississippi, University, MS
[2]Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS

Background & Objectives: Broad spectrum of pharmacological activities and therapeutic applications of berberine, an isoquinoline quaternary alkaloid, make it an important natural product candidate for modern drug and pharmaceutical research. Berberine is a prominent constituent of several medicinal plants used in Indian and Chinese traditional/indigenous systems of medicine. Chemically standardized extracts containing berberine are now available as over the counter herbal remedy for different clinical indications. Recent studies have shown promising antidepressant actions of berberine in animal models. Computational analysis suggested monoamine oxidases (MAO) as potential targets. MAOs are involved in numerous neurophysiological functions that are affected by the neurological disorders such as depression, Alzheimer's disease and Parkinson's disease. Berberine was evaluated *in vitro* as an inhibitor of human monoamine oxidase-A and -B to explore its potential applications for the treatment of neurological disorders.
Methods: Berberine chloride was procured commercially and evaluated *in vitro* for inhibition of recombinant human MAO-A and -B by spectrophotometric kynuramine deamination assay. The IC_{50} values were determined at saturated substrate concentrations. Interaction and binding characteristics of berberine with MAO-A and -B were determined by substrate-kinetics assays, enzyme-inhibitor complex formation and equilibrium-dialysis dissociation analysis.
Results: Berberine showed significant inhibition of MAO-A and -B with IC_{50} values of 43.7±1.1 and 7.4±1.0 μM respectively. Berberine showed 11.63 fold selectivity for inhibition of MAO-B (Ki 1.924± 0.282μM) than -A (Ki 22.383± 3.450 μM) with mixed-type inhibition kinetics. Binding of berberine with MAO-A and -B was not time-dependent and reversible, as determined by enzyme-inhibitor binding and dissociation-dialysis assays.
Discussion and Conclusions: The study shows berberine as a selective inhibitor of MAO-B. Berberine interacts with the MAO through competitive reversible-binding mechanism. Selective MAO-B inhibitory properties of the berberine suggest its potential for eliciting selective pharmacological effects that might be useful in treatment of neurological disorders.

A 8.14- "High Throughput Screening for the Investigation of Blood-Brain Barrier Integrity Enhancers"
Quoc-Viet Duong, Ashley DePaula, Courtney Flick, Trista LeBeouf, Youssef Mousa, Jeffrey N. Keller, Amal Kaddoumi
The University of Louisiana at Monroe, LA

Background: Alzheimer's disease (AD) is known for its hallmark features such as increased levels of tau hyperphosphorylation and amyloid-beta (Aβ) deposition. Cerebral vascular dysfunction constitutes an important feature of AD as well, which includes amyloid angiopathy and clearance impairment of Aβ across the blood-brain barrier (BBB). In the search for potential drugs that may enhance or maintain the BBB integrity and function, in this study, compound libraries

were evaluated utilizing an in-vitro high throughput screening assay (HTS). From the screening, 3 hits that exhibited properties to enhance the BBB model integrity were selected and will be further investigated in vivo in animal models.
Methods: An in-vitro BBB model utilizing $bEnd_3$ cells was constructed for HTS of Sigma LOPAC®1280 compounds library. Lucifer Yellow (LY) permeation across the cell monolayer was measured to monitor the integrity of the model. Compounds were identified as hits if they were able to enhance the monolayer integrity. Next, hit compounds were secondary screened for their effect on BBB model function. Utilizing the same cell line, western blot analysis for the transporter proteins P-gp and LRP-1, and the tight junction proteins ZO1, Occludin, and Claudin-5 was performed. Finally Aβ transport study was conducted to measure the effect of hit compounds on Aβ transport across the membrane.
Results: The HTS assay identified 3 unique compounds that decreased LY permeation, indicating enhancement of the in-vitro BBB model integrity. Western blot analysis showed these hit compounds to deferentially alter transport and tight junction protein expression. In addition, hit compounds enhanced the Aβ transport across the monolayer in this model.
Conclusion: From the HTS, we identified 3 hit compounds that have shown to increase the cell-based BBB model integrity and function, suggesting these compounds are compelling candidates for further in vivo investigation in AD animal models. The results from this work are expected to identify compounds that have the potential to rectify the compromised BBB observed in AD and related disorders.

A 8.15- "The National Center of Neuromodulation for Rehabilitation (NC NM4R)"
Steven A Kautz, PhD
Medical University of South Carolina, Charleston, SC

Background: The NC NM4R will be a national resource for researchers using the exciting tools of NM4R to develop the next generation of rehabilitation interventions and to understand and study neuroplastic nervous system changes associated with rehabilitation. The center builds on outstanding expertise in non-invasive brain stimulation, rehabilitation, operant conditioning of brain and spinal networks, and animal models of NM4R.
Methods: Exceptional resources already in place at MUSC will support the center. These include a number of directly relevant core resources supporting neurorehabilitation research associated with an NIH/NIGMS Center of Biomedical Research Excellence (COBRE) in Stroke Recovery and the specially designed equipment that enabled MUSC to become the first institution in the world to image TMS in the scanner with fMRI. A rich portfolio of NM4R research at MUSC offers robust opportunities for collaboration with external investigators. The overarching goal is to exert a sustained, powerful influence on the research field of NM4R. We seek to fulfill that goal through translational activities and programming to achieve the following specific aims: 1. Train researchers in NM4R; 2. Provide scientific programming in NM4R; 3. Develop a research community in NM4R; and 4. Advance the field of NM4R.
Conclusion: NM4R is a teachable expertise – one that MUSC has a proven history of exporting to the world and will build upon. The NC NM4R will build critical mass over the next 5 years by training 100+ researchers through workshops and/or on-site training, giving current and future researchers access to the comprehensive translational multidisciplinary training necessary (but not currently available) to turn emerging concepts into evidence-based treatments.
Acknowledgment: This work was funded by NICHD Grant P2CHD086844 (to SAK) and by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM109040.

A 8.16- "Oleocanthal Enhances Amyloid−β Clearance and Reduce Inflammation in the Brains of TgSwDI Mice"
Yazan S. Batarseh, Hisham Qosa, Amal Kaddoumi
The University of Louisiana at Monroe, Monroe, LA

Background: Mediterranean diet (MD) is considered one of the most health promoting diets adopted initially by the Mediterranean population. Recent studies, showed a link between MD and lowering the incidence of mild cognitive impairment and Alzheimer's disease (AD). Clinical and preclinical studies have suggested several health promoting effects for the dietary consumption of extra-virgin olive oil (EVOO), a major component of MD, that could protect and decrease the risk of developing AD. Moreover, recent studies have linked this protective effect to oleocanthal, a phenolic secoiridoid component of EVOO. Here we provide evidence to support the role of oleocanthal in enhancing the clearance of beta amyloid (Aβ), a major pathological hallmark in AD, and reducing the overall inflammatory burden in the brain.
Methods: TgSwDI mice were intraperitoneally treated with oleocanthal or normal saline for duration of 4 weeks. Western blot analysis was constructed for Aβ transport and degradation involved proteins. Double immunohistochemistry was also performed for Aβ with astrocytes and microvessels. Brain efflux index was calculated by stereotaxic Aβ -125I micro-injection. ELISA was performed to quantify Interleukin -1β (IL-1β) levels.
Results: Oleocanthal treatment significantly decreased Aβ load in the hippocampal parenchyma and microvessels. Further mechanistic studies

demonstrated oleocanthal to increase the expression of important amyloid clearance proteins at the blood-brain barrier (BBB) including P-glycoprotein (P-gp) and low density lipoprotein receptor-related protein 1 (LRP1), and to activate the ApoE-dependent amyloid clearance pathway in the mice brains. Furthermore, Oleocanthal was able to reduce astrocytes activation and IL-1β levels.

Conclusion: The reduction in Aβ levels and microvessels deposition could be explained, at least in part, to the enhanced Aβ clearance across the BBB via the up-regulation of P-gp and LRP1, and the ApoE-dependent pathway. In addition, oleocanthal demonstrated an anti-inflammatory effect by reducing astrocytes activation and IL-1β brain levels, which emphasize the importance of considering oleocanthal as a potential therapeutic agent in AD.

Bioinformatics, Poster Session B

Friday, September 13, 10:45 a.m.-12:15 p.m.

B 1.1- "Assessment of mRNA 3'Untranslated Region (3'UTR) Dynamics During Neuroplasticity"

Benjamin Harrison, Jeffrey Petruska, Eric Rouchka
University of Louisville, Louisville, KY

mRNA 3'-untranslated regions (3'UTRs) play an important role in regulating gene functions by modifying cellular localization, stability and/or translational efficiency of transcripts during normal biological functions (e.g., development, nervous system functions) and disease states (e.g., UTR shortening in cancer). 3UTRs, and current estimates suggest there may be up to 5,000 genes with unannotated 3-end sequencing studies and EST-tag datasets. The resulting putative alternate 3 ends were then assigned to their respective gene loci to develop a modal of all possible 3UTR sequences, of which more than 200 are extended when plasticity is induced. Using position weight matrices, analyses of the UTR sequences extended during neuroplasticity revealed strongly over-represented motifs for neuron-specific miRNAs and RNAbps with known roles in nervous system pathologies.
These studies demonstrate the utility of our method, by employing a comprehensive database of putative poly-adenylation sites, to assess existing RNA-seq data sets for both UTR shortening and lengthening events. In addition, our analyses of RNA-seq profiles of neural plasticity suggest a role for 3'UTR dynamics during axon growth.
Acknowledgment: This work was funded by NIH grant P20GM103436

B 1.2- "Assessment of Major RNA-Seq Assembly Approaches for Identification of Long Noncoding Rnas in Different Tissue Types"

Dan Li, Carolyn Arthur, Mary Yang
The University of Arkansas at Little Rock, Little Rock, AR

Background: It is well known that long noncoding RNAs (lncRNAs) function as regulators and play critical roles in diverse biological processes. RNA sequencing (RNA-Seq) techniques and computational methods allow for the reconstruction of incompletely characterized transcripts and enable genome-wide discovery of lncRNAs. Comprehensive identification of lncRNA is essential for downstream functional analysis. Here, we developed and compared two lncRNA identification pipelines, based on reference-guided and *de novo* assembly, respectively.
Methods: We used STAR/Cufflinks as the reference-guided method and GMAP followed by Trinity as the *de novo* method. Our approaches were applied to five RNA-Seq datasets from different types of human tissues. LncRNAs were obtained by coding potential assessment. Furthermore we analyzed tissue-specific expression of lncRNA and differential expression of lncRNA in normal versus tumor tissues.
Results: We found that the *de novo* method tended to discover lncRNAs having less variance in transcript length distribution, splice pattern, and H3K4me3 modification across different tissue types, as compared to those discovered by the reference-based approach. Furthermore, the overlap rate of lncRNAs identified by these two approaches was less than 42% for all five RNA-seq datasets. The expression levels of majority lncRNAs we identified were low, which is consistent with previous reports. Moreover we found that the average expression level of lncRNAs in normal tissues is higher than that in tumor samples.
Conclusion: Combination of datasets from the de novo and reference-based approaches yields a more comprehensive set of lncRNAs. Concordant lncRNAs from both approaches leads to a more conservative and higher confidence lncRNA set for further biological analysis.
Acknowledgment: This work was supported by grants from P20RR016460, P20GM103429, 1R15GM114739 and the Arkansas Science and Technology Authority (ASTA).

B 1.3- "Combinatorial Therapy of Rolipram and pNGF for Traumatic Brain Injury"

Christian Macks[1], So Jung Gwak[1], Michael Lynn[2], Mark Kindy[3], Jeoung Soo Lee[1]
[1]Department of Bioengineering, Clemson University, Clemson, SC
[2]Department of Neurology, USC Medical School, Greenville, SC
[3]Dept of Pharmaceutical Sciences, College of Pharmacy, University of South Florida, Tampa, FL

Background: Traumatic brain injury (TBI) represents one of the leading causes of disability and death following injury[1]. The presentation of TBI involves both a primary and a secondary injury. The primary injury is a direct result of the traumatic event, and is accompanied by inflammatory response. The progression of inflammatory response is marked by the production of various cytokines that either act in neuroprotective or neurotoxic roles[1]. Several key potentially neurotoxic cytokines are inhibited by increasing levels of cyclic adenosine monophosphate (cAMP)[2]. Rolipram, a hydrophobic drug used in treatment of traumatic CNS injury, prevents the degradation of cAMP and is able to inhibit production of potentially neurotoxic cytokines[3]. Additional treatment for TBI is the administration of exogenous nerve growth factor (NGF), which has shown neuroprotective function and can reduce edema following primary injury[2]. We propose a novel polymeric carrier system combining both the delivery

of a hydrophobic drug and plasmid NGF to microglia present at the injury site. we designed amphiphilic copolymers composed of poly (lactide-co-glycolide)-g-polyethylenimine (PgP), which has demonstrated its ability for efficient nucleic acid delivery[4]. Here, we present the rolipram loading efficiency and transfection efficiency and duration of PgP/pNGF polyplexes in B-35 neuroblastoma cells.
Methods: The PgP/pBLAST44-hNGF (NGF) polyplexes were transfected in C8B4 microglia in both non-serum and 10% serum conditions. Transfection was performed with PgP/pNGF at N/P ratios 25/1 and 30/1 and at subsequent time points culture media was harvested. NGF concentration was determined via ELISA assay, and cytotoxicity using MTT assay. To evaluate the rolipram loading efficiency, varying amount of rolipram was dissolved in ethanol and then added in PgP (1 mg/ml) solution and incubated overnight to allow the ethanol evaporation. The amount of rolipram in PgP solution was measured by HPLC (Waters System) using a Waters Symmetry C18 column with mobile phase water:acetonitrile (60:40).
Results: In both non-serum and 10% serum conditions, transfection efficiency of PgP/pNGF polyplexes at N/P ratios 25/1 and 30/1 was significantly higher than that of PEI at N/P 5/1. Furthermore, transfection efficiency of PEI/pNGF polyplexes shows a dramatic decrease compared to PgP/pNGF polyplexes in 10% serum condition. The duration of NGF expression after transfection with PgP/pNGF polyplexes persisted upto 5 days post transfection. The rolipram loading efficiency of PgP was calculated as follows. % Loading efficiency = (Amount of Rolipram loaded/amount of Rolipram added) X 100. The amount of Rolipram loaded to PgP solution (1 mg/ml) was about 0.86 mg ±0.029. This represents over 4 times higher than Rolipram's solubility in water (0.2 mg/ml), and loading efficiency was about 86%.
Conclusions: We demonstrated that PgP polymeric micelle is a promising carrier for different forms of plasmid DNA to B35 neuroblastoma cells in 10% serum condition. We also demonstrated persistent elevation of NGF expression over a course of 120 hours. The ability of PgP to load Rolipram was established. In the future Rolipram loading will be tested with other PgP molecular weights. Currently, we are generating hypoxia condition for traumatic brain injury model and synergistic efficiency of rolipram loaded PgP/pNGF in C8B4 microglia cells in gaseous hypoxia condition will be evaluated.
Acknowledgement: Research reported in this publication was supported by NIGMS of the National Institutes of Health under award number 5P20GM103444-07.
References:
1. Kelso et al, Prog. In Mol. Biology and Trans. Science, 96: 85-132 (2011), 2. Tian et al, Brain Res., 1440: 47-55 (2012), 3. Schaal et al, PLOS One, 7: 1-22 (2012), 4. Lee et al. *Trans SFB* p.917 (2010).

B 1.4- "Identification of Successful Mentoring Communities Using Network-based Analysis of Mentor-Mentee Relationships Across Nobel Laureates"

Julia H. Chariker, Yihang Zhang, John R. Pani, Eric C. Rouchka
University of Louisville, Louisville, KY

Background: High achievements in intellectual innovation have been measured in part with the awarding of prestigious honors, such as the Nobel Prize. Transmission of the high-level scientific knowledge and skill underlying intellectual innovation is achieved in large part through academic mentorship.
Methods: Successful mentors and mentoring communities were identified in the realm of high-level science through the construction and analysis of a network connecting Nobel Prize winners from 1901 to 2014 based on doctoral student-dissertation advisor relationships.
Results: Nobel laureates had a greater number of Nobel laureate ancestors, suggesting that high-level skill is transmitted over generations. Similarly, several mentors were identified with high numbers of Nobel laureate descendants, and subnetworks composed entirely of Nobel laureates extended across as many as four academic generations. Three major mentoring communities were identified centering on highly successful mentors.
Conclusion: Biographical and historical accounts of these communities suggest that two characteristics of successful mentoring communities are (1) high degrees of interaction and collaboration between students and experienced scientists within large scientific communities and (2) disciplined practice of domain-specific skills that promote scientific innovation and discovery.
Acknowledgment: This work was funded by the National Institute for General Medical Sciences (NIGMS), National Insititutes of Health (NIH) Grant P20GM103436 (Nigel Cooper, PI).

B 1.5- "Integrated Modeling for an Enhanced Predictive DILI Model"

Kristin McEuen[1,2], Leihong Wu[1,2], Minjun Chen[1,2], Shradda Thakkar[1,2], Weida Tong[1,2]
[1]The University of Arkansas at Little Rock, Little Rock, AR
[2]The University of Arkansas for Medical Sciences, Little Rock, AR

Background: Drug induced liver injury (DILI), though rare, can result in serious clinical outcomes, such as acute liver failure, the need for liver transplant, and even death. Despite rigorous toxicity testing during

developmental phases, drugs causing DILI still enter first-in-human trials and are a leading cause of drug failure and withdrawal from the market. An *in silico* model capable of identifying a drug as DILI in the early stages of development could greatly reduce the cost of developing and regulating drugs as well as improve public health by reducing DILI incidence. However, DILI is a complex disease and current models are inadequate.
Methods: In this study, we used simple voting and a decision tree algorithm to integrate results from four unique DILI models, including the "Rule-of-Two", the DILI prediction system, a QSAR model, and a modified QSAR model.
Results: Combining the three highest performing models resulted in improved DILI prediction.
Conclusion: This approach provided added insight into the prediction of DILI, highlighting the importance of including diverse data and algorithms in DILI predictive models.
Acknowledgment: This work was funded by the Oak Ridge Institute for Science and Education.

B 1.6- "Sequencing and Analyzing Ribosomal Frameshifting of *Trichomonas vaginalis* virus 4 Isolates along with Treatment of Ribavirin on *T. vaginalis* Cells"
Lizhuo Ai, Hirni Patel, Cory Toyota
Millsaps College, Jackson, MS

Background: Trichomoniasis, the most common nonviral sexually transmitted disease with estimated 2 million new infections every year in the world, caused by a flagellated protozoan parasite *Trichomonas vaginalis* that hosts on human genitourinary tract. *Trichomonas vaginalis* harbors up to four strains of non-segmented double stranded RNA *Trichomonas vaginalis* virus (TVV1-4). *T. vaginalis virus* encodes two genes, an upstream capsid open reading frame (CP ORF) and a downstream fusion RNA-dependent RNA polymerase (RdRp ORF). Studies state TVV1 and TVV3 might have ribosomal frameshifting of -1 bp, while TVV2 are more likely to have -2 bp frameshifting during translation of RdRp in fusion with its capsid. However, there are not many available known genome sequences or many studies done on TVV4 ribosomal frameshifting. Here, we begin to investigate TVV4. A nearly complete genome of TVV4 from a clinical isolate has been sequenced. We hope to determine the relative susceptibility of the different TVVs to ribavirin, a nucleoside analog anti-viral drug.
Methods: We used the only three known consensus sequence of TVV4, and designed sequencing primers. We also used cell cultivation, RNA isolation, RT PCR, qRT PCR, electrophoresis, mass spectrometry, and DNA isolation and purification.
Results: One whole genome of a TVV4 isolate was sequenced successfully. Expecting frameshifting region on the new sequence is sent off for translating for further analysis.
Conclusion: New samples of TVV4 genome have been sequenced in this research for future studies and references. We can analyze more about the ribosomal frameshifting for *T. vaginalis* virus and find out which mechanism of framshifting, whether -1bp or -2 bp, TVV4 has.
Acknowledgement: This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

B 1.7- "From *In silico* to *in vitro*: Rapid Verification of Bioinformatically Identified Promoters and Terminators"
Jace Bradshaw, AlleaBelle Gongola, Nathan Reyna
Ouachita Baptist University, Arkadelphia, AR

Background:. For the last four years, students at Ouachita Baptist University have been isolating and characterizing mycobacteriophages that infect the common host Mycobacterium smegmatis mc2155 as part of the HHMI SEA-PHAGES program. However, since this is done *in silico,* these predictions still need biological confirmation—a difficult and time-consuming process. The goal of this project is two fold: first, to examine characteristics of the regulatory elements found in mycobacteriophage using novel cloning techniques; second, to investigate the validity of commonly used algorithms to ensure that the data produced from genomic analysis can be properly interpreted.
Methods: Fourteen putative promoters from mycobacteriophage Mendokysei were identified through bioinformatic analysis using DNA Master. Additionally, six terminators were identified using ARNOLD. Promoters were then verified and quantified using the expression vector pClone Red (BBa_J119137). We created a novel construct (pGR-Blue) specifically for terminator verification named PGR-Blue. Both vectors utilize Golden Gate Assembly, allowing us to go from in silico identification to lab verification in as little as four days.
Results: New insight on gene regulation were revealed by discovering the locations of hairpin terminators and SigA-like promoters in relation to their polycistronic gene cassettes. Our results provide insight into effective utilization of bioinformatic methods and comparative genomics in addition to the understanding of mycobacteriophages and associated organisms.
Conclusion:. Identification of novel terminators requires bioinformatic analysis of novel genomes. However, as increasing amounts of bioinformatic software are developed, each with a unique algorithm utilized for prediction, more discrepancy between putative results occurs. Because this process is

somewhat subjective and done *in silico*, these predictions need biological confirmation. An important aspect of our project was the development of a novel plasmid (pGR-Blue) and protocols used for the physical verification of regulatory sequences
Acknowledgment: This project was supported by grants from the National Center for Research Resources (P20RR016460) and the National Institute of General Medical Sciences (P20GM103429) from the National Institutes of Health and the J.D. Patterson Summer Research Fellowship.

B 1.9- "Surface Signature Analysis of the Binding Pattern of Sesquiterpene Lactones to Nf-Kb"

Ujwani Nukala[1,2], Paola E. Ordóñez[1,3,4], Shraddha Thakkar[1,2], Darin E. Jones[4], Monica L. Guzmán[5], Cesar M. Compadre[1]
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[5]Division of Hematology/Oncology, Department of Medicine, Weill Cornell Medical College, New York, NY

Background: Sesquiterpene lactones (SLs) are naturally occurring compounds that have shown potent anti-leukemic activity, and have the ability to target leukemic stem and progenitor cells. It has been postulated that, the SLs exert their effect by inhibiting the rapid-acting primary transcription factor NF-κB by alkylating cysteine-38 in the DNA binding loop and cysteine-120 in the nearby E' region which makes specific interactions with the DNA impossible. NF-κB is a heterodimer complex of p50 and p65 subunits that interact with the DNA, regulating the expression of several genes. The aim of this study is to study the binding pattern of sesquiterpene lactones to NF-κB.
Methods: For the drug-receptor interactions apart from geometrical complementarity, it also requires matching interactions between the drug and the receptor. In an effort to study this complementarity, we performed the surface signature analysis of the SLs and the region around cysteine-38 of the NF-κB chain A. We used MOLCAD program to construct the surface of the ligands and the receptor site.
Results: Electrostatic surface signature analysis showed that the cysteine-38 residue provides an electron rich region for nucleophile interaction of SLs with NF-κB and the lipophilic surface signature analysis of NF-κB active site showed that tyrosine-36 and cysteine-38 residues are providing a lipophilic region for hydrophobic interaction of SLs with NF-κB.
Conclusions: This study shows that NF-κB and the SLs have matching lipophilic and electrostatic surface signatures, i.e. a strong lipophilic surface, and a strong electron rich area. Thus, these characteristic surface patterns would explain why the various types of substantially different SLs interact with NF-κB. These findings could be also used to identify additional naturally occurring compounds with potential anti-leukemia activity and to guide synthetic approaches to develop more potent or specific compounds.

B 1.10- "Performance Evaluation for Gene Set Analysis Approaches for RNA-seq Data"

Yasir Rahmatallah, Galina Glazko
University of Arkansas for Medical Sciences, Little Rock, AR

Background: Transcriptome sequencing (RNA-seq) is steadily replacing microarrays for high-throughput studies of gene expression. Gaining insights into the biological processes underlying phenotypic differences is the major challenge in analyzing the expression of thousands of genes. For this purpose, gene set analysis (GSA) emerged as the method of choice, in particular because it incorporates pre-existing biological knowledge in the form of functionally related gene sets into the analyses.
Methods: We consider several statistically different GSA approaches (competitive and self-contained) that were adapted from microarrays practice or specifically designed for RNA-seq data. We perform a comprehensive evaluation of their performance in terms of Type I error rate, power, robustness to the sample size and heterogeneity, as well as the sensitivity to different types of selection biases on simulated and real RNA-seq data.
Results: We show that the performance of various methods depends on the statistical hypothesis they interrogate and not on the platform type for which they were designed for. We found that competitive methods have lower power as well as robustness to the samples heterogeneity than self-contained methods, leading to poor results reproducibility. Moreover, we found that the power of unsupervised competitive methods is highly affected by the balance between up- and down-regulated genes between two phenotypes in the tested gene sets.
Conclusion: Our evaluation provides a concise guideline for selecting proper GSA approaches, and warns against using others in their current form.
Acknowledgment: Support has been provided in part by the National Center for Advancing Translational Science award UL1TR000039 and the IDeA Networks of Biomedical Research Excellence (INBRE) program, with grants from the National Center for Research Resources (P20RR016460) and the National Institute of General Medical Sciences (P20 GM103429) from the National Institute of Health (NIH). Large-scale

computer simulations were implemented using the High Performance Computing (HPC) resources at the UALR Computational Research Center supported by the following grants: National Science Foundation grants CRI CNS-0855248, EPS-0701890, MRI CNS-0619069 and OISE-0729792. F.E.S. thanks the Engineering and Physical Research Council (EPSRC EP/H048871/1) for support.

B 1.11- "Autophagy Regulates Muscle Contraction Induced Myokine Synthesis"

Simran Batth, Tarek Abd-elhamid, Jose Subauste, Angela Subauste
University of Mississippi Medical Center, Jackson, MS

Background: Exercise has emerged as an intervention against conditions such as diabetes and cardiovascular disease. The underlying mechanism is still unclear. In response to contraction muscle secretes proteins (myokines) that mediate some of the metabolic benefits associated with exercise. Autophagy is a highly conserved process of recycling that has been shown to be acutely upregulated by exercise. In this study we demonstrate that myokine synthesis and secretion in response to contraction is dependent on autophagy activation.
Methods: We constructed a short hairpin for the autophagy protein ATG5 in an inducible lentiviral vector. An *in vitro* contraction protocol was used in C2C12 myotubes. For *in vivo* studies we used the mouse model of autophagy related beclin 1 (Becn1) knockout.
Results: Downregulation of ATG5 leads to a blunted response to contraction for myokines IL6 and LIF. Omega 3 fatty acids have been shown to activate autophagy. We demonstrate that treatment with omega 3 fatty acids while activating autophagy also upregulate contraction induced myokine synthesis (IL6, LIF). Using Becn1 -/+ mice we demonstrated that after a bout of exercise LIF and IL6 protein levels were downregulated in the Becn1 -/+ mice when compared to controls.
Conclusion: Myokine synthesis and secretion in response to muscle contraction is dependent on autophagy activation. Furthermore we show that the effect of omega 3 fatty acids on autophagy is capable of enhancing the effect on myokine synthesis. This could potentially place omega 3 fatty acid supplementation as an intervention aimed at maximizing the metabolic effects of exercise.
Acknowledgement: This work was supported by the Department of Medicine, UMMC and by the National Institute Of General Medical Sciences of the National Institutes of Health under Award Number P20GM104357.

Cancer,
Poster Session B
Friday, September 13, 10:45 a.m.-12:15 p.m.

B 2.1- "Design and *In Vitro* Evaluation of modified Tocosol™ Paclitaxel Nanodispersion Using a Quality-by-Design Approach"

Ahmed Abu-Fayyad[1], Paul W. Sylvester[1], Jennifer L. Carroll[2,3], James A. Cardelli[2,3], Sami Nazzal[1]
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[2]Department of Microbiology and Immunology, Louisiana State University Health Sciences Center, Shreveport, LA
[5]Feist-Weiller Cancer Center, Louisiana State University Health Sciences Center, Shreveport, LA

Background: Tocosol™ Paclitaxel (PTX) was introduced as an alternative delivery vehicle to the Cremophor EL Taxol® Formulation for cancer therapy. In Tocosol™, vitamin E α-Tocopherol, the core solubilizer for PTX, is emulsified using PEGylated Vitamin E TPGS to obtain a ready-to-use injectable nanoemulsion. Due to its comparable objective response rate to Taxol® in a Phase III clinical trial on women with metastatic breast cancer, future development of this product were placed on hold. It has also been demonstrated that the tocotrienol (T_3) isomers of vitamin E have potent anticancer activity and were shown to potentiate the activity of PTX. Therefore, our core hypothesis is that substituting α-tocopherol (α-T) with T_3 in the Tocosol™ nanoemulsion will significantly enhance the anticancer activity of the dispersion and would present a novel and alternate treatment option to nab-paclitaxel (Abraxane®) against pancreatic cancer.
Methods: A Nanoemsulion of PTX was developed with the aid of a 3-factor, 3-level Box–Behnken experimental design. Eight responses were investigated, including hemolytic effect and particle size. The cytotoxicity of the optimized T_3/PTX nanoemulsion was tested in vitro against AsPC-1, BxPC-3, MIA PaCa-2 and Panc-1 pancreatic ductal adenocarcinoma cells.
Results: From the Box–Behnken experimental design it was shown that the ideal PTX:T_3 ratio was 0.16:1. Optimized dispersion had a particle size <200 nm, zeta potential >I-25 mVI, and PTX entrapment efficiency >70%. The nanoemulsion and its co-admixture with calcium gluconate was physically stable over two months when refrigerated. No in-vitro hemolysis was observed for the selected formulation. The IC50 values for PTX solubilized with T_3 were lower than PTX formulated with α-T in BxPC-3 and MIA PaCa-2 cancer cell lines with values of 25 nM and 41 nM for γ-T_3, 22.5 nM and 45.5 nM for δ-T_3, and 50.4 nM, 63.4 nM for α-T respectively. However, no significant activity between formulations were observed in AsPC-1 and Panc-1 cell lines.
Conclusion: Results from this study suggest that replacement of α-tocopherol with T_3 with/without PEGyalted T_3 as emulsifier may enhances the activity of the Tocosol™ PTX nanoemulsion. The new formulation may provide an alternate treatment option to the currently approved nab-paclitaxel (Abraxane®). Additional in vivo study are nonetheless warranted to confrm the observed responses. *Research reported in this abstract was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103424.*

B 2.2- "Pharmacological Clearance of Senescent Cells by ABT263 Mitigates Total Body Irradiation-Induced Premature Aging of the Hematopoietic System in Mice"

Yingying Wang[1,2], Jianhui Chang[1], Lijian Shao[1], Wei Feng[1], Remi-Martin Laberge[3], Marco Demaria[3], Judith Campisi[3,4], Yi Luo[1], Nukhet Aykin-Burns[1], Kimberly Krager[1], Martin Hauer-Jensen[1], Aimin Meng[2], Daohong Zhou[1]
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[5]These authors contributed equally to this work

Senescent cells (SCs) accumulate in many tissues and organs with age and after genotoxic stress such as total body irradiation (TBI), which accelerates aging phenotypes. The clearance of SCs using a transgenic approach delayed several age-associated disorders in a progeroid mouse model, suggesting SCs play a causative role in certain aging-related patholgies. Thus, the pharmacological clearance of SCs using a 'senolytic' drug that can selectively kill SCs might extend health span. To test this idea, we screened collection of compounds and identified ABT-263 (a specific Bcl-2 and Bcl-xl inhibitor) as a potent senolytic drug. Here, we show that ABT-263 selectively kills SCs in culture in a cell type- and species-independent manner by inducing apoptosis. Oral administration of ABT-263 to mice after exposure to sub-lethal TBI effectively depleted SCs from several tissues, including the depletion of senescent hematopoietic stem cells (HSCs) from the bone marrow (BM). Strikingly, this depletion mitigated TBI-induced premature aging of the hematopoietic system. Our results provide the first proof of concept that selective clearance of SCs is

pharmacologically achievable *in vivo* and beneficial. Therefore, senolytic drugs have the potential to be developed as novel anti-aging agents as well as new radiation mitigators.

B 2.3- "CDK8 Inhibition Potentiates Anti-ER and Anti-HER2 Therapies and Prevents Targeted Drug Resistance in Breast Cancer"

M.S. McDermott[1], C. Lim[1], L. Ivers[2], N. O'Donovan[2], S. Altilia[1], M. Chen[1], A. Chumanevich[1], J.F. Catroppo[3], B. Gyorffy[4], D. Oliver[1], M. Shtutman[1], I.B. Roninson[1], E.V. Broude[1]

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Background: Breast cancer is a disease, the treatment of which is based primarily on targeted therapeutics. In particular, hormonal therapy targets the estrogen receptor (ER) in ER-positive cancers, whereas cancers that overexpress HER2 are treated with several HER2-targeted drugs. However, many patients do not respond to targeted therapies or respond initially and then develop resistance. Identification of new "druggable" mediators of the oncogenic effects of the current breast cancer drug targets could yield critical improvements in the treatment of targeted therapy-resistant cancers. The common endpoint of essentially all the signal transduction pathways that are mediated by cancer drug targets is the effect on cellular gene expression that leads to the stimulation of tumor growth. Here we investigated if the inhibition of cyclin-dependent kinase 8 (CDK8), a pleiotropic transcription regulator that potentiates the induction of gene expression by several transcription factors, would affect the response of breast cancer cells to targeted therapies.
Methods: CDK8 expression in breast cancers was analyzed by immunohistochemical staining of breast tissue arrays. Prognostic correlations of CDK8 expression were analyzed through microarray data mining using KMplot.com. For functional studies, the following cell lines were used: MCF7, T47D (ER+), BT474 (ER+ HER2+), SKBR3, HCC1954, HCC1419, JIMT-1, and derivatives of SKBR3 and HCC1954 selected for lapatinib resistance. CDK8 inhibition was achieved using selective small-molecule inhibitors, Senexin A and Senexin B (Senex Biotechnology) and with lentiviral vectors expressing CDK8 shRNA. The effects of CDK8 inhibition on gene expression were measured using quantitative reverse transcription-PCR and by Agilent microarray hybridization. To assess synergy of drug interactions relative cell growth in cells treated with increasing concentrations of individual drugs and fixed-ratio drug combinations was compared to untreated cells by MTT assay. CI (combination index) values were calculated using CompuSyn software. The effects of different inhibitors on the development of estrogen independence were measured upon long-term estrogen deprivation over 12-20 days; cells were stained with crystal violet. The effects on the development of lapatinib resistance were analyzed similarly over 25 days.
Results: Immunohistochemical staining of breast tissue arrays and bioinformatics analysis of gene expression microarray data of breast cancer patients revealed that CDK8 is overexpressed in breast cancer and that higher CDK8 expression correlates with the failure of systemic therapy. CDK8 inhibition by selective small-molecule inhibitors or by shRNA knockdown inhibited the mitogenic effects of estrogen and estrogen-dependent transcription in estrogen receptor (ER)+ breast cancer cell lines. CDK8 inhibitors had a cytostatic effect on different ER+ cell lines, and this effect was synergistic with that of an anti-estrogen fulvestrant. CDK8 inhibitors also prevented the emergence of long-term estrogen independence (LTED) in different ER+ cell lines. We also tested the effects of CDK8 inhibitors in combination with the HER2 and EGFR tyrosine kinase inhibitor lapatinib and an anti-HER2 monoclonal antibody (a biosimilar of trastuzumab) in different HER2+ cell lines. CDK8 inhibition produced a synergistic decrease in cell growth with both HER2 inhibitors. This synergy was observed in different HER+ cell lines regardless of their ER status. Strikingly, CDK8 inhibition overcame both intrinsic and acquired resistance to HER2-targeted drugs and prevented the development of lapatinib resistance in long-term culture.
Conclusion: These results suggest that CDK8 inhibition in breast cancer cell lines potentiates the effects of ER- and HER2-targeted drugs and prevents the development of resistance to targeted therapies. Upon confirmation in animal models, these findings will warrant a program of clinical development of CDK8 inhibitors in combination with targeted drugs to improve the efficacy of breast cancer therapy.
Acknowledgment: This work was funded by Susan G. Komen PDF15329865 Grant; American Cancer Society Institutional Research Grant (ACS IRG) 124275-IRG-13-043-01-IRG and COBRE Center for Translational Therapeutics Grant 5P20GM109091.

B 2.4- "Polymeric Micelle for pTK and GCV Delivery to Spinal Cord Tumor"

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Background: Spinal cord tumors are neoplasms of the central nervous system (CNS). Intramedually spinal cord tumor (IMSCT) are relatively rare, accounting for five to 10 percent of all spinal tumors[1]. However, these tumors exert non-mechanical back pain, especially middle or lower back pain. Treatment can vary depending on the type of tumor and chemotherapy, radiation therapy, and surgery are most common. However, there is no effective clinical treatment for IMSCT. The therapy of HSV-thymidine kinase (TK) and ganciclovir (GCV) is a powerful strategy among suicide gene therapy for tumor therapy. The goal of this work is to develop a polymeric micelle delivery system for acceleration of the suicide gene (pTK)/GCV therapeutics for the efficient treatment of SCT.
Methods: Poly (lactide-co-glycolide)-g-polyethylenimine (PgP) were synthesized and characterized by ^{1}H-NMR/GPC[2]. The suicide effect of PgP/pTK polyplex with GCV on C6 cells was evaluated after transfection of PgP/pTK polyplex at N/P ratio of 60/1 with various dose of GCV in the presence of 10% serum and the suicide effects were analyzed by MTT assay. To evaluate suicide effect of PgP/pTK polyplex with GCV, we generated rat spinal cord tumor model. C6 cells were injected in the T5 spinal cord and then PgP/pTK polyplexes were injected in the 5 days tumor cell injection and GCV (50 and 100 ug/ml) was injected intraperitonially every day. PgP/pGFP only, PgP/pTK only, PEI/pTK with GCV (100 ug/ml), and GCV only were used as controls. At 14 days, the tumor were harvested, sectioned, and the suicide effect of PgP/pTK with GCV were analyzed using TUNEL assay.
Results: Poly (lactide-co-glycolide)-g-polyethylenimine (PgP) were synthesized using PLGA (25kDa) and branched PEI (25kDa) and characterized by ^{1}H-NMR/GPC[2]. The molecular weight of PgP was approximately 48,791 Da by GPC. Particle size of PgP/pDNA was measured by Dynamic light Scattering and was approximately 200 nm at all N/P ratios and surface charge of polyplexes was positive above N/P ratio of 16/1. The suicide effect of PgP/pTK with/without GCV were evaluated after transfection of PgP/pTK at N/P ratio 60/1 and the lowest cell viability was observed at PgP/pTK with GCV (100 ug/ml). At 14 days, PgP/pTK-GCV (100 ug/ml) polyplex showed highest number of apoptosis-positive cells in spinal cord tumor by the TUNEL assay.
Discussion and Conclusion: We demonstrated that the PgP micelle is an effective nucleic acid delivery carrier in C6 cells *in vitro* and in rat spinal cord tumor model *in vivo*. Currently, we are studying the effect of suicide pTK and GCV gene therapy on tumor size and survival of spinal cord bearing rat after intratumoral/systemic injection of PgP/pTK following intraperitoneal injection of GCV. In the future, we will study biodistribution and immune response after systemic injection of PgP/pTK.
Acknowledgements: Research reported in this publication was supported by NIGMS of the National Institutes of Health under award number 5P20GM103444-07.

B 2.5- "Tetrahydrobiopterin (BH4) – Mitochondria Crosstalk in Radiation-induced Skin Injury"

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Division of Radiation Health, University of Arkansas for Medical Sciences, Little Rock, AR

Background: Skin tissue is a major recipient of significant collateral damage during radiotherapy. To address the prevention and treatment of ionizing radiation (IR) -induced normal tissue injuries, it is essential to unravel the molecular and biological mechanisms responsible. IR causes functional and morphologic changes in human epidermis and dermis by altering intracellular redox homeostasis and mitochondrial function. These changes can be persistent contributors to long-term effects of IR.
Methods: Mitochondrial respiration studies were done using Seahorse Extracellular Flux Analyzer XF96 in human immortalized and primary skin cell cultures. A transgenic mouse model was utilized, in which *de novo* BH4 biosynthesis is limited, to determine whether skin cells are more prone to IR-induced cytoskeletal changes when BH4 levels are decreased.
Results: Our preliminary data obtained by exposing immortalized keratinocytes (HaCaT) to IR at various doses (2–8 Gy) revealed increased mitochondrial respiration at 4 h in a dose-dependent manner. However, 72 h following IR, the cells' basal respiration decreased and their spare respiratory capacity was significantly abrogated, suggesting that IR exposure results in an early mitochondrial adaptive response that eventually declines due to IR damage to mitochondria. Earlier investigations of key players in redox-regulated effects of IR pointed toward IR-induced decreases in BH4 bioavailability. BH4 is an essential cofactor for enzymes involved in keratinocyte differentiation. BH4 may be a significant player during IR-induced normal tissue responses due to the oxidative-susceptible nature and its critical cofactor function it performs in NOS enzymes. Results determined that IR exposed dermal fibroblasts had increased levels of stress fiber formation. This increase was more prominent in dermal fibroblasts isolated from mice that displayed decreased BH4 bioavailability.

Conclusion: Collectively, these results suggest that IR induces mitochondrial dysfunction and impaired BH4 metabolism may be linked in metabolic crosstalk and contribute to short and long term radiotherapy effects on skin tissue.
Acknowledgement: This work was funded by Centers of Biomedical Research Excellence (COBRE) – Center for Host responses to Cancer Therapy at UAMS from NIGMS under grant number P20 GM109005.

B 2.6- "Bone Marrow-Derived Mesenchymal Stem Cells and Preadipocytes Cultured with Breast Tumor Cells Favor Cytokine Secretion"
Leah Figurski, Yuya Kudo, Linda Vona-Davis
West Virginia University, Morgantown, WV

Background: Women who are obese have a higher risk of breast cancer compared to those who are lean. Research has shown that adipocyte-epithelial interactions are mediated by tumor-enhancing factors such as cytokines, chemokines, and growth factors. The objective of our study was to identify novel secreted cytokines and growth factors common to adipocytes and breast tumor cells using protein array analysis.
Methods: Mesenchymal stem cells or their differentiated preadipocytes derived from either bone marrow
or adipose tissue were placed in transwell cultures with the invasive breast tumor cell line MDA-MB-231. Cultures were incubated for several days and the conditioned media was collected to compare protein expression levels using a cytokine antibody array. Signals were visualized on x-ray film, scanned, and quantitated by densitometry.
Results: Within a week, stem cells derived from either bone marrow or adipose tissue differentiated into preadipocytes, as evident by the induction of adipogenesis after staining with oil red O solution. More cytokines and growth factor expression was observed when mesenchymal stem cells or adipocytes were cultured in the presence of breast cancer cells. 79/120 proteins on the array were either up or downregulated by two-fold or greater.
Conclusions: Stem cells derived from either bone marrow or adipose tissue and their differentiated preadipocytes when cultured with invasive MDA-MB-231 breast tumor cells secrete more cytokines and growth factors in the conditioned media. Additional studies are needed to uncover which soluble factors are the most important as novel targets in the link between obesity and breast cancer.
Acknowledgements: This work was funded by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence.

B 2.7- "Analysis of the DNA-Cleaving Efficiencies and Mechanism of Bifunctional DNA-Cleaving Reagents"
Leon H. Karekezi, Marcus E. Powell, Courtney B. Mullins, Gurjit Kaur, Lauren M. Hoth, B. Woods Curry, Emily H. Stewart, Jonathan P. Giurintano, Wolfgang H. Kramer
Millsaps College, Jackson, MS

Background: Current Photodynamic therapy uses sensitizers to generate singlet oxygen which causes cell death. The hypoxic environment of most cancer tissues makes oxygen a limiting reagent for this approach and several methods have recently been developed to circumvent this problem.
Methods: The photoinduced homolytic N-O bond cleavage of N-Heteroaromatic compounds with an N-alkoxy substituent (onium salts) leads to the formation of a heteroaromatic radical cation and an alkoxy radical. Both of these species have been shown to induce DNA cleavage, each with a different mechanism. The synthesis of the nitrogen onium salts includes the oxidation of the heteroaromatic nitrogen and subsequent O-alkylation. To increase the DNA cleaving effiency by enhancing ground-state association we synthetically attached a known DNA-binder, 1,8-naphthalimide.
Several binfunctional compounds have been synthesized and their photochemistry has been investigated.
Results: Here we present the DNA cleaving efficiency of a series of bifunctional DNA-cleavers which has been analyzed by gel electrophoresis and CD spectroscopy. Interestingly, the bifunctional compounds appear to be double-strand cleavers. A thermal effect is explained by molecular docking studies. The influence of quenchers has been analyzed and indications of the reaction mechanism are discussed. It appears as though oxygen is inhibiting the photocleavage.
Conclusion: The bifunctional compounds can be used to efficiently initiate DNA-cleavage. Oxygen appears to inhibit the reaction and thus the compounds could exhibit and increased activity in hypoxic tissues.
This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

B. 2.8- "Alternate Protein Targets & Drug Designing for Breast and Prostate Cancers"
Pradip K Biswas
Laboratory of Computational Biophysics & Bioengineering, Department of Physics, Tougaloo College, Tougaloo MS

Hormone therapy resistance and recurrence are the two major challenges in breast & prostate cancers as

they together cause more than 700,000 deaths per year worldwide and affect about 2.4 million new people each year. Estrogen Receptor (ER) alpha and Androgen Receptor (AR), are the two main nuclear hormone proteins that are responsible for the progressions of breast and prostate tumors, respectively, and represent the main targets for hormonal therapies. However, about 70% of breast and prostate tumors develop resistances in hormonal therapies and they sustain growth in hormone-independent manner. In order to develop inhibitors that could address the issue of hormonal therapy resistance, we elucidate protein-protein and protein-DNA interfaces of ER and AR and identify and validate alternate targets for drug designing.
Using the crystal structures of ER and AR DNA and Ligand binding domains, molecular modeling, molecular dynamics simulations, and bioinformatics we identified the hydrogen-bonding contacts and the sequence motifs that are responsible for dimerization and/or DNA recognition. The crucial amino acids of a motif are then grafted on stable helices (alanine or leucine) in order to develop peptidic inhibitors.
In ER ligand binding domain, out of the three sequence motifs found to be responsible for dimerization, we are using DXXTD (480-484) and LQXXHQXXAQ (497-506), as templates for designing peptide-based and small molecule inhibitors for ER dimerization – an essential process in ER mediated transcription. In-vitro testing of peptide inhibitors developed based on LQXXHQXXAQ sequence motif is found to inhibit ER in the presence of hormone. In AR, we didn't find any suitable target in the ligand binding domain but found a suitable target in AR DNA binding domain: This LCAXRXD motif (578-584) has been grafted on alanine and glutamine helices and in-silico and in-vitro testings are in progress. In-vitro testing
Author acknowledges financial support from MS-INBRE funded by NCRR/NIH-5P20RR016476-11 and NIGMS/NIH-8P20GM103476-11.

B. 2.9- "A Mixed Immuno-Chemotherapy Against Colon Cancer: Modeling and Simulations"
Qing Wang[1], Zhijun Wang[1], David J Klinke[2]
[1]Shepherd University, Shepherdstown, WV
[2]West Virginia University, Morgantown, WV

Background: A chemotherapy agent oxaliplatin in combination with interleukin-12 was recently used to eliminate pre-existing liver metastatic colorectal cancer and protect from relapse in a murine model. In this study, we aim to develop a predictive simulation platform for optimizing anti-tumor immunity using combined treatment strategies.
Methods: To better understand the effects of the combination therapy, we developed a multi-scale mathematical model using impulsive ordinary differential equations to describe the interaction between the immune system and tumor in response to the therapy. Model parameters were calibrated to published experimental data using a genetic algorithm.
Results: The multi-scale model captures three categories (non-responders, partial responders and responders) of mice tumor growth in response to the combination therapy against a tumor re-challenge and was used to explore the impact of changes of dosage and timing of the mixed immune-chemotherapy on tumor growth subject to tumor re-challenge in mice.
Conclusion: This study shows that increased timing and/or dosage of the mixed immune-chemotherapy may improve tumor control for partial-responders and/or non-responders. Overall, this study illustrates how mechanistic models can be used for in silico screening of the optimal therapeutic dosage and timing in combined cancer treatment strategies.
Acknowledgment: This work was funded by the West Virginia INBRE from the NIGMS under grant number P20GM103434.

B 2.10- "Reactions of Oxaliplatin Analogs with DNA Nucleotides at Variable pH and Concentration"
Rippa Sehgal, Dr. Kevin Williams
Western Kentucky University, Bowling Green, KY

Background: Oxaliplatin is one of three FDA-approved platinum anticancer drugs. Reaction with DNA occurs primarily at guanine residues and secondarily at adenine residues for oxaliplatin and other platinum drugs. We have previously studied oxaliplatin and an analog with additional steric hindrance in the amine ligand and found that the analog had different reactivity with methionine.
Methods: We have prepared oxaliplatin and the analog and have reacted each platinum compound with both guanine and adenine nucleotides at pH 4 and pH 7. These reactions have been characterized by NMR spectroscopy over time to observe the formation of products.
Results: Reactions are generally faster at pH 7 than pH 4 due to the deprotonation of the phosphate group. The dominant products are usually those with two nucleotides coordinated to one platinum center even under conditions of excess platinum. Both oxaliplatin and its analog react with similar rates, and for each platinum compound the reaction with GMP is faster than that with AMP.
Conclusions: Reaction of the second nucleotide with a platinum center is faster than reaction of the first because of the chelate formed by the oxalate ligand. The extra methyl groups on the oxaliplatin analog do not appear to slow reaction with nucleotides considerably. The pH generally affects the rate but does not substantially affect the product distribution.
Acknowledgement: This work was supported by a KBRIN Investigator Development Award (NIH General

Medical Sciences INBRE award 2P20GM103436-14) and by a Research and Creative Activities Program (RCAP) award from Western Kentucky University.

B 2.11- "Dysbiosis Induced Impairment of Immune Surveillance in Non-Gastrointestinal Tumors"

Samir Jenkins, Carl Cerniglia, Kieng B. Vang-Dings, Robert J. Griffin, Ruud P.M. Dings
The University of Arkansas for Medical Sciences, Little Rock, AR

Background: Gut microbiota profoundly shapes immunity in humans. Dysbiosis, a disturbance in the quantity and composition of gut microbiota, leads to aberrant immune development and functioning and subsequently increases the risk of various diseases, including cancer. However, the effects of dysbiosis on leukocyte extravasation in non-gastrointestinal (non-GI) primary tumor microenvironment (TME) have not been explored.
Methods: We measured and analyzed the induction of dysbiosis by antibiotics, changes in microbiota, endothelial adhesion molecules (EAMs), tumor growth curves, serum cytokine levels, and leukocyte extravasation in non-tumor tissues and in tumors by e.g., 16S rRNA sequencing, real-time imaging and in vivo flow cytometry, immunofluorescence and PCR.
Results: We found that EAMs such as ICAM-1, VCAM, and E-selectin, crucial for leukocytes to bind and adhere to tumor endothelial cells and transmigrate into the tumor, are very dynamic during dysbiosis.
Conclusion: Our preliminary results show that EAM levels are very dynamic during tumor establishment and progression and are influenced by various factors including cytokines, such as those produced by the microbiota. Overcoming this dysbiosis-induced impairment should enhance tumor immunity. Effective interference has the potential to increase treatment efficacy and subsequently improve cancer patient survival and quality of life.
Acknowledgment: This work was in part funded by a pilot grant (to R.P.M.D.) from COBRE P20 GM103625 (PI Smeltzer): "The Center for Microbial Pathogenesis and Host Inflammatory Responses".

B 2.12- "Design, Synthesis and Evaluation of a Novel Doxorubicin-Peptidomimetic Conjugate for the Specific Delivery of Doxorubicin to HER2 Positive Breast Cancer Cells"

Sandeep Pallerla[1], Ted Gauthier[2], Rushikesh Sable[1], Seetharama D. Jois[1]
[1]University of Louisiana at Monroe, Monroe, LA
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Background: Doxorubicin (DOX) is the cornerstone in the therapy of many carcinoma types. However, due to its lack of specific targeting to tumor cells it causes irreversible cardiac toxicity. Various strategies were applied to improve DOX selectivity by conjugating the drug with peptides, antibodies, hormones, etc. In the current approach, we have attempted to conjugate peptidomimetic (comp 5) that is highly specific for HER2 positive cancer cells to DOX via a glutaric anhydride as a linker.
Methods: Docking studies were used to optimize the linkage of DOX with compound 5. Surface plasmon resonance (SPR) studies and confocal microscope experiment were performed to examine whether the conjugate is binding to the HER2 receptors of SKBR-3 cells and to study the intracellular distribution of the conjugate respectively.
Results: NMR and mass spectroscopy results suggest that conjugate was successfully synthesized via C-terminal conjugation. Antiproliferative assay results indicate conjugate is having potency along with selectivity. SPR experiment suggests that conjugate binds to the HER2 domain IV. Confocal microscopy images suggest conjugate can successfully enters the cells.
Conclusion: The conjugate was successfully synthesized with glutaric anhydride as a linker. This novel conjugate has antiproliferative activity along with selectivity.
Acknowledgment: Funding for this research was from the National Institute of General Medical Sciences of the National Institutes of Health under grant number 8P20GM103424

B 2.13- "BVES and BCAR3: A Potential Tight Junction/Focal Adhesion Signaling Pathway"

Shenika Poindexter
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Background: Blood Vessel Epicardial Substance (BVES) -a tight junction associated protein- is silenced via promoter hypermethylation in all stages of colon cancer. Interference with BVES function in human corneal epithelial cells promotes increased migration, decreased trans-epithelial resistance, and reduced expression of epithelial markers including E-cadherin and Zo-1: characteristic for epithelial to mesenchymal transition (EMT). In contrast, expression of BVES in mesenchymal-like (Lim 2405 and SW620) colorectal cancer cell lines results in mesenchymal to epithelial transition (MET). These data suggests that BVES loss in cancer may contribute to cancer progression via promotion of mesenchymal states. We have determined that BVES regulates RhoA signaling, but incompletely understand how BVES modulates these networks. Therefore, identifying BVES interacting proteins is imperative to mapping BVES regulated intracellular signaling pathways.
Method: We employed a yeast two-hybrid screen with the intracellular domain of BVES (aa-115-360) as bait to probe a placental cDNA library. This screen yielded 67 interacting proteins. One of these interacting protein is Breast Cancer Anti-estrogen Resistance 3

(BCAR3), a known GEF with oncogenic activity that has been shown to interact with RhoA. BCAR3 has been shown to modulate its signals via complex formation with p130Cas, a focal adhesion protein. We hypothesize that the BCAR3 regulates BVES dependent RhoA activity in colon cancer.
Results: We confirmed the interaction via directed Y2H and co-IP. To determine the functional significance of this interaction, we used complimentary approaches of overexpression and knockdown of BCAR3 in BVES-expressing CACO2 and non-BVES-expressing SW620 cells and screened for changes in BVES-dependent phenotypes. Lentiviral mediated transduction of these lines with WT BCAR3 and theBCAR3/p130Cas uncoupling mutant, R743A, produced no morphological change in the CACO2 cells but promoted membrane ruffling in SW620 ; in contrast, BCAR3 knockdown resulted in epithelial-like appearance suggesting that BCAR3 is epistatic to BVES. We next developed a BVES inducible SW620 cell line that stably overexpresses BCAR3 and performed cell attachment assays. Following induction of BVES, 80% of cells attached to the surface of the culture dish at Day 11 as compared to non-induced SW620s. 1) We have identified a BCAR3 as a BVES interacting protein. BVES) BCAR3 appears to antagonize BVES by promoting EMT.
Conclusion: This interaction potentially reveals a unique tight junction to focal adhesion signaling pathway important in regulating EMT. Clarifying the role of BVES and BCAR3in EMT may lead to therapeutic strategies targeting this interaction in colon cancer.
Acknowledgement: This work was funded by Vanderbilt T32 CURE Supplement.

B 2.14- "Xanthohumol Inhibits the Proliferation of Neuroblastoma NG-108"

Xingchi Chen, Wanqing Sun, Nanhu Quan, Lin Wang, Courtney Cates, Ji Li
Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, MS

Background: A nature compound, Xanthohumol (Xn), has exhibited antioxidant, anti-inflammatory, antibacterial and antiviral activities from previous studies. Markedly, several authors have described the antiproliferation activity of Xn in different cancer cell lines, such as prostate cancer cell lines and ovarian cancer cell lines. However very few studies have evaluated the inhibitory effects of Xn on neuro-cancer cells. This study aims to investigate the effect and mechanisms of Xanthohumol (Xn), a nature prenylated chalconoid from hops and beer, on the proliferation and apoptosis of mouse NG-108 cells.
Methods: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and typan blue staining were used to measure cell viability. The intracellular levels of reactive oxygen species (ROS) was measured by the florescent probe 2',7'-dichlorofluorescein diacetate (H2DCF-DA). Flow cytometry was performed to measure the cell cycle distribution. Protein expression was detected by western blot analysis.
Results: Among the different doses examined, Xn showed the best inhibitory effect on the proliferation of NG-108 cells with IC50 of 40uM. Xn treatment arrested the cell cycle at G1/S stages. Further studies demonstrated that Xn treatment triggered the phosphorylation of the estrogen regulated kinase (ERK1/2) and p38 mitogen activated protein kinase (MAPK). However, the phosphorylation of c-Jun N-terminal protein kinase (JNK) was not altered by Xn significantly. Intriguingly, the inhibitor of ERK1/2 but not p38 MAPK abrogated the phosphorylation of both ERK1/2 and p38 MAPK and augmented the cell death of NG-108 by Xn
Conclusion: Xn treatment inhibits the proliferation of NG-108 cells via activating ERK1/2 signaling pathways by retarding G1/S stages. Xn could be a potential therapeutic natural product for neuroblastoma.
Acknowledgment: This work was supported by American Heart Association 14IRG18290014, American Diabetes Association Basic Sciences Grant 1-14-BS-131, NIH R21AG044820 and R01AG049835.

Cardiovascular Research, Poster Session B

Friday, September 13, 10:45 a.m.-12:15 p.m.

B 3.1- "An Insight into the Antioxidant Responses to Exogenous Oxidative Stress Agents and a Glimpse into the Role of Catalase in the Reproductive Fitness of tahe Gulf-Coast Tick (*Amblyomma Maculatum*)"

Deepak Kumar, Shahid Karim
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Background: Ticks are important ecto-parasites and blood is the only nutritious meal taken by ticks. Aside from dealing with the host's hemostatic and immune mechanism, ticks must cope with the potentially toxic molecules in their large blood meal. Blood digestion may contribute to significant oxidative stress resulting from the release of heme, free iron radicals, H2O2, and other stress-inducing molecules. Severe and prolonged oxidative stress can trigger apoptosis and necrosis in ticks. To overcome the toxic effects of reactive oxygen species (ROS), ticks utilize a battery of antioxidant molecules available in their repertoire, including catalase. We hypothesized that the induction of endogenous and exogenous ROS activates the tick antioxidants machinery to mitigate oxidative stress.
Methods: The relative gene expression of select antioxidant targets was determined by real time quantitative PCR in tick tissues after injecting them with H_2O_2 and Paraquat. A reverse genetics approach was used to silence the gene expression of tick catalase to assess the functional significance of cat in tick hematophagy, and reproductive fitness. Transcriptional gene expression and native microbiota quantification were also performed using qRT-PCR.
Results: The selected tick antioxidants transcript level increased from 2-18 fold in the tick tissues injected with H_2O_2 and Paraquat. The gene knock-down of cat showed the depletion of transcript level and increase in reactive oxygen species level but it did not interfere with tick hematophagy or phenotype. The transcript levels of various tick antioxidants in cat knocked-down tissues were differentially regulated indicating the presence of alternate pathway. Interestingly, cocktail of cat dsRNA and cat inhibitor impaired the tick reproductive fitness.
Conclusion: Our results support the presence of a robust antioxidant system and demonstrate the significance functional role of tick catalase in mitigating the reactive oxygen species generated during the prolonged tick feeding on the vertebrate host.

B 3.2- "Disruption in PKC Mediated K Channel Regulation Contributes to Enhanced Serotonin Mediated Carotid Vessel Constriction from the Fawn Hooded Hypertensive Rat"

Gurjit Kaur, Mallikarjuna R Pabbidi
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Background: Previous studies indicate Fawn Hooded Hypertensive (FHH) rats exhibit cerebral damage due to diminished cerebrovascular myogenic function associated with elevated BK channel function. Introgression of the 2.4 Mbp region of BN chromosome 1 into the FHH rat restored myogenic response and BK channel function in a FHH.1^{BN} congenic rat. However, results obtained from resistance vessels cannot be extrapolated to conduit vessels due to structural and functional differences. Thus, the present study addresses the hypothesis that carotid vessels of FHH rats exhibit altered serotonin (5-HT)-mediated contraction compared to FHH.1^{BN} rats.
Methods: Tension myography was used to measure conduit vascular function from FHH and FHH.1^{BN} rats.
Results: We found that carotid vessels of FHH rats exhibited greater 5-HT-mediated contraction than FHH.1^{BN} rats. Activation of BK channels (NS1619: 5 to 30μM) relaxed vessels that are pre-constricted with 5-HT in both strains but to a lesser extent (1.17 times less) in FHH rats. On the other hand, inhibition of BK channels (Paxilline or Iberiotoxin: 100nM) had no effect in either strain. Inhibition of voltage gated calcium channels (VGCC, Diltiazem: 10 μM) or store operated calcium channels (SOC, 2-APB: 75μM) relaxed vessels pre-constricted with 5-HT similarly in both strains. Furthermore, activation (PMA: 100nM) or inhibition (GF109203X: 3μM) of PKC increased and decreased 5-HT mediated constriction respectively in FHH.1BN rats but has minimal effect in FHH rats.
Conclusion: These results suggest that a mutation in the genes located in 2.4Mbp region in FHH rats may contribute to a disruption in the PKC and its regulation on K channels, that may contribute to enhanced 5-HT-mediated contraction.
Acknowledgement: This research has received partial funding support from the American Heart Association, Scientist Development Grant (13SDG14000006), and the IOSP program which is supported by a grant from the National Science Foundation (NSF) Integrative Organismal Systems (IOS) Award No. IOS-1238831.

B 3.3- "Diabetes Resistant Tissue Engineered Vascular Grafts"

Jhilmil Dhulekar, M.S., Eric Wright, Zoltan Hajdu, M.D., Dan Simionescu, Ph.D., Agneta Simionescu, Ph.D.
Department of Bioengineering, Clemson University, Clemson, SC

Background**:** Diabetes Mellitus is a major risk factor for cardiovascular disease (CVD) and currently affects 387 million people worldwide as of 2014, with numbers rising steadily. Hyperglycemia and insulin resistance, in combination with dyslipidemia, oxidative stress, and inflammation, significantly increase the risk of vascular tissue damage. The primary cause of this damage in diabetic patients is the interaction of elevated levels of blood glucose and lipids with proteins such as collagen and elastin from the blood vessel wall via oxidation and cross-linking processes, which result in the formation of advanced glycation end products (AGEs). The presence of AGEs impairs wound healing and causes severe inflammation, fibrosis, and tissue stiffness. Consequently, the fate of reparative surgery and tissue transplantation is more difficult in diabetic patients.
The field of tissue engineering has great potential in treating cardiovascular diseases. Engineered vascular grafts made of synthetic materials have been successful in large-diameter applications (>6mm) but tend to fail when used in small-diameter applications (<5-6 mm) because of poor patency and early occlusion. There are more than 1 million vascular procedures in the United States each year involving small-diameter vessels, which is why finding an alternative conduit is important. Previous research has demonstrated that the application of a tannic acid derived polyphenol, known as penta-galloyl glucose (PGG) stabilizes collagen and elastin, and provides anti-inflammatory and anti-oxidant properties, which can inhibit vessel wall stiffening, calcification, inflammation and thereby allow the vascular grafts to resist diabetes-related damage. A successful vascular graft would possess mechanical strength and compliance to bear long-term hemodynamic stresses; non-immunogenicity; operative suturability, and complete incorporation into the host tissue with sufficient graft healing. In order to achieve these requirements, it is necessary to understand the structure and components of the native blood vessel and be able to translate these properties into the engineered blood vessel conduit.
Methods: Porcine kidneys were obtained from a local abattoir, and the renal artery branches were isolated and removed from the kidneys. The arteries were placed in a 0.1M NaOH solution for 3 hours under agitation to completely remove all cellular components. The decellularized arteries were then sterilized with peracetic acid and treated with PGG. To confirm complete decellularization, H&E and DAPI staining was performed and DNA Quantification using a Qiagen DNeasy kit and gel electrophoresis was performed to confirm removal of DNA. Additionally, mechanical testing was performed; including stress-strain analysis with MTS, burst pressure testing, and suture retention strength. The prepared vascular grafts will be seeded with adipose derived stem cells (ADSCs), conditioned in a bioreactor and implanted into a rat abdominal aorta model to compare the effect of diabetic conditions on PGG-treated vascular grafts versus untreated grafts and ADSC remodeling of the tissue.
Results**:** Histological analysis confirmed complete decellularization of the tissues. Mechanical testing for stress-strain analysis, burst pressure, and suture retention load demonstrated appropriate strength and compliance properties to withstand physiological conditions for *in vivo* studies. PGG-treated grafts demonstrated reduced stiffening, calcification, and inflammatory cell infiltration in previous *in vivo* studies when implanted sub-dermally in rats.
Conclusion**:** Results have demonstrated that the decellularization method is an effective technique to create viable, tissue engineered blood vessel scaffolds that are capable of being remodeled using adipose-derived stem cells to create a functional vascular graft. Treating the grafts with PGG not only increases the overall strength and stability of the tissue, but also makes the tissue resistant to the harsh inflammatory and oxidative environment created by hyperglycemia during diabetes. The ongoing *in vivo* studies with an abdominal aorta diabetic rat model will further understanding of the effect of diabetes on PGG-treated tissue engineered vascular grafts as a potential solution to treating arterial diseases in diabetic patients.
Acknowledgements**:** This work was funded by the NIH COBRE Grant and NIH RO1 Grant.

B 3.4- "Effects of Radiation and Sunitinib in the Rat Heart"

Marjan Boerma, Vijayalakshmi Sridharan, Martin Hauer-Jensen
The University of Arkansas for Medical Sciences, Little Rock, AR

Background: Radiation therapy for the treatment of thoracic cancers is associated with the development of radiation-induced heart disease, especially in cancer survivors who received a high dose of radiation to the heart. The novel anticancer agents, tyrosine kinase inhibitors (TKI) are known to produce acute cardiac dysfunction. Because tyrosine kinases modulate cell survival, cardiac toxicity of TKI is thought to be caused in part by cell death in the heart. Thoracic irradiation is often combined with TKI treatment. However, it is unknown whether radiation may modulate the adverse cardiac effects of TKI.

Therefore, this study investigates the effects of radiation and the TKI sunitinib in the rat heart.
Methods: Male Sprague-Dawley rats received localized image-guided irradiation to the heart at a fractionated dose of 9 Gy for 5 days. From the first day of irradiation, rats were orally administered 8 mg/kg body weight or 15 mg/kg body weight sunitinib or vehicle, every day for two weeks. Cardiac function was examined with echocardiography before and at 2 weeks after irradiation. Cardiac tissue was obtained at 2 weeks after irradiation to assess oxidative stress, apoptosis and autophagy. Cardiac mitochondria were examined under electron microscopy, and opening of the mitochondrial membrane permeability transition pore was assessed by mitochondrial swelling assay.
Results: Sunitinib alone decreased the GSH/GSSG ratios at both low and high dose and elevated 4HNE adducts only at the high dose (15 mg/kg). Radiation did not consistently modify these markers of oxidative stress. While radiation alone and sunitinib alone enhanced Bax/Bcl2 ratios, ratios did not further increase in the combined group, while sunitinib (15 mg/kg) inhibited the radiation-induced increase in numbers of apoptotic cells. Electron microscopy revealed mitochondrial damage in the combined treatment group. Moreover, sunitinib caused some calcium-induced mitochondrial swelling, which was increased further by radiation. Nonetheless, no severe changes were seen in cardiac function in any of the treatment groups.
Conclusions: Radiation enhanced the early effects of sunitinib on cardiac mitochondria, but had limited effects on early markers of cardiac cell death, oxidative stress, or cardiac function after sunitinib treatment.
Acknowledgments: This work was funded by NCI grant RE03701 (to M.B.) and NIGMS grant P20 GM109005 (to M.H-J.).

B 3.5- "Epicardial Fat and Markers of Hypertension"

Janae Jackson[1], Chris Adams[2], Nepal Chowdhury[3], Todd Gress[2], Carla Cook[1], Paulette Wehner[2], Ellen Thompson[2], Mohammed Waqas[2], Caitlin Kocher[1], Nalini Santanam[1,2]
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Background: Cardiovascular disease (CVD) is the number one killer of men and women. Hypertension is also a "silent killer" as it damages the heart and arteries. Epicardial fat (EF) is a visceral fat that surrounds the heart. When a person becomes obese the epicardial fat becomes thicker and their risk for CVD increases. In this study we show that EF biomarkers are associated with hypertension.
Methods: Blood and epicardial fat (EF) were collected from patients with and without coronary artery disease (CAD) during their heart surgeries. Additional methods used were ELISA and Human cytokine/chemokine Multiplex array in plasma using the Luminex200 system and RT^2 Profiler PCR Array for human hypertension related genes on EF mRNA.
Results: There were significant changes in p-values (<0.05) in the circulating levels of cytokines such as IFN- α -2, IL-1ra, and IL-1α in CAD patients with hypertension than those without. RT^2-PCR array showed significant increases in genes such as *ACE2* in EF of CAD patients both with and without hypertension. There was a significant correlation between EF thickness with increased risk to hypertension.
Conclusion: This study has identified novel EF biomarkers that will be validated for its importance in risk to hypertension.
Acknowledgement: JJ and NS acknowledge the support by AHA GRA: 15UFEL23700000, JCESOM translational pilot grants (NS) and NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence.

B 3.6- "COBRE Translational Research in Hypertension & Renal Biology"

Nina R. Majid
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Background: Because hypertension and associated kidney and cardiovascular diseases are prevalent in Louisiana, Tulane University established the Tulane Hypertension & Renal Center of Excellence (THRCE). The center was possible via funding from National Institutes of Health (NIH) Center of Biomedical Research Excellence (COBRE) program. The objective was to create a center of excellence in hypertension and renal research that would foster research on the basic mechanisms responsible for hypertension and on the interactions between environmental and genetic pathophysiological causes of hypertension.
Results: Over the years, the NIH COBRE grants awarded to THRCE has helped provide research opportunities for emerging leaders in hypertension and renal research and established enriched research environment to encourage and support multidisciplinary research in hypertension and related fields.
COBRE Phases I & II have helped THRCE support 16 junior faculty members (of which 10 received NIH research funding), 19 postdoctoral fellows, 12 graduate students, and 29 medical students. COBRE Phase III currently supports 33 Tulane investigators, 1

Administrative Core, 4 Research cores, and 3 pilot projects.
The Research Cores includes the molecular, imaging and analytical core with state-of-the-art equipment and resources, the transgenic animal core facility, the phenotyping core, and the clinical/translational core facility. The services provided by these cores contribute to a highly stimulating research and mentoring environment, strengthen hypertension and renal related research capability at our institution, and enhance the Center's research infrastructure.
Conclusion: With hypertension affecting the health of millions of Americans, Tulane COBRE research is vital to combat this disease; the Cores provide resource and services to support investigators thus enabling key research in hypertensive renal & cardiovascular diseases.
Acknowledgment: Center funded by the NIH/National Institute of General Medical Sciences under Award Number P30GM103337.

B 3.7- "Localizing Cardiac Magnetic Dipole Sources in Fetus Using Inverse and Forward Solutions on Fetal Magnetocardiography"

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Background: Cardiac cells in the fetal heart generate electric currents within the fetal heart. These currents are not confined to the fetal heart itself, but also spread through the tissues surrounding the fetal heart. All these currents together create a magnetic field observed over the maternal abdomen. Fetal magnetocardiography (fMCG) is a non-invasive technique to measure the magnetic fields outside the maternal abdomen and to extract the signals of the fetus' cardiac activity. Currently, fMCG is used for clinical and diagnostic purposes for fetal heart as well as monitoring fetus' development throughout gestation period. One of the main aspects of fMCG that needs to be studied and addressed is the orientation of the fetal heart. Tracking magnetic dipole sources and having the knowledge of the fetal heart orientation can be helpful for various research problems i.e. identifying the propagation velocity, developing a more accurate fetal movement algorithm and extracting clearer brain signals, etc.
Methods: In this study, we aimed to fit magnetic dipole to current dipole created by cardiac tissues and track the magnetic dipole sources to infer the information of the heart's orientation. We adapted the spherically symmetric conductor model introduced for MEG by Mosher et al. [1] and proposed a mathematical model to fit magnetic dipoles and track their sources. Our model uses inverse solutions on fMCG data for source localization and forward solution to produce magnetic fields from identified source points. The model picks candidate source points and produce magnetic fields at the sensors. Then the model evaluates the data fits and provides the source information for the best-fit.
Results: We have tested the applicability and performance of our method on 14 data sets that was obtained by SARA (SQUID Array for Reproductive Assessment) system at University of Arkansas for Medical Sciences (UAMS). SARA is an MEG system with a high spatial–temporal resolution with 151 sensors especially designed for fetal assessment that is currently used for heart and brain studies [2]. We observed that for the 14 data sets we have used, the highest explained variance for the best-fit was less than 5% while the most of the data has an explained variance around 1% or less.
Conclusion: Our initial results suggest that spherical conductor model can be used for the cardiac magnetic signals as well and fitting magnetic dipole to the current dipole is feasible.
Acknowledgement: This work was supported by grants from NCRR (P20RR016460) and NIGMS (P20 GM103429) at NIH
References:
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[2] Hari Eswaran, Curtis L Lowery, James D Wilson, Pam Murphy, Hubert Preissl, "Fetal magnetoencephalography -- a multimodal approach.", Brain Research. Developmental Brain Research. 2005; 154:57–62.

B 3.8- "Role of Sphingosine-1-phosphate in High Blood Pressure Programmed by Intrauterine Growth Restriction in Mouse"

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Background: Intrauterine growth restriction (IUGR) is a risk factor for hypertension and cardiovascular (CV) disease in later life, but the underlying mechanisms remain unclear. The bioactive sphingolipid metabolite sphingosine-1-phosphate (S1P) is critically involved in CV development in the fetus, and plays a significant role in the regulation of CV health in adulthood. S1P receptor (S1PR) type 1, 2 and 3 are widely expressed in CV system which S1PR1 has a protective role against kidney injury, while S1PR3 is involved in controlling BP. Yet, the contribution of S1P on BP in IUGR is unknown. In the present studies, we tested the hypothesis that IUGR alters renal S1P receptors expression during- and post-nephrogenesis, which contributes to high BP in male IUGR mouse.
Method: C57bl/6J mice underwent sham or reduced uterine perfusion (RUP) at day 13 of gestation with delivery at full term. Birth weight of control and IUGR offspring (from RUP dam) was measured. Kidneys

were isolated from 2 day old male pups or adult 24 week old male control and IUGR for determination of S1P receptor protein expression by western blot. We also assessed the acute effect of S1PRs agonist (FTY720) on BP of male IUGR at 24 week old via carotid catheter in the conscious state.
Result: IUGR offspring had a lower birth weight than control ($p<0.05$). S1PR3 protein expression was increased in 2 day old IUGR kidneys (2.4 fold vs control, N=3, $p< 0.01$). At 24 weeks of age, S1PR3 protein levels were decreased (0.75 fold vs control, N=4, $p< 0.05$) in IUGR kidneys. Protein expression levels of S1PR1 and S1PR2 were not different between control and IUGR kidneys. Male IUGR offspring had a significantly higher BP compared to male control (control: 112.1±2.1, IUGR: 125.0±3.7 mmHg; N=7, *P*<0.05). Acute administration of FTY720 (1 mg/kgBW i.p, Fingomod), did not significantly alter BP in control (106.0 ± 5.7 mmHg) but significantly decreased BP in IUGR (105.7±2.3 mmHg, $p< 0.05$). A dose response to FTY720 (10 mg/kgBW) decreased BP in both control (94.0±2.0 mmHg, $p< 0.05$) and IUGR (99.3±2.3 mmHg, $p< 0.05$).
Conclusion: Together our data suggest that IUGR programs an alteration of renal S1PR3 expression in both during- and post-nephrogenesis thereby contributing to an increase in sensitivity to S1PRs agonist. Thus, S1P signaling is a putative mechanism underlying the hypertension of IUGR offspring.
Acknowledgment: This work was funded by NIH COBRE (pilot) grant GM104357 (to S.I)

B 3.9- "The Efficacy of Soluble Guanylyl Cyclase (Sgc) Stimulators and Activators in a Rat Model of Preeclampsia"

Ta'Shariah Robinson, Ellen Gillis, Joey Granger, Eric George, Michael Garrett, Jennifer Sasser
Departments of Pharmacology and Toxicology and Physiology and Biophysics, The University of Mississippi Medical Center, Jackson MS

Background: Preeclampsia is a pregnancy disorder that adversely affects both the mother and the fetus and is characterized by the onset of hypertension and proteinuria after the twentieth week of gestation. While there are currently no effective treatments, agents that improve nitric oxide (NO) - cyclic guanosine monophosphate (cGMP) signaling and endothelial function may be therapeutic targets to treat patients with preeclampsia. Recent studies suggest that soluble guanylyl cyclase (sGC) stimulators and sGC activators can enhance reduced sensitivity to NO and increase the enzymatic activity of sGC, respectively. The purpose of this study was to determine the efficacy of a sGC stimulator and a sGC activator on the maternal syndrome and fetal outcomes in the pregnant Dahl S rat model of preeclampsia.
Methods: Baseline blood pressure (BP) was recorded (DSI telemetry), and rats were placed in metabolic cages for 24 hr urine collection before mating. On day 14 of pregnancy, rats were randomly placed on either the control diet (n=3), the BR5918 sGC stimulating diet (80mg/kg/day, n=5), or the BR5919 sGC activating diet (16mg/kg/day, n=4). Uterine artery resistance index (UARI) was measured by Doppler ultrasound (Vevo 770) on day 18, urine was collected again on day 19, and blood and tissues were harvested on day 20. Plasma and urinary cGMP concentrations were measured using a commercially available EIA kit (Cayman Chemical).
Results: Only the sGC activator enhanced the sGC activity as evidenced by increased cGMP in the urine and plasma (see Table); however, both treatments improved the maternal syndrome as measured by a decrease in blood pressure and proteinuria. These treatments did not affect UARI, litter size, or pup weight.
Conclusion: These preliminary findings demonstrate reductions in blood pressure and proteinuria following treatment with either a sGC activator or stimulator with no adverse effects on fetal growth. Therefore, our findings support further investigation into the therapeutic effects of sGC stimulators and activators for the treatment of preeclampsia.

B 3.10- "Pericyte Fate Depends on Stem Cell Type and Age in Cultured Microvascular Networks"

Walter L. Murfee, Mohammad S. Azimi, Amy L. Strong, Bruce A. Bunnell
Tulane University, New Orleans, LA

Background: The development of stem cell based therapies for manipulating the microcirculation requires a better understanding of stem cell fate and function during angiogenesis. Our laboratory recently developed a novel *ex vivo* tissue culture model that enables time-lapse investigation of mechanistic cell-cell interactions at specific locations across intact blood and lymphatic microvascular networks. The objective of this study was to demonstrate the usefulness of the rat mesentery culture model for evaluating the effects of aging and cell source on stem cell differentiation into vascular pericytes.
Methods: DiI-labeled cells were seeded onto adult Wistar rat mesenteric tissues and cultured in alpha-MEM + 1% serum for up to five days according to four experimental groups: 1) adult human bone marrow-derived stem cells (hBMSCs), 2) aged hBMSCs, 3) adult human adipose-derived stem cells (hASCs), and 4) aged hASCs. After 1 day, cells displayed changes in cell morphology indicative of tissue integration.
Results: By day 5, aged hBMSCs were observed in typical pericyte location wrapped along blood capillaries during angiogenesis and the number of vessels covered by DiI-positive hBMSCs was increased in the aged versus adult cell group. No change in hASC cell fate was observed between the respective adult and aged populations.

Conclusion: Our results suggest that age and origin influence the ability of stem cells to become vascular pericytes and establish the rat mesentery culture model as a valuable tool for *ex vivo* screening of type-specific differences in stem cell fate across an intact microvascular network.
Acknowledgment: This work was supported by COBRE funding from NIGMS through the Tulane Center for Aging under grant number P20GM103629. Additional support was provided by the Tulane Hypertension and Renal Center for Excellence funded by NIGMS P30GM103337.

B 3.11- "Role of ER Stress in Development of Hypertensive-Diabetic Nephropathy"

Zhen Wang, Jussara M. do Carmo, John E. Hall
Department of Physiology and Biophysics, Mississippi Center for Obesity Research, University of Mississippi Medical Center, Jackson, MS

Background: Hypertension (HT) is common in diabetes and substantially increases the risk for diabetic nephropathy. To investigate the mechanisms by which HT and diabetes interact to promote nephropathy, 6 month-old male Goto-Kakizaki (GK) rats, a model of spontaneous type 2 diabetes, were used in this study.
Methods: HT was induced by aorta coarctation (AC) between the renal arteries to determine the impact of hypertension plus diabetes in the right kidney (above the coarctation) and normal or slightly reduced blood pressure plus diabetes in the left kidney (below the coarctation). Blood pressures (BP) above and below the AC were measured by telemetry and femoral artery catheter, respectively. Glomerular filtration rate (GFR) and urinary albumin excretion (UAE) in left and right kidneys were examined separately by collecting urine from each kidney. To examine the role of endoplasmic reticulum (ER) stress in development of nephropathy, GK rats with AC were treated with ER stress inhibitor, tauroursodeoxycholic acid (TUDCA, 200 mg/kg/day IP), from 2 to 8 wks after AC.
Results: Eight weeks after AC, BP above the AC and urinary albumin excretion (UAE) were significantly increased from baseline (BP: 155±4 vs 108±2 mmHg; UAE: 39.2±14.8 vs 0.9±0.7 mg/24h, n=6, $p<0.05$). We observed 17% and 15% increase in protein expression of ER stress markers CHOP and GRP 78 in the HT-diabetic right kidney compared to the normotensive-diabetic left kidney. After 6 wks of TUDCA treatment, CHOP expression from the right kidney was significantly reduced 47% compared to untreated rats, and BP above the AC was significantly lower (135±4 vs 155±4 mmHg, n=7, $p<0.05$ compared to untreated group). TUDCA treatment also increased GFR (0.9±0.1 vs 0.6±0.1ml/min/g of kidney weight, n=6, $p<0.05$) and decreased UAE (5.6±1.5 vs 18.4±5.8 ug/min, n=4-6, $p<0.05$) when comparing right kidneys between treated and untreated GK-AC rats.
Conclusion: These results suggest that ER stress contributes to kidney injury when HT is superimposed on diabetes. Pharmacological inhibition of ER stress may attenuate increases in BP and kidney injury in hypertensive-diabetic nephropathy.
Acknowledgments: This work was funded by NHLBI-PO1HL51971, AHA 14POST18160019 and by an Institutional Development Award (IDeA) from the NIGMS P20GM10435776.

Cell Signaling,
Poster Session B
Friday, September 13, 10:45 a.m.-12:15 p.m.

B 4.1- "The Effects of Oleic Acid Concentration on Cholesterol Absorption in the Larval Zebrafish Enterocyte"

Laura Faith Stevens, Jennie Spencer, James Walters
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Background: High levels of dietary lipids are associated with dyslipidemias. The presence of fatty acids, specifically oleic acid (OA), has been shown to facilitate the entry of dietary cholesterol into enterocyte cells in the intestine. Two proposed mechanisms for signaling the uptake of cholesterol, stoichiometric or signaling, were examined. For this study we utilized the vertebrate model organism *Danio rerio*, or zebrafish.
Methods: Larval zebrafish were fed diets of 1000 µM, 100 µM, 10 µM, and 1 µM OA added to low fat diets with equal amounts of native and TopFluor-Chlolesterol (TF-Chl) available. After a 5.5 hour incubation, a lipid extraction was performed and the results were developed on a thin layer chromatography (TLC) with two solvent systems. TLC plates were analyzed with Gel Analyzer 2010 software to determine pixel intensity of the separation bands. Band separation allowed for distinguishing of esterified TF-Chl from unesterified TF-Chl.
Results: Analysis of relative fluorescent units (RFU) showed that 100 µM OA had the highest uptake of esterified TF-Chl. The trend of uptake between 100 µM and 1 µM appears stoichiometric.
Conclusion: OA mediated cholesterol absorption in larval zebrafish occurs under 1 µM OA, intervals between 0 µM and 1 µM will distinguish the model of absorption.
Acknowledgment: This work was funded by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence.

B 4.2- "C/EBPδ Modulates Oxidative Stress and Mitochondrial Dysfunction to Promote Post-Radiation Survival"

Sudip Banerjee[1], Nukhet Aykin-Burns[1], Kimberly J. Krager[1], Stepan B. Melnyk[2], Martin Hauer-Jensen[1, 3], Snehalata A. Pawar[1]
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[2]Arkansas Children's Hospital Research Institute, Little Rock, AR
[3]Surgical Services, Central Arkansas Veterans Healthcare System, Little Rock, AR

Background: Ionizing radiation (IR) generates reactive oxygen species (ROS) which induces oxidative stress and DNA strand breaks and are the two underlying mechanisms of tissue injury. Cells deficient in antioxidants may be unable to prevent the IR-induced oxidative stress and undergo IR-induced apoptosis. CCAAT enhancer binding protein delta (*Cebpd*, C/EBPδ) is a transcription factor implicated in the regulation of oxidative stress, DNA damage response and inflammation, however its role in the IR response is not known. We recently reported that *Cebpd*-deficiency sensitizes mice to IR-induced lethality however the molecular mechanism of C/EBPδ-mediated protection needs investigation. We hypothesize that the underlying sensitivity of *Cebpd*-knockout (KO) mouse embryonic fibroblasts (MEFs) is due to an impaired ability to detoxify IR-induced ROS.
Methods: *Cebpd*-KO and *Cebpd*-wild type (WT) MEFs were exposed to IR and seeded for clonogenic survival assays or harvested at various timepoints for 1) preparation of whole cell extracts (WCEs); 2) for measuring apoptosis by Annexin V and ROS levels by flow cytometry;
3) measuring glutathione (GSH); and 4) for detecting 4-Hydroxynonenal (HNE)-protein adducts by immunoblotting of WCEs. Cellular bioenergetic profiles of both genotypes were compared using a sea-horse X-Flux analyzer.
Results & Conclusions: Irradiated KO MEFs expressed elevated levels of hydroperoxides and mitochondrial superoxides which correlated with increased apoptosis and decreased clonogenic survival. This decrease in clonogenic survival of KO MEFs was partially rescued by pretreatment with polyethylene glycol-conjugated CuZn superoxide dismutase and catalase prior to irradiation. Irradiated KO MEFs showed significant reductions in basal and ATP-linked respiration, spare capacity respiration and proton leakage indicative of mitochondrial dysfunction post-irradiation. We also found that unirradiated and irradiated KO MEFs expressed lower GSH Methionine and Cysteine levels, which correlated with oxidative damage visualized by increased expression of HNE-protein-adducts compared to respective WT MEFs. These results demonstrate a novel role of C/EBPδ in modulating oxidative stress and IR-induced mitochondrial dysfunction. Future studies will focus on the identification of molecular targets of C/EBPδ that regulate these processes.
Acknowledgment: This work was funded by Centers of Biomedical Research Excellence (COBRE)-Center for Host responses to Cancer Therapy at UAMS from NIGMS under grant number P20GM109005.

General Biomedical Sciences, Poster Session B

Friday, September 13, 10:45 a.m.-12:15 p.m.

B 5.1- "The *Trichomonas vaginalis Virus* Among Mississippi and ATCC *T. vaginalis* Isolates"

Allison K. Judge, John. C. Meade, Cory G. Toyota
Millsaps College, Jackson, MS

Background: Trichomoniasis is the most common non-viral STD worldwide. The disease is caused by the parasite *Trichomonas vaginalis*, which itself can be infected with a virus, known as *T. vaginalis virus*, or TVV. Previous studies indicate that *T. vaginalis* strains infected with TVV are more easily treatable with the antibiotic ribovarin; however, it is also speculated that prolonged irritation can occur when TVV infected *T. vaginalis* is cured with the antibiotic. Four types of TVV have been identified, which are simply called TVV 1, 2, 3, and 4.
Methods: RTPCR was used to type over 40 strains of *Trichomonas vaginalis*. Sanger sequencing was utilized to confirm PCR results.
Results: Eight ATCC strains of *T. vaginalis* were successfully confirmed through Sanger sequencing, as well as one strain local to the Jackson area. Of the 16 viruses confirmed among the nine strains, six were TVV1, three were TVV2, six were TVV3, and one was TVV4.
Conclusion: These results allow us to utilize previous experiments to speculate how each TVV may have affected *T. vaginalis* infection, treatability, and other behaviors.
Acknowledgements: This work was supported by the Misissippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476

B 5.2- "Leptin Stimulation of GnRHr in Gonadotropes: Is Musashi involved?"

Angela K. Odle, Melody Allensworth-James, Melanie MacNicol, Angus MacNicol, Gwen V. Childs
University of Arkansas for Medical Sciences, Little Rock, AR

Background: The appetite-regulating hormone leptin is produced in many tissues of the body, including the pituitary. Pituitary gonadotropes lacking LepRb signaling capability have decreased GnRHr protein. These studies investigate whether (1) leptin can stimulate GnRHr protein expression in gonadotropes, and (2) whether or not leptin regulates the translational regulator Musashi.
Methods: Control male and female mouse pituitaries were stimulated with leptin to determine the effects of leptin on GnRHr or Musashi expression. Pituitaries from mice lacking the leptin signal on gonadotropes (Cre-LH, LepR Exon $17^{fl/fl}$) were assayed for *gnrhr* or *msi* mRNA.
Results: Leptin stimulates GnRHr expression in both males and females in a dose-dependent manner. This increase is seen generally as well as specifically in LH-labeled cells. Conversely, leptin suppresses MSI1 expression in both male and female cultures. Males mice lacking LepRb on gonadotropes have decreased *msi* mRNA (both isoforms), while females have increased *msi* mRNA.
Conclusion: Using control and gonadotrope-specific leptin receptor-null animals, we determined that (1) leptin stimulates GnRH receptor proteins, (2) leptin suppresses Musashi in gonadotropes, and (3) deletion of the leptin receptor on gonadotropes disrupts *msi* mRNA levels. These studies add mechanistic insight into the complex interplay of leptin and the hypothalamic-pituitary-gonadal axis.
Acknowledgments: NIH R03 HD059066 and NIH 1R01HD059056, NIH NIGMS P20 GM103425, Core facilities funded by NIH NCRR P20 RR020146 and NIH P30 NS047546 at UAMS.

B 5.3- "Development of Four-Way Junctions (4wjs) as Novel Therapeutic Inhibitors of the DNA-Binding Cytokine High Mobility Group B1 (HMGB1)"

Douglas Iverson[1], Paul Stoulig[1], Tamara Bell[2], Crystal Serrano[1], Sana Salongi[1], Anushobha Regmi[1], Dexter Dean[1], Filbert Totsingan[3], Anthony J. Bell Jr.[1]
[1]The University of Southern Mississippi, Hattiesburg, MS
[2]Alcorn State University, Lormon, MS
[3]New York University, New York, NY

Background: Until the turn of the century, HMGB1 was known as an abundant architectural DNA-binding protein. In the nucleus, HMGB1 binds preferentially to DNA 4WJs to modulate chromatin remodeling and genetic recombination/repair. It is now clear that HMGB1 can also function as a redox sensing proinflammatory cytokine. And several studies link unintended HMGB1 signaling with pathogenesis in a variety of diseases and immune disorders such as: cancer, lupus, rheumatoid arthritis and sepsis. The objective of these studies is to develop novel 4WJs as therapeutic inhibitors against HMGB1. Two classes of 4WJs are being developed: *i)* intramolecular (intra-) DNA and *ii)* hybrid junctions composed of DNA and peptide nucleic acids (PNAs).
Methods: Nondenaturing gel electrophoresis is used to monitor the global conformation of intra-DNA and hybrid PNA-DNA 4WJs. Circular dichroism (CD) is used to measure the secondary structure and conformational stability of 4WJs. Nuclease resistance assay are used to evaluate the stability of 4WJs against endo- (DNase I) and exonucleases (Exo III and Lambda Exo). Electrophoretic mobility shift assays (EMSAs) are used to measure the binding

affinity of 4WJs toward HMGB proteins.
Results: Nondenaturing gel electrophoresis shows that intra-DNA and hybrid PNA-DNA 4WJs form "immobile" structures that mimic the DNA control, J1. CD reveals that intra-DNA 4WJs are composed of B-DNA helices. Whereas, hybrid PNA-DNA junctions are composed of mixed A- and B-form helices, the A-helix content correlates with PNA content. Thermal denaturing scans and nuclease resistance assays indicate that intra-DNA and hybrid junctions composed of multiple PNA strands are significantly more: *i)* stable (DT_m > 15°C) and *ii)* resistant to endo- (DNase I) and exonucleases (Exonuclease III) *vs.* J1. EMSAs indicate that intra-DNA and single-PNA hybrid 4WJs bind HMGB proteins with high affinity.
Conclusion: These studies clearly show that novel 4WJs can be developed with enhanced stability and nuclease resistance vs. all-DNA junctions. Moreover, the majority of novel 4WJs maintain high binding affinity toward HMGB proteins. Future experiments will focus on measuring the serum (human) stability of lead 4WJs.

B 5.4- "Regulation of Biofilm Formation in the Symbiotic Bacterium *Xenorhabdus nematophila*"
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Millsaps College, Jackson, MS

Background: The bacterium *Xenorhabdus nematophila* engages in a mutualistic relationship with *Steinernema carpocapsae* nematodes, and together these partners invade and kill a variety of insect larvae, mostly of the Lepidopteran order. The global transcriptional regulator Lrp is critical to both mutualism and pathogenesis behaviors in *X. nematophila*. Due to the pleiotropic effects of Lrp activity, as well as the multifactorial nature of the symbiotic interactions, the exact contributions of Lrp to these behaviors are as-yet unknown. Given that biofilm formation is often an indicator of attachment to host tissues, we chose to investigate the role of Lrp in *in vitro* biofilm formation as an initial step toward characterizing the role of Lrp in host association.
Methods: We investigated *X. nematophila in vitr*o biofilm formation using an *lrp* mutant strain, as well as strains constitutively expressing low or high levels of *lrp*. We measured the effect of Lrp on biofilm formation under a variety of different environmental conditions using a standard crystal violet staining method.
Results: No biofilms were observed in the *lrp* mutant strain under the conditions tested, however a surface-attached biofilm did appear with increased magnitude as *lrp* expression levels increased between strains. Interestingly, a pellicle also formed at the air-liquid interface of *lrp*-expressing strain cultures, and preliminary data indicate that this pellicle may form in the low *lrp*-expressing strain before the high *lrp*-strain.
Conclusion: We found that though Lrp is necessary for *in vitro* biofilm formation in *X. nematophila*, the overall levels of Lrp protein may influence surface attachment and pellicle formation differently, indicating that the underlying mechanisms of formation of these biofilms may be different. Future work will focus on the role(s) of biofilm formation in host association.
Acknowledgment: This work was funded by MS-INBRE research development grant P20 GM103476.

B 5.5- "Molecular Dynamics Simulation Study of VECAR in Water: Data Analysis"
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Background: VECAR are bolaamphiphilic molecules which consist of hydrophilic chromanol and carnosine ends linked together by a hydrophobic hydrocarbon chain of varying length. VECAR is an unsymmetric bolaamphiphilic molecule. The hydrophilicity of carnosine end is stronger than that of chromanol end. Atomistic molecular dynamics simulations are performed using GROMACS v.4.6 for aqueous systems of VECAR molecules of three different chain lengths (n=4, 13, and 22). Here, n is the number of repeating methylene units.
Methods: Data analysis was carried out using FORTRAN and data analysis tools of VMD and GROMACS. The atomistic structure of each self-assembly was analyzed by calculating time averages of the radial distribution function, the density profile, and the end-to-end chain-length distribution. In order to study the assembling process, we also calculated changes of various energies, the mean square displacement, the number of hydrogen bonds, and the size distribution of self-assemblies as function of time.
Results: Radial distribution function shows a well-defined micelle structure of small self-assemblies composed of VECAR molecules with chain lengths (n=13 and 22). The strongly hydrophilic carnosine end forms the outermost shell exposed directly to water and the weakly hydrophilic chromanol end form another shell just underneath the carnosine layer due to the unsymmetric bolaamphiphilicity of VECAR. The hydrophobic chains were sequestered inside of the micelle. Density profile of micelles shows that the mass density within micelles decreases from that of bulk systems for a given chain-length VECAR. Furthermore, the mass density decreases as the chain length increases, in both aqueous and bulk systems. We also found that small-size micelles remain stable longer for VECAR molecules with a longer chain at a given molecular concentration.
Conclusion: We found a clear dependence on the hydrocarbon chain length of the self-assembly shape, the micelle stability, and the atomic distribution within each assembly. We also found that VECAR with a longer chain formed a well-defined small-size micelles which are more stable. In the future, we will explore the feasibility of these small-size micelles as a drug delivery system.

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B 5.6- "Decarboxylative Photocyclization of Pyromellitimide w-Carboxylic Acids"

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Background: The decarboxylative photocyclization is an elegant photochemical way to synthesize small to medium sized heterocycles. The photochemical starting material is a phthalimide chromophor and a w-carboxylic acid. The products are pyrrolizidine-annelated structures. Several functional groups are tolerated and ring sizes up to 36 members have been isolated with appropriate precursors. An interesting Memory of Chirality effect via a 1,7-triplet biradical has been observed during the course of the photochemical cycilzation to yield pyrrolo-[1,4]-benzodiazepines. The synthetic potential of the reaction is diminished by the incorporation of the phthalimide nitrogen into the product.
The pyromellitchromphor has so far been used extensively in polymeric films such as Kapton in the aerospace industry. Kapton has a high thermal stability, good mechanical properties, low dielectric constant, low coefficient of thermal expansion and high radiation resistance. In photosynthetic systems pyromelliimdes are used as electron-acceptor moeities. No synthetic applications involving the pyromellit chromophor have been reported so far.
Methods: Simple condensation products of w-carboxylic acids (with spacer lengths of 1 to 10) gave the photochemical starting materials which were irradiated at 300nm in basicaqueous solution.
Results: The photochemical reaction led to the formation of cyclization products. The possible regio- and stereoisomers made product analysis complex. Yields were small to poor due to undesired side reactions. The irradiation solution developed a strong color which might be due to a CT-complex or the radical anion of pyromellitimide.
Conclusion: the pyromellit chromophor can be ued for the decarboxylative photocyclization. The complex product mixtures makes is difficult to predict products but optimization of the reaction conditions can improve the overall yield of desired products. This opens a new field of preparative photochemistry of this formerly considered photostable chromophor.

Acknowledgment: This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

B 5.7- "Inhibition of Retinoic Acid Receptor Beta Enhances the Neuronal Differentiation of Mouse Embryonic Stem Cells"

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Background: Previous research has shown that when Embryonic Stem (ES) cells are treated with retinoic acid, neuronal differentiation is increased. However, the efficiency of neuronal differentiation in ES cells treated with RA is not optimal because RA also induces cell differentiation along other cell lineages. We hypothesize that this effect is due to the interaction of RA with its different receptors (alpha, beta, and gamma), and that binding of RA with the Retinoic Acid Receptor beta (RARb) may decrease the efficiency of neuronal differentiation in ES cells.
Methods: We employed LE135, an inhibitor for RARb, in conjunction with RA to treat mouse ES cells. Cells cultured as monolayers were treated with LE135 (1 or 5 mM) in the presence or absence of RA (1 mM). Control cells were treated with vehicle only (ethanol). After 48 hours of treatment, cells were cultured for 4 days in Neurobasal medium supplemented with B27 in order to induce neuronal maturation. The effect of these treatment regimens was evaluated by immunofluorescence using an antibody against the neuronal marker Beta Tubulin III.
Results: We observed an increase in ES to neuronal differentiation in cells treated with a combination of RA and LE135, as compared to cells treated with RA or LE135 alone. The maximum efficiency of neuronal differentiation was obtained by employing 1 mM RA plus 5 mM LE135.
Conclusion: Our results suggest that RARb represses neuronal lineages and that its pharmacological inhibition may be a novel method to increase the efficiency of neuronal differentiation in culture.
Acknowledgements: This work was by funded by NINDS Grant 1R15NS091949-01 (to E.M.) and by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM103424.

B 5.8- "Synthesis and Antioxidant Activity Studies of Tocotrienol Analogues with Variable Tail Length"

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Background: γ-Tocotrienol (γ-T3) and δ-tocotrienol (δ-T3), two vitamin E homologues, are some of the most effective and low-toxic radioprotective agents identified to date. Ionizing radiation (IR)-triggered overproduction of reactive oxygen species (ROS) with concordant induction of DNA damage and cell death is one of the major mechanisms of normal tissue injury during radiotherapy. Because of the strong antioxidant action of T3s, they can protect against direct cell damage by scavenging IR-induced ROS and chain-terminating free radical lipid peroxidation (LPO). Due to their high lipophilicity, T3s likely exert their antioxidant activity within the cell membranes. Recent studies have indicated that the anti-ROS and anti-LPO actions of vitamin E compounds occur specifically at cell membrane's hydrophobic-water interface; and the mobility of the molecules in the cell membrane is also important for the antioxidant potency. We hypothesize that the location and mobility of T3s in cell membrane are correlated to the length of their tail unit. To test this hypothesis, we synthesized a series of δ-T3 analogues with varying isoprenyl units (from one to four) to explore relationship between the tail length and antioxidant activity.
Methods: δ-T3, which was isolated from annatto oil, was used as starting material for the synthesis of all the desired analogues. The murine hematopoietic progenitor cell line (32D) was used for the antioxidant activity assay. Analogues were tested for their ability in reducing H_2O_2-induced reactive oxygen species (ROS) production. ROS levels were analyzed by using flow cytometry to measure MFI of DCF, a highly fluorescent oxidation product of nonfluorescent H_2DCFDA.
Results: Synthetic modifications focusing on the farnesyl tail of δ-T3 resulted a series of homologues with tails containing one, two, or four isoprenyl units. These homologues were tested for their ability to reduce H_2O_2-induced ROS production in 32D cells. At 10 μM, all the analogues displayed little antioxidant activity under normal conditions (without H_2O_2 treatment) but significantly inhibited H_2O_2-induced ROS production; and they are equally potent to γ-T3 and δ-T3.
Conclusion: The lipophilic farnesyl tail of T3s is believed to be important for their ROS scavenging actions within cell membrane. Thus, with modifications on the tail unit, we expected to see changes on structure-antioxidant activity relationships (SAR). Further studies will include antioxidant activity at various concentrations and their anti-LPO activity.
Acknowledgment: This work was supported by National Institute of General Medical Sciences of the NIH under grant number P20 GM109005.

B 5.9- "The Role of Bolaamphiphilic Character in Self-Assembly of VECAR Molecules: A Molecular Dynamics Simulation Study"

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Background: VECAR are antioxidant molecules comprised of two hydrophilic molecular groups, a carnosine derivative and chromanol, covalently linked by a hydrophobic alkyl spacer of varying length. The goal of this study is to gain atomistic insights into the self-assembly behavior of bolaamphiphilic VECAR molecules in solution.
Methods: Molecular dynamics simulations are performed using GROMACS. Three VECAR molecules with alkyl chain length n=4, 13, and 22 were investigated. The time evolution, and energetic and geometrical characteristics of the self-assembled structures were evaluated using graphic and numerical analysis tools.
Results: The study of small self-assemblies of VECAR (with n=13 and 22) showed a well-defined micelle structure with hydrophobic alkyl spacers located close to its center, the strongly hydrophilic carnosine end located at the outermost surface, and the weakly hydrophilic chromanol end located just under the region occupied by the carnosine groups. The shape of the cross-section of these self-assemblies was elliptical, with one of the semi-axes lengths comparable to the alkyl spacer length. The larger VECAR assemblies demonstrated complex structures in accordance with other studies of bolaamphiphiles.
Conclusion: In addition to providing microscopic understanding of the assembly structure and the dynamics of the self-assembly process of VECAR molecules in water, the results of this study provide atomistic insights into the general behavior of bolaamphiphiles that was observed in experiments or coarse-grained simulation studies.
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B 5.10- "Increased Expression of Dkk-1 in Human Bone Marrow Stromal Cell Populations Following Chemotherapy Exposure"

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Background: Wnt signaling has been shown be a critical regulator or bone formation and hematopoiesis within bone and marrow tissues. Dkk-1, a secreted inhibitor of Wnt signaling, has been implicated in these processes and has also been shown to regulate the survival of various tumors. We have screened MSCs and osteoblasts for the expression of various Wnt ligands and regulating molecules using qPCR and show that Dkk-1 is elevated following exposure to chemotherapy. MSCs and osteoblasts are critical components of the bone marrow microenvironment, highlighting the potential for Dkk-1 mediated alterations in bone marrow function following chemotherapy exposure.
Methods: Primary human MSCs and osteoblasts were generated from de-identified bone aspirates from healthy donors or purchased from PromoCell (Heidelberg, Germany), respectively. MSC and osteoblast phenotypes were determined by alizarin red and oil red staining, as well as flow cytometry. qPCR was utilized to evaluate Wnt activating and regulating molecule gene expression following exposure to etoposide, melphalan, or 5-Fluorouracil. Western blotting and ELISA were utilized to confirm elevations in Dkk-1 expression. Fluorescent imaging was utilized to evaluate active β-catenin levels in MSCs following chemotherapy exposure.
Results: Exposure of MSCs and osteoblasts to chemotherapy altered gene expression of Wnt3a, Wnt4, and Dkk-1. Although alterations in Wnt3a and Wnt4 did not correlate with changes in protein abundance, Dkk-1 was elevated at a protein level. Dkk-1 could only be visualized in the presence of GolgiStop (BD Biosciences), suggesting that DKK1 is primarily regulated at a transcriptional level. Active β-catenin was not elevated following exposure to chemotherapy.
Conclusion: Dkk-1, an inhibitor of Wnt signaling, is increased in MSCs and osteoblasts following chemotherapy exposure. This highlights a potential mechanism of disrupted of hematopoiesis and bone formation which is commonly observed in patients receiving such regimens clinically. Future work will evaluate the effects of chemotherapy induced Dkk-1 expression on bone formation and hematopoietic support by MSCs and osteoblasts, and determine whether inhibition of Dkk-1 will help alleviate these sequelae.
Acknowledgment: This work was funded by National Institutes of Health (NHLBI) R01 HL056888 (LFG), National Cancer Institute (NCI) RO1 CA134573NIH (LFG), P30 GM103488 (LFG), WV CTR-IDEA NIH 1U54 GM104942, the Alexander B. Osborn Hematopoietic Malignancy and Transplantation Program, and the WV Research Trust Fund.

B 5.11- "Short- and Long-Term Effects of Exposure to Low Doses of High-LET Radiation in the Mouse Lung"

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Background: The health effects of exposure to high-LET radiation and the underlying mechanisms are poorly understood. Studies show that space missions can result in the development of pulmonary pathological states. Our initial study demonstrated that exposure to low doses of ^{56}Fe resulted in minor pro-fibrotic changes exhibited primarily as increased expression of chemokines and interleukins, detected at the beginning of the fibrotic phase (22 weeks post exposure). At the same time-point, epigenetic alterations were exhibited as global DNA and repetitive elements-associated hypermethylation and transcriptional repression of repetitive elements.
Methods: In this study, we evaluated the effects of total body exposure to ^{56}Fe (0.5 Gy) and protons (0.1 Gy) alone or in combination and compared them with the effects caused by γ-radiation in C57BL/6 male mice 4 weeks after exposure.
Results: We show that exposure to ^{56}Fe, proton or in combination did not result in significant histopathological changes and minor pro-fibrotic molecular alterations in the murine lung. At the same time, combined exposure to ^{56}Fe and protons resulted in alterations in the methylation and expression of transposable elements and satellite DNA. These effects were also associated with alterations in DNA methylation machinery.
Conclusion: We show the exposure to high-LET radiation can result in significant epigenetic alterations detected in the mouse lung short (4 weeks) and long (22 weeks) term after exposure. Furthermore, combined exposure to ^{56}Fe and protons results in more pronounced epigenetic effects that can lead to development of genomic instability and cancer.
Acknowledgement: This work was supported by NIH Grant 1P20GM109005, NSBRI Grant RE03701, and ASGC Grant NNX13AB29A.

B 5.12- "Biochemical And Molecular Methods For The Detection Of Endogenous Cftr Expression"

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Background: CFTR is a membrane protein that function as a chloride channel on surface of many epithelial cells. Defective CFTR is the cause of genetic disease cystic fibrosis. CFTR is expressed endogenously at a level that is normally difficult to detect by traditional biochemical methods such as western blotting and immunohistochemistry. In order to detect the endogenous CFTR levels we first aimed to increase the sensitivity of CFTR immunoblots by altering the concentrations of SDS and methanol in transfer buffer. SDS facilitates the removal of proteins from polyacrylamide gels (PAGE) and methanol allow there binding to nitrocellulose membrane.
Methods: Endogenous (Capan-1, CFPAC) and exogenous (LLC-PK_1, CFBE) CFTR expressing epithelial cell lines were treated with 5 mM sodium butyrate for 60 h to up-regulate the CFTR expression. CFTR protein levels were determined by western blotting and immuprecipitation methods. Specificity of CFTR immunoprecipitates was confirmed by peptide (antigen) inhibition of anti-CFTR antibody. Real-time RT-PCR was used to determine the CFTR mRNA transcripts.
Results: The addition of 0.015% SDS in transfer buffer enhanced the detection of exogenous but not endogenous CFTR expression levels in western blotting. Methanol concentrations (5 to 20%) didn't influence the sensitivity of CFTR immunoblots. Endogenous CFTR expression levels, however, could be detected by immunoprecipitation with anti-CFTR antibody. At molecular level, the endogenous CFTR mRNA expression can also be detected and quantified by real-time RT-PCR.
Conclusions: Endogenous CFTR expression cannot be detected by traditional western blotting procedure. Endogenous CFTR protein and mRNA expression, however, can be detected by immunoprecipation and real-time RT-PCR methods.
Acknowledgements: This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM103476 and Faculty Research Award to GDH.

B 5.13- "MC4R in Forebrain Neurons Contributes to Leptin's Acute Effects to Stimulate Peripheral Glucose Uptake"

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Background: We previously showed that an intact brain melanocortin 4 receptor (MC4R) is required for leptin to exert its antidiabetic actions. However, the brain regions where MC4R activation contributes to the powerful effects of the leptin-MC4R axis to improve glucose uptake in peripheral tissues are still unknown. We examined whether rescue of MC4R specifically in forebrain neurons (LoxTB/CamK2-Cre mice, n=7) of whole-body MC4R deficient mice (LoxTB-$MC4R^{-/-}$ mice, n=5) is associated with increased peripheral glucose uptake after acute intracerebroventricular (ICV) leptin injection. Wild-type (WT) littermates were used as controls (n=4). Methods: Mice were implanted with an ICV cannula (lateral ventricle) and after 10 days of recovery, leptin (2 mg/2 ml) was injected 15 minutes before mice received a bolus dose of [^{14}C] 2-deoxyglucose (2-DG, 12 mCi, ip). Blood samples were collected at 10, 20, 30 and 40 min post 2-DG injection and tissues were harvested for 2-DG uptake. Results: LoxTB-$MC4R^{-/-}$ mice were heavier (42±1 vs 36±3 and 27±2 g) with slightly higher blood glucose levels (110±5 vs 92±8 and 95±9 mg/ml) compared to LoxTB/Camk2-Cre and WT mice, respectively. After ICV leptin injection glucose uptake by the brain, brown adipose tissue, skeletal muscle and white adipose tissue was significantly higher in LoxTB/Camk2-Cre and WT mice (69±4 and 81±2; 118±5 and 155±17; 23±5 and 43±2; 14±2 and 25±8 mmol/100 mg tissue/min) compared to loxTB-$MC4R^{-/-}$ mice (44±12, 86±5, 16±3 and 9±3 mmol/100 mg tissue/min). Conclusion: These results suggest that MC4R in forebrain neurons may play an important role in contributing to the acute effects of leptin on peripheral glucose homeostasis. Acknowledgment: This work was funded by NHLBI-PO1HL51971 (to J.E.H.), an Institutional Development Award (IDeA) from the NIGMS P20GM104357 and by AHA SDG5680016 (to J.M.C)

B 5.14- "Tiny Cells for a Tiny Animal"

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Cell lines are a useful tool for scientists that provide access to biochemical and genetic studies that otherwise would not have been possible. Cell lines have been created from many organisms and are widely used in biological research, but while protocols exist for recovering cells from many other organisms, there is not yet a report for deriving a cell line using Tetranychus urticae commonly known as the Two-Spotted Sider Mite. Two-Spotted Spider Mites are a species of mite that destroys plants. They are a common pest of agricultural crops, and have shown remarkable pesticide resistance. This cell culture

protocol expands the list of tools that scientist can use in the research of Two-Spotted Spider Mite biology. Specifically we will be able to perform reporter assays to explore gene regulatory pathways in this organism. Methods used in this study are derived from researchers who did a similar experiment where cells were isolated from Drosophila (fruit fly) embryos. Dr. Perrimon et al. published their methods in "Primary Cell Cultures from Drosophila Gastrula Embryos" (2011). The protocol I have developed involves harvesting Tetranychus urticae embryos, homogenizing them with media, and allowing them to grow in plates. I have managed to keep a living tissue culture for cells from Tetranychus urticae. To verify I conducted DAPi DNA staining to detect the presence of cells, and a polymerase chain reaction using Tetranychus urticae specific primers. I was also able to successfully transfect the cells with GFP proving that the cells will be useful for further genetic experiments.

B 5.15- "Role of SIRT3 in the Pathobiology of Pulmonary Fibrosis"

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Background: Idiopathic Pulmonary fibrosis (IPF) is driven by excessive myofibroblast activation and resulting collagen deposition compromises lung function. Oxidative stress promotes fibroblast-to-myofibroblast differentiation (FMD). Inhibition of oxidative stress and activation of antioxidant pathways has been shown to attenuate fibrosis. We hypothesize SIRT3 may play a role in preventing TGFβ1-induced FMD by activation of MnSOD and other substrates and that inhibition of SIRT3 may thereby potentiate lung fibrosis.
Methods: qRT-PCR and Western blot (WB) techniques were used to measure the expression of SIRT3 and downstream, as well as fibrotic markers, in Normal Human Lung Fibroblasts (NHLF) in the presence of TGFβ1. qRT-PCR was performed on NHLF cultured in the presence of Resveratrol and in the presence or absence of TGFβ1 to assess changes in expression of SIRT3. NHLF were transfected with siRNA targeting SIRT3 in order to determine its effect on myofibroblast differentiation. qRT-PCR for expression of cytokines and fibrotic markers was performed on SIRT3-knockout (KO) and wild-type (WT) mice exposed to Adenovirus-TGFβ1-induced lung injury.
Results: We demonstrated in NHLF TGFβ1 reduced the expression of SIRT3 and MnSOD. Resveratrol induced SIRT3 expression and ameliorated metabolic changes induced by TGFβ1. Targeting of SIRT3 induced expression of markers of chronic inflammation and fibrosis. By contrast, promotion of SIRT3 expression attenuates the effect of TGFβ1 on myofibroblast differentiation.
Conclusions: Our preliminary studies suggest a possible role for SIRT3 in the pathobiology of pulmonary fibrosis.
Acknowledgment: Research supported by NIH Award P20GM103629.

B 5.16- "Saturated Fat-Dependent Ubiquitination: Potential Role in Obesity-Associated Non-Alcoholic Fatty Liver Disease"

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Background: Cells maintain protein homeostasis, or proteostasis, in the endoplasmic reticulum (ER) by tightly regulating protein synthesis, folding, and degradation. The accumulation of misfolded proteins in the ER and resultant activation of the unfolded protein response (UPR) have been implicated in the pathophysiology of several liver diseases. Previous studies from our laboratory have demonstrated that saturated fats induce ER stress and UPR activation in H4IIE liver cells and in the liver in vivo. In this study, we provide preliminary data suggesting that differential handling of proteins by the ubiquitin proteasome system may play a role in UPR activation by saturated fats.
Methods: We treated H4IIE liver cells with 250 μM palmitic acid (16:0), 250 μM oleic acid (18:1), or both for 4 hours. We measured protein ubiquitination in cell lysates by western blot analysis. UPR activation was determined by qPCR analysis.
Results: Incubations with 16:0 resulted in increased protein ubiquitination and UPR activation (n=5). In contrast, incubations with 18:1 did not increase protein ubiquitination nor result in UPR activation (n=5). Co-incubation of 16:0 with 18:1 resulted in levels of protein ubiquitination and UPR activation that were not significantly different from control levels (n=5).
Conclusion: These preliminary observations that saturated fat increases ubiquitination in liver cells suggests that impaired protein degradation may mediate deranged proteostasis, ER stress, and UPR activation in obesity-associated liver disease.
Acknowledgment: This work was funded by DK072017 NIDDK and by the American Society for Cell Biology Visiting Professorship Award.

B 5.17- SC BioCRAFT Bioengineering and Bioimaging Core – 2015 Southeast Regional IDeA Conference

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The overarching theme of SC BioCRAFT is regenerative medicine, which is a highly interdisciplinary field; expertise and support in bioengineering methods as well as cell and molecular biology and bioimaging are required to succeed. Junior investigators in our IDeA state need core support to further their research and compete nationally for grant funding. From our experience in Phase I of our COBRE program, we identified four bioengineering thrust areas where targeted PIs need support. These thrust areas, which the Bioengineering and Bioimaging (B&B) Core supports, are: 1) Biomaterials Characterization, 2) Bioreactor Design and Bioactive Matrix Syntheses, 3) Biomechanics, and 4) In Vivo Bioimaging.
To provide this support, the B&B Core maintains a broad range of state-of-the-art instruments and facilities and provides technical support, consultation, and training for their use for each of the focus areas. In addition, the B&B Core develops, organizes, and presents training workshops on the use of selected core facilities. For example, a workshop on high-performance liquid chromatography (HPLC) was presented this past summer, which was in high demand by our graduate students. Future workshops are planned. To keep up with current research needs, the B&B Core has acquired new instruments this year MicroCT SkyScan 1176, Bruker; IVIS Lumina XR series II, Perkin Elmer; Ultra Sound imaging system Vevo 2100, Fuji Film, Visual Sonics Inc, and home built electrospinning set up, which are already in high demand by the COBRE PIs as well as other Clemson University investigators.
The B&B Core also supports undergraduate education by providing instrument and technical support for undergraduate laboratory classes (e.g., laboratory support for our Tissue Engineering and Biomaterials courses) and for Creative Inquiry undergraduate research projects.
In summary, the B&B Core provides resources, facilities, and training to support education and research related to tissue engineering, regenerative medicine, and drug delivery-system design for designated COBRE PIs and other research groups both within and outside the state of South Carolina, with the overall objective of increasing the state's competiveness in biomedical research.
Acknowledgment: B&B Core is supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant numbers 5P20RR021949 and P20GM103444.

B 5.18- "A Potential Role for TtSNX4 in macronuclear Degradation in *Tetrahymena thermophila* Conjugation"

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Background: Autophagy a process by which cells degrade cellular material for recycling in the absence of nutrients. Recent evidence has suggested that this process is not limited to cases of nutrient depravation, but that selective degradation of specific organelles in the presence of nutrients may also occur. However the mechanisms by which organelles are marked for degradation remain poorly understood. The ciliate, *Tetrahymena* thermophila represent an ideal system to study organelle autophagy since *Tetrahymena* degrade their nuclei by an autophagy like mechanism as part of their normal mating program. In this study, we identify a gene, TtSNX4 that appears to localize to the degrading macronuclues of mating *Tetrahymena* and may play a role in its degradation by regulating the activity of lysosomes.
Methods: We generated Tetrahymena that expressed TtSNX4-GFP fusion protein using biolistic transformation. We then used fluorescence microscopy to determine the localization of this fusion protein during Tetrahymena development. We determined its localization to the degrading macronucleus via DAPI staining and Lysotracker. Finally we determined the role of autophagosomes in this localization using 3 methyladenine (3-MA) to disrupt autophagosome formation.
Results: TtSNX4 expression was verified by western blot analysis and fluorescence microscopy. TtSNX4 localized to the degrading macronucleus and to lysosomes. This was evident from co-staining of degrading nuclei with Lysotracker and TtSNX4-GFP. Importantly, this localization appeared to be dependent on autophagosome formation since depletion of autophagosome via treatment with 3-MA disrupted the localization of TtSNX4 to the nucleus but not the lysosome.
Conclusion: This study suggests that TtSNX4 may be required for macronuclear degradation through the regulation of lysosome-autophagosome interactions.
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B 5.19- "Electrospinning Natural Polymer Fibers"

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Background: Recent evolutions in the field of biomaterials have focused on developing materials that can facilely interface with biological systems to treat or replace tissues or functions of the body. Natural polymers including polysaccharides have been investigated as suitable biomaterials to mimic the environment of body tissues and facilitate tissue regeneration. Electrospinning natural polymers like alginate and chitosan yields nanofibers that have shown promise as tissue scaffolds and drug delivery vehicles. However, these renewable biopolymers contain inherent charges, which make it difficult to electrospin these polymers, thus researchers continue to investigate the optimal electrospinning conditions for biopolymer fiber formation.
Methods: In this project, the formation of nanostructures was explored using alginate and chitosan solutions with a variety of additives that could assist nanofiber formation, including co-solvents, co-polymers, and neutralizing solutions. Other electrospinning parameters were also explored including distance to the target and flow rate.
Results: It was found that chitosan fibers formed most easily when they were electrospun with another polymer while alginate fibers were able to form when electrospun with a co-solvent system. Changing of other electrospinning parameters generally had little effect on the formation of fibers.
Conclusion: This study lead to valuable results for the production of natural electrospun fibers. Several natural polymers electrospin best when an additive, either a co-solvent or a co-polymer, is added to the biopolymer solution. Positive results were also obtained when chitosan was electrospun with a neutralizing polymer.
Acknowledgment: The authors would like to thank Dr. M. Miller, Microscopy Facility at Auburn University, for assistance with the SEM micrographs.

B 5.20- "Synthesis and Antioxidant Activity of δ-tocoflexol, a δ-tocotrienol Analogue Designed to have Improved Pharmacokinetic Profile"

Xingui Liu, Lijian Shao, Satheesh Gujarathi, Cesar Compadre, Martin Hauer-Jensen, Peter Crooks, Daohong Zhou, Guangrong Zheng
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Background: Tocopherols (Ts) and tocotrienols (T3s) are members of vitamin E family, each composed of four homologues (i.e., α, β, γ, and δ). T3s have been shown to have some bioactivities that are often not seen with Ts; and γ-T3 and δ-T3 are often the most potent homologues. However, both γ-T3 and δ-T3 have limited bioavailability and short plasma elimination half-lives compared with Ts, partially due to their weak binding affinity to α-tocopherol transfer protein (α-TTP). α-TTP is the protein responsible for transferring the vitamin E compounds out of the liver, where they are subject to metabolism, into the systemic circulation. We designed a new class of T3 analogues, named tocoflexols, with potentially increased binding affinity for α-TTP. Our purpose herein is to synthesize δ-tocoflexol.
Methods: δ-Tocoflexol was synthesized starting from DeltaGold®, a tocopherol free vitamin E supplement from annatto oil (contains ~70% δ-T3 and ~7% γ-T3), through a series of chemical transformations. Antioxidant activity of δ-tocoflexol was tested by measuring its ability in reducing H_2O_2-induced reactive oxygen species (ROS) production in mouse hematopoietic progenitor cells (32D). ROS levels were analyzed by using flow cytometry to measure MFI of DCF, a highly fluorescent oxidation product of nonfluorescent H_2DCFDA.
Results: An 8-step synthetic route was developed for the synthesis of δ-tocoflexol. Antioxidant activity test showed that at 10 μM, δ-tocoflexol and γ-T3 displayed little antioxidant activity under normal conditions (without H_2O_2 treatment) but significantly inhibited H_2O_2-induced ROS production; and δ-tocoflexol and γ-T3 are equally potent.
Conclusion: δ-Tocoflexol was successfully synthesized and tested for its antioxidant activity. Further studies will include α-TTP binding assay and pharmacokinetics.
Acknowledgment: This work was supported by National Institute of General Medical Sciences of the NIH under grant number P20 GM109005.

B 5.21- "Autophagy Regulates Muscle Contraction Induced Myokine Synthesis"

Simran Batth, Tarek Abd-elhamid, Jose Subauste, Angela Subauste
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Background: Exercise has emerged as an intervention against conditions such as diabetes and cardiovascular disease. The underlying mechanism is still unclear. In response to contraction muscle secretes proteins (myokines) that mediate some of the metabolic benefits associated with exercise. Autophagy is a highly conserved process of recycling that has been shown to be acutely upregulated by exercise. In this study we demonstrate that myokine synthesis and secretion in response to contraction is dependent on autophagy activation.
Methods: We constructed a short hairpin for the

autophagy protein ATG5 in an inducible lentiviral vector. An *in vitro* contraction protocol was used in C2C12 myotubes. For *in vivo* studies we used the mouse model of autophagy related beclin 1 (Becn1) knockout.
Results: Downregulation of ATG5 leads to a blunted response to contraction for myokines IL6 and LIF. Omega 3 fatty acids have been shown to activate autophagy. We demonstrate that treatment with omega 3 fatty acids while activating autophagy also upregulate contraction induced myokine synthesis (IL6, LIF). Using Becn1 -/+ mice we demonstrated that after a bout of exercise LIF and IL6 protein levels were downregulated in the Becn1 -/+ mice when compared to controls.
Conclusion: Myokine synthesis and secretion in response to muscle contraction is dependent on autophagy activation. Furthermore we show that the effect of omega 3 fatty acids on autophagy is capable of enhancing the effect on myokine synthesis. This could potentially place omega 3 fatty acid supplementation as an intervention aimed at maximizing the metabolic effects of exercise.
Acknowledgement: This work was supported by the Department of Medicine, UMMC and by the National Institute Of General Medical Sciences of the National Institutes of Health under Award Number P20GM104357.

Genomics,
Poster Session B
Friday, September 13, 10:45 a.m.-12:15 p.m.

B 6.1- "Telomere Shortening is Associated with Cartilage Transcriptome of Patients with Osteoarthritis"

Malwina Czarny-Ratajczak, James Eastwood, Vinod Dasa, John Zhang, Melody Baddoo, Tiffany Chynces, S. Michal Jazwinski
Tulane Center for Aging, Department of Medicine, Tulane School of Medicine, New Orleans, LA

Background: Osteoarthritis is one of the diseases causing the most serious disabilities worldwide, especially in the elderly population. The idiopathic form of this disorder is associated with age and with genetic and epigenetic factors, which interact with environmental factors and contribute to osteoarthritis development and progression. In this study we show that the silencing effect of telomeres on genes located nearby is disrupted by telomere shortening in the cartilage of patients with osteoarthritis. Furthermore, these telomere-proximal genes are significantly overexpressed and cause transcriptional noise in the tissue.
Methods: We analyzed patients with knee osteoarthritis and conducted our experiments on unaffected and affected cartilage collected from the same joint of each patient. Relative telomere length was measured via qPCR in affected and unaffected chondrocytes obtained from fifty patients during joint replacement surgery. We evaluated with RNA-Seq twenty transcriptomes from unaffected and affected chondrocytes collected from ten patients. RNA-Seq was completed on the Ion Torrent Platform and results were analyzed with STAR, Bowtie2, HTSeq and R.
Results: We detected severe shortening of telomeres in affected cartilage samples. Transcriptome analysis revealed significant enrichment in overexpressed genes from the telomere-proximal regions of chromosomes in these tissues. Some of these genes have not been implicated previously in the pathogenesis of osteoarthritis; however, their activity may cause significant changes at the protein level in the affected cartilage and contribute to disease progression.
Conclusions: Our data indicate that telomere shortening could be a new important mechanism contributing to cartilage aging and osteoarthritis pathology. Osteoarthritis is an age-related disorder and we propose a new model of cartilage aging, which could potentially be applicable to the other age-related diseases characterized by telomere shortening.
Acknowledgment: This research was funded by NIGSM, Mentoring Research Excellence in Aging and Regenerative Medicine grant P20GM103629 to S.M.J as a pilot project for M.C-R. RNA-Seq was performed in the COBRE Genomics and Biostatistics Core at the Tulane Center for Aging, supported also by grant P20GM103629. Tulane's Cancer Crusaders Next Generation Sequence Analysis Core, funded by grant Cancer Crusaders and P20 GM103518 from the NIHGMS contributed to RNA-Seq data analysis.

B 6.2- "Chromatin Structural Changes in Gene Regulation"

Yvonne Fondufe-Mittendorf
University of Kentucky, Lexington, KY

Background: The positioning of nucleosomes with respect to the DNA sequence plays an important role in regulating transcription. Studies in yeast reveal that the overwhelming majority of nucleosome positions are encoded in the genome by specific signals that favor or disfavor nucleosome positions. We recently showed that this code exist in archaeal genome and must have evolved together with the packaging of the DNA into the nucleus. However, the extent to which nucleosome occupancy and dynamics is modulated to regulate specific gene expression patterns is limited. Moreover, the extent to which chromatin architectural proteins binding at the entry/exit of the nucleosome dyad, regulate nucleosome positioning and stability is not known.
Methods: We study how chromatin architectural proteins, H1, MeCP2, HMGD1 and PARP1 regulate chromatin dynamics in transcription regulation. We used high-resolution nucleosome positioning analysis to determine the functional location of CAP-chromatin structures. Furthermore, we used biophysical FRET and biochemical studies to measure the direct effect of CAPs on the wrapping/unwrapping of DNA on nucleosomes.
Results: We show that CAPs bind to specific genomic locations correlating with their functional transcriptional outcomes. We also show that CAPs control specific chromatin structures that play a role in in not only transcription regulation but also how the genes are ultimately spliced.
Conclusion: This study shows that the CAPs regulate chromatin structures, and thus are active participants in regulating chromatin-mediated gene regulation. Such information is critical in the functional understanding of chromatin structural organization during gene regulatory processes.
Acknowledgment: This work was funded by NIEHS Grant 1R01ES024478-01A1; NSF 1517986, IRSF (to Y.N.F-M) and by an Institutional Development Award (IDeA) from the NIGMS under grant number 8P20GM103486-09.

Infectious Disease/Immunology, Poster Session B

Friday, September 13, 10:45 a.m.-12:15 p.m.

B 7.1- "Specific Mucin Response to Human Paramyxovirus Infections"

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Background: Human Metapneumovirus (hMPV) and Respiratory Syncytial Virus (RSV) are paramyxoviruses that cause respiratory diseases ranging from mild cold-like symptoms to more severe conditions like bronchiolitis and pneumonia. No vaccine has been yet developed for these infections. Despite the structural and epidemiological similarities between these two viruses, the immune response they elicit in the infected organism is different. The innate immune response is the immediate defense after a pathogen insult. In that regard, the physiological barrier formed by mucus layering the epithelial is critical to control the entrance of alien microorganisms, including viruses. Mucins (MUC) are the main component of mucus, they are glycosylated and high molecular weight proteins. However, the mucin response in viral infections is still largely unknown. Therefore, in this work we comparatively determined the expression of MUC response by RSV and hMPV in human epithelial cells.
Methods: Epithelial cells (A549 cells) were infected with hMPV or RSV at an MOI of 3. The expression of MUC was determined by qRT-PCR. The comparative cycle threshold ($\Delta\Delta$CT) was used to quantitate the expression of MUC and normalized to GAPDH as endogenous reference. One way ANOVA followed by Tukey post-test was used to determine the differences between the two viruses.
Results: hMPV induced the expression of MUC2, MUC5B and MUC1 in higher levels than RSV. While RSV infection led to higher induction of MUC8, MUC15, MUC20, MUC21 and MUC22 when compared to hMPV. Both viruses induced similar magnitude of MUC3, MUC5AC, MUC16 and MUC19.
Conclusion: These data indicate that the MUC response in human epithelial cells is differentially induced by RSV and hMPV. The differential expression of mucins upon RSV and hMPV infections could relate to the different immune response induced by these two major human respiratory paramyxoviruses. This contribution is relevant because understanding the induced immune response will help to a better design of specific antiviral therapies for each of these viruses.
Acknowledgment: This research work was supported by Grants from the National Center for Research Resources (P20RR020159-09) and the National Institute of General Medical Sciences (P20GM103458-09) from the National Institutes of Health.

B 7.2- "The *msaABCR* Operon Plays a Role in Antibiotic Susceptibility in *Staphylococcus aureus* Biofilm"

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Department of Biological Sciences, The University of Southern Mississippi, Hattiesburg, MS

Backgrounds: Community-acquired, methicillin-resistant *Staphylococcus aureus* strains cause severe infections among healthy individuals. Many of these infections are recalcitrant to antimicrobial therapy. Formation of biofilm within the host tissue and indwelling medical devices is one major contributing factor for their resistance to treatment. Biofilm formation is a complex developmental process that is regulated by several global regulators. One of those global regulators is the *msaABCR* operon that is involved in biofilm development, antibiotic resistance and cell death in *S. aureus.* In this study we tested the impact of *msaABCR* deletion in the susceptibility of several antibiotics using three different in-vitro biofilm assays.
Method: We used minimum biofilm eradication concentration (MBEC) assay, in-vitro catheter based assay, and microfluidics BioFlux system to monitor the effect of different antibiotics. this study, we selected antibiotics that are used to treat the biofilm-associated infections. We used daptomycin, linezolids, clindamycin, vancomycin, rifampicin and gentamycin. We also tested the combination of rifampicin and gentamycin with daptomycin, linezolids and vancomycin to make this study more relevant to clinical settings.
Results: Deletion of *msaABCR* had no impact on the daptomycin, linezolids, clindamycin, and gentamycin susceptibility in planktonic conditions, but showed at least two-fold reduction in sensitivity to rifampicin and vancomyin. However, all these antibiotics alone or in combination showed significant effect on biofilm formation in the *msaABCR* deletion mutant. The MBEC assay showed statistically significant effect on the mutant's biofilm (18 fold) compared to the wild type in the presence of 40 μg/ml of daptomycin. Daptomycin concentrations above 40 μg/ml reduced the mutant's biofilm to an undetectable level, whereas concentrations up to 160 μg/ml did not affect the wild type biofilm. None of the in vitro catheters colonized with WT strain were cleared even after continuous exposure of daptomycin (40 μg/ml) for 4 days, whereas the mutant's biofilm was completely cleared by day 4. Both wild type and mutant strains showed increased susceptibility to antibiotics when used in combination. The wild type strain showed 32-fold and 8-fold increased susceptibility in daptomycin and rifampicin when used in combination relative to

individual use. The mutant showed an even higher increase with 80-fold for daptomycin and 16-fold for rifampicin. Using microfluidic bioflux system, we found that the mutant biofilm was effectively cleared in the presence of 5 μg/ml daptomycin and 0.3 μg/ml rifampicin, which is clinically relevant in terms of treatment using these antibiotics. Likewise, the mutant's biofilm was significantly reduced to an undetectable level, when treated with daptomycin-gentamycin, linezolid-gentamycin, and linezolid-rifampicin.
Conclusion: In conclusion, since deletion of *msaABCR* operon limits the biofilm formation in *S. aureus,* this limitation was correlated with the increased susceptibility with different antibiotics treatment using all in-vitro models of biofilm formation.

B 7.3- "Role of the SOS Response in the Resistance of *Listeria monocytogenes* to Conditions Encountered within the Gastrointestinal Tract"

Brandy Roberts[1], Amber Thompson[1], Morgan L. Wright[1], Hossam Abdelhamed[2], Jessica G. Wilson[1], Janet R. Donaldson[1]
[1]Mississippi State University, Mississippi State, MS
[2]College of Veterinary Medicine, Mississippi State University, Mississippi State, MS

Background: *Listeria monocytogenes* can survive broad ranges of environmental conditions, including temperature, pH, salt concentrations and oxygen availability, making it very difficult to eliminate. Previous studies have suggested an important link between the SOS response and resistance to the gastrointestinal tract. In order to determine whether this response is involved in bile resistance, mutants defective in the SOS response were constructed and survival was assessed following exposure to conditions that mimic those encountered within the gastrointestinal tract.
Method: Mutants were constructed in the background of the *Listeria monocytogenes* strain F2365 using the pHoss1 suicide plasmid, which utilizes a double recombination event to generate an in-frame deletion. The *dinB* (DNA polymerase), *kat* (catalase-like heme binding protein), and *lexA* (SOS response regulator – transcription repressor) genes were deleted. Mutants were exposed to 0, 1, 5 and 10% bile under both aerobic and anaerobic conditions and viability was compared to the wild-type strain F2365 over a 6h time period.
Results: Mutants were analyzed in normal growth conditions; no significant difference was exhibited between the strains in comparison to the wild-type F2365 strain. Additionally, F2365 and the *dinB, kat,* and *lexA* mutants had similar growth in the presence of porcine bile under aerobic conditions. The *dinB* and *kat* mutants exhibited similar survival under anaerobic conditions in the presence of bile as the wild-type strain. However, the Lmf2365Δ*lexA* had a decrease in survival (87% v. 100%) compared to the wild-type and other mutants in 1% bile (p=0.0041). All showed similar decline at 10% bile.
Conclusion: These results indicate that removal of *lexA* impairs bile resistance under physiologically relevant anaerobic conditions. These results suggest that the regulation of the SOS response is key for survival and is not due to an increase in mutagenesis, as the *dinB* mutant did not have an improved survival. Additional studies are needed to decipher the mechanism by which the stress response differs under anaerobic conditions, as this enhances virulence.

B 7.4- "An Investigation of Nitrogen Metabolism in the Dimorphic Fungus *Histoplasma capsulatum"*

Logan Blancett, Caitlin Cooksey, Thomas Buford, Glen Shearer
The University of Southern Mississippi, Hattiesburg, MS

Background and Objective: *Histoplasma capsulatum (Hc)* is the etiological agent of histoplasmosis, a common cause of respiratory mycoses in humans. *Hc* is a dimorphic organism existing as a mold (M) at 25°C and once inhaled by host (37°C) undergoes a dimorphic shift to the yeast (Y) phase. This dimorphic shift is essential for the pathogenesis of the organism within the host. It is most commonly found in the United States along the Mississippi and Ohio River Valley regions where high levels of bird and bat excrements can be found. In previous experiments it was found that *Hc* differentially utilizes sole nitrogen sources and also shows a preference of sources in both the M and Y phases respectively. Our experimental objectives are to investigate the nitrogen regulatory protein *AreA* and determine its function in Nitrogen Catabolite Repression.
Methods: To begin our experimentation we grew *Hc* on various sole nitrogen sources to see which nitrogen sources are more energetically favorable. Next, we began investigating the transcript level of *AreA* via quantitative RT-PCR. We then investigated some down-stream effector genes whose response should be regulated in an AreA-dependent fashion.
Results: We can conclude that *AreA* is up-regulated when subjected to a no nitrogen environment opposed to a sufficient environment containing ammonia. It has also been found that Dal5 and Gap1 are up-regulated as well.
Discussion and Conclusions: These results show that *AreA* is needed in greater quantities when subjected to a poor nitrogen environment. Future directions include knocking-down *AreA* to see the response of Dal5 and Gap1 to test whether they are solely AreA-dependent genes.
Acknowledgements: This work was supported by the Mississippi INBRE funded by grants from the National Center for Research Resources (5P20RR016476-11)

and the National Institute of General Medical Sciences (8 P20 GM103476-11) from the National Institutes of Health.

B 7.5- "Identifying Enriched CDR3 Sequences in Systemic Lupus Erythematous Patients"

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Background: Systemic lupus erythematosus (SLE) is an autoimmune disorder with symptoms varying from malar rash to renal failure. One common SLE hallmark is increased antibody levels, and several studies have documented potential antigens: DNA, RNA, Ro, La, etc. Antibodies consist of 4 chains: 2 heavy (IGH) chains and either 2 κ (IGK) or 2 λ (IGL) chains. Within the IGH region, complementarity-determining-region 3 (CDR3) is primarily responsible for antigen recognition and represents a prime target for antigen-binding studies. In study, we analyze the CDR3 regions of SLE patients and present clusters of enriched sequences which may be utilized in future studies to determine SLE-associated antigens.
Methods: We obtained deep-sequencing data from peripheral blood mononuclear cells for SLE patients. The population was screened for patients with a confirmed SLE diagnosis, at least one time point in flare (≥6 SLEDAI criteria), and a non-flare time point. The CDR3 sequences within this population were clustered using the CD-HIT web server's hierarchical clustering feature. A flare distribution calculation was developed to identify highly enriched sequences, and these target clusters were realigned using ClustalOmega and visualized in Jalview to determine sequence homology. VDJ gene combinations were also analyzed for association with SLE.
Results: Our analysis narrowed our results from 128,000+ sequences down to 148 sequences in 22 clusters. 5 target clusters were identified which presented with good consensus and high flare distribution. We further identified that terminal sequence consensus aligned well with J segment. An analysis of the VDJ combination patterns demonstrated an association between DJ combination and SLE flare.
Conclusion: This study demonstrates that the CDR3 region of IGH is involved in systemic lupus erythematous flare. Hierarchical clustering using CD-HIT, combined with ClustalOmega and Jalview analysis for sequence homology, yielded 5 clusters of CDR3 sequences with high flare distribution and consistent sequence homology. Further, DJ combination was also demonstrated to play a role in the autoimmune response to SLE, as certain DJ combinations were more highly associated with SLE flare. The sequence clusters and DJ combinations reported can be utilized for future antigen-identification studies.
Acknowledgment: This research was supported by grants from NCRR (P20RR016460) and NIGMS (P20 GM103429) at NIH. The authors express their thanks to the Oklahoma Medical Research Foundation.

B 7.6- "Memory Tfh cells promote rapid antibody production after secondary *Plasmodium yoelii* infection"

Daniel J. Wikenheiser, Jason S. Stumhofer
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Background: The antibody (Ab)-mediated immune response contributes to protection against a secondary blood-stage *Plasmodium yoelii* infection. Follicular helper T (Tfh) cells promote B cell survival and facilitate somatic hypermutation and affinity maturation within germinal centers, resulting in the production of high-affinity Ab in a primary infection. The presence of memory T cells is important for the rapid induction of an effector response in secondary infections; however, it is unclear what role memory T cells play in supporting Ab production in a secondary response. Here, we report the identification of a splenic-resident population of $CXCR5^+$ T cells that possess a central memory phenotype ($CXCR5^+$ Tcm) that is maintained greater than 90 days post-*P. yoelii* infection.
Methods: $CXCR5^+$ Tcm and isotype-switched memory B cells were adoptively transferred into naive congenic or *Cd28*$^{-/-}$ mice, followed by *P. yoelii* infection. We utilized flow cytometry and ELISA to assess the functional outcome of the donor cell phenotype and Ab production, respectively.
Results: Transfer of $CXCR5^+$ Tcm resulted in early plasma cell and germinal center B cell differentiation, as well as class-switched parasite-specific Ab production relative to control mice that did not receive transferred cells. Furthermore, transferred $CXCR5^+$ Tcm cells displayed a greater propensity for up-regulating Bcl6 compared to transferred $CXCR5^-$ Tcm cells, indicating their ability to differentiate into Tfh cells after re-activation.
Conclusions: Ultimately, mice receiving $CXCR5^+$ Tcm cells controlled parasitemia more quickly than mice receiving memory B cells alone or no transferred cells. Collectively, these data suggest *Plasmodium*-specific $CXCR5^+$ Tcm can differentiate into functional Tfh cells upon re-exposure to antigen, and participate in secondary humoral responses, leading to rapid production of protective, isotype-switched Abs.
Acknowledgment: This work was funded by NIH grant G1-38592-04-05/211 awarded to J.S.S.

B 7.7- "Induction of an Atypical Lymphoid Progenitor Lin$^-$Sca-1$^+$c-kit$^-$ Cells in Spleen During Acute Infection with *Plasmodium yoelii*"

Debopam Ghosh, Jason S. Stumhofer
Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR

Background: Hematopoietic stem and progenitor cells (HSPCs) function to replenish the immune cell repertoire under steady-state conditions, and in response to inflammation due to infection or stress. While the bone marrow serves as the primary niche for hematopoiesis, extramedullary mobilization and differentiation of HSPCs occurs in the spleen during acute *Plasmodium* infection – a critical step in the host immune response. Here, we identified an atypical HSPC population in the spleen of C57BL/6 mice, with a Lineage$^-$Sca-1$^+$c-kit$^-$ (LSK$^-$) phenotype that proliferates in response to infection with non-lethal *Plasmodium yoelii* 17X.
Methods: LSK$^-$ cells from *Plasmodium* infected Ubc-GFP Tg was adoptively transferred into naïve or infection matched congenic mice and the transferred cell population was analyzed by flow cytometry based on their GFP expression. The functional significance of the progenitor cell population was assessed by ELISpot and flow cytometry based cell-phenotyping.
Results: Infection-derived LSK$^-$ cells upon transfer into naïve congenic mice were found to differentiate predominantly into mature follicular B cells. However, when transferred into infection-matched hosts, infection-derived LSK$^-$ cells gave rise to B cells capable of entering into a germinal center reaction, and developed into memory B cells and plasma cells that were capable of producing parasite-specific antibodies. Differentiation of LSK$^-$ cells into B cells in vitro was enhanced in the presence of parasitized RBC lysate, suggesting that LSK$^-$ cells expand and differentiate in direct response to the parasite.
Conclusion: Collectively, these data identify a population of atypical lymphoid progenitors that differentiate into B-lymphocytes in the spleen, and are capable of contributing to the ongoing humoral immune response against *Plasmodium* infection.
Acknowledgement: This work was supported by the Arkansas Biosciences Institute, NIH grant P20 GM103625 (J.S. Stumhofer). A special thanks to Andrea Harris for her technical assistance as part of the UAMS flow cytometry core.

B 7.8- "Recruitment of NLRP10 and NLRP12 in Lipid Raft Entities Following Cigarette Smoke Challenge in Murine Macrophages"

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[2]Department of Computer Science, Jamia Millia Islamia, New Delhi, India
[3]Departments of Comparative Biomedical Sciences, Louisiana State University, Baton Rouge, LA

Background: Lipid rafts are detergent-insoluble micro-domains in the plasma membrane of the cells. Lipid rafts contribute in diversity of cellular process including cell signaling, apoptosis and cellular trafficking during viral and bacterial infections. NOD-like receptors (NLRs) are the cytosolic sensors and membrane recruitment of some NLR family members (NOD1 and NOD2) is important for their response against a wide array of adjuvants. NLRP10 and NLRP12 are the two members of NLR family which play an important role in both inflammasome activation and T cell mediated responses against microbial pathogens. Our hypothesis that lipid rafts play a critical role in regulating NLRP10/NLRP12 mediated responses against cigarette smoke (CS) challenge is novel and will lead to development of new therapeutic strategies.
Methods: Our *in silico* studies using ClusPro 2.0 online docking server showed the possibility of interaction of NLRP10 and NLRP12 with caveolin-1 and flotillin-1 (proteins associated with caveolae and lipid rafts, respectively). We therefore conducted *in vitro* studies using confocal imaging to confirm membrane recruitment of NLRP10 and NLRP12 in CS challenged RAW 264.7 macrophages and THP-1 cells. We used *Vybrant® lipid raft labeling kit* (Life Technologies, NY, USA); anti-NLRP10; anti-NLRP12; and CS condensate (CSC, Murty Pharmaceuticals) to challenge the cells for our studies.
Results: Expression of both NLRP10 and NLRP12 was induced in response to CSC. We also observed CS mediated increase in the expression of caveolin-1 and flotillin-1. Interestingly, NLRP10 and NLRP12 were observed to co-localize with lipid raft entities in CSC challenged cells. However, we were unable to detect any significant interaction between NLRP10 and NLRP12 by confocal imaging. We are therefore conducting immunoprecipitation experiments and proximity ligation assays (PLA) to validate our findings. CS challenge also induced inflammatory responses in RAW264.7 macrophages and THP-1 cells.
Conclusion: Our findings show that lipid rafts regulate NLRP10 and NLRP12 mediated inflammatory responses against CS challenge.
Acknowledgment: This work was supported by LBRN

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B 7.9- Optimal Antibody-Mediated Immunity to Chlamydia Genital Infection Requires Interferon-γ Activation of an Effector Cell Population

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Background: *Chlamydia trachomatis*, an obligate intracellular bacterial pathogen, is responsible for more than 1.4 million new sexually transmitted infections each year in the United States. *Chlamydia muridarum* mouse genital infection replicates many characteristics of human *C. trachomatis* infection and has been used extensively to model protective immunity to genital chlamydia infection. Using the *C. muridarum* model, we previously demonstrated a key role for antibody in immunity to reinfection. Those studies showed that antibody decreased shedding of infectious bacteria 100-1000 fold, and significantly shortened the duration of genital infection. Although the protective effect of antibody is profound, the mechanism of antibody-mediated protection has not yet been fully elucidated. A necessary corollary for antibody-mediated protection is the recruitment and/or activation of an effector cell population to genital tract tissues by $CD4^+$ T cells. Our current studies strive to identify the effector cell and the role of $CD4^+$ T cell activation in antibody-mediated immunity to chlamydial genital infection. Our results provide important insight into immunity to genital chlamydial infection and may help to explain the limited protective immunity afforded by the numerous experimental chlamydial vaccines.
Methods: Because interferon-gamma (IFNγ) is a major activating cytokine secreted by $CD4^+$ T cells, we utilized $IFN\gamma^{-/-}$ mice and T cell depletion methods to determine the effect(s) of IFNγ on the protective antibody response. Flow cytometry was used to determine if the absence of $CD4^+$ T cells affected recruitment of immune cells during primary infection, a time when the genital tract is being "primed." To characterize the inflammatory activation state of the genital tract, we measured transcript levels of inducible nitric oxide synthase (iNOS) and arginase 1 (Arg1) at various times post-infection via qRT-PCR.
Results: The absence of IFNγ does not greatly impact the resolution of primary genital infection, but does markedly diminish antibody-mediated immunity to reinfection. Furthermore, depleting $CD4^+$ T cells does not affect the infiltration of immune cells during primary infection, suggesting that activation rather than recruitment of a cell type is responsible for antibody-mediated protection to reinfection.
Conclusion: Our studies demonstrate an important role for IFNγ in antibody-mediated protection to chlamydia genital tract reinfection. Antibody-mediated protection does not appear to be afforded by recruitment of an effector cell per se, but rather due to $CD4^+$ T cell activation of an effector cell. Current T cell depletion studies aimed at determining the iNOS/Arg1 activation state of the genital tract should help to elucidate the precise mechanism of antibody-mediated protection.
Acknowledgment: This work was supported in part by a grant from NIGMS P20GM103625, UAMS Center for Microbial Pathogenesis and Host Inflammatory Responses, and by funding through the UAMS College of Medicine

B 7.10- "Cigarette Smoke Mediated Regulation of Epigenetic Signatures on *NF-κB* Proximal Promoter Region in Murine Macrophages"

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Introduction: Chronic Obstructive Pulmonary Disease (COPD) is the third leading cause of death in the United States. Active or passive cigarette smoke (CS) exposure is the principal cause of its occurrence. Transcription factor NF-κB plays a key role in regulating inflammatory responses in COPD patients. However, expression of NF-κB can itself be under epigenetic control. We hypothesized that acetylation of lysine-9 of Histone 3 (H3K9ac) at NF-κB proximal promoter region (PPR) is critical for its regulation in response to CS challenge. We also investigated the effect of histone deacetylase (HDAC) inhibitor benzyl Isothiocyanate (BITC) on H3K9ac at NF-κB PPR.
Methods: RAW 264.7 murine macrophages were exposed to CS or DMSO (control) for 24 hours. Following treatment/challenge the cells were harvested, washed with PBS and fixed using formaldehyde. Cells were then lysed using Chromatin Immunoprecipitation (ChIP) Lysis Buffer and sonicated to achieve 700-800 bp. DNA fragments. ChIP was performed using anti-H3K9ac and isolated DNA was eluted, reverse cross-linked and purified using ethanol-chloroform precipitation. Eluted samples were analysed and quantified using Applied Biosystems 7000 Real-Time PCR System at LSU Genomic facility. Primers specific to NF-κB PPR were designed using OligoPerfect™ Designer**.** Expression of NF-κB/p65 protein in CS or DMSO challenged macrophages was further determined with the help of immunoblotting.
Results: We observed increase in H3K9ac at NF-κB PPR flowing CS challenge in murine macrophages. A

considerable increase in NF-κB/p65 protein expression following CS challenge was also found. Furthermore we noticed abrogation of protein levels and CS-induced acetylation at lysine 9 of histone 3 at NF-κB PPR following treatment with BITC in our samples.
Conclusions: Our data suggests that NF-κB induction in response to CS-exposure is possibly regulated by HDACs at epigenetic level by H3K9ac at NF-κB PPR. Future studies will focus on further analyzing our results using ChIP assays using other Histone H3 and H4 modifications on NF-κB gene in response to CS challenge.
Acknowledgments: This work was supported by LBRN Summer Award to SB and GK; Young Clinical Scientist Award (FAMRI- 123253_YCSA_Faculty); NIH R15 (7 R15 ES023151 02) and Southern University Foundation Grant (COSC0016) to SB.

B 7.11- *"Rickettsia Parkeri* Modulates Tick Superoxide Dismutase to Survive Within the Tick Vector"

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Background: The Gulf Coast tick *(Amblyomma maculatum)* is a competent vector of *Rickettsia parkeri*, a pathogen similar to *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever. Tick antioxidant and pathogen relationship was a largely unexplored area in tick research. In this study, we investigated the role of superoxides in physiology and *Rickettsia parkeri* colonization inside of tick tissues. We hypothesized that *R. parkeri* manipulates tick's superoxide dismutases in a manner that is beneficial to the bacteria's survival within the tick vector.
Methods: RNAi-mediated gene silencing approach was used to assess the functional role of superoxide dismutase in *R. parkeri*-infected and uninfected *A. maculatum*. Estimation of transcriptional expressions in tick tissues, determination of overall microbial load and rickettsial load in SODs silenced tick tissues were assessed with qRT-PCR. Superoxide dismutase enzymatic activity and malondialdehyde (MDA) lipid peroxidation were used to assess the redox status.
Results: The mitochondrial and cytoplasmic superoxides complement each other in each SOD knockdown tissues using the dsRNA method. The knockdown of mitochondrial (Mn-SOD) and cytosolic (Cu/Zn-SOD) significantly increased tick oxidative stress in tick midgut tissues. The total native microbial load in naïve tick appeared to florish with Mn-SOD depletion, whereas Cu/Zn-SOD depletion resulted in an increase in the native midgut microbial load, and a decrease in the salivary glands. *R. parkeri*-infected ticks induced the transcriptional gene expression of Cu/Zn-SOD whereas Mn-SOD remained unaffected. Cu/Zn-SOD was then selected for further RNAi analysis in R. parkeri-infected ticks. It was observed that depletion of cytosolic SOD negatively impacted the colonization of *R. parkeri* in both tick tissues.
Conclusion: *R. parkeri* induces superoxide dismutase to survive against oxidative stress in tick tissue from superoxide anions formed as a tick defense against invading pathogens. Furthermore, depletion of Cu/Zn-SOD significantly impaired colonization of *R. parkeri* in tick tissues.

B 7.12- "Inhibition of HSV-1-Associated Ocular Neovascularization by Cyclin-Dependent Kinase Inhibitors"

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Herpes simplex virus type 1 (HSV-1) infects greater than 90% of humans worldwide and during ocular infection produces inflammation and angiogenesis that can lead to blindness. In the United States, HSV infection is the leading cause of infection-induced blindness; nearly 40,000 new cases are reported and 300,000 cases are treated yearly. Cyclin-dependent kinases, mostly known for their involvement in the cell cycle and transcription, are involved in HSV transcription and replication. Cyclin-dependent kinase 9 (CDK9) and its downstream target, serine 2-phosphorylated RNA polymerase II, are essential in HSV-1 transcription and replication however, to date there is little literature on the role of CDKs in HSV infection of the eye or on the efficacy of CDK inhibitors in preventing HSV-1-associated ocular neovascularization and its consequences. We are testing the hypothesis that cyclin-dependent kinase 9 inhibitors (Flavopiridol, FP and 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole, DRB) decrease angiogenesis and clinical pathology associated with ocular HSV-1 infection. To date, we have demonstrated that these inhibitors decrease vascular endothelial cell migration, invasion, tubule formation *in vitro* and angiogenic factor-induced angiogenesis in chick embryo and mouse Matrigel angiogenesis models. We have further determined that FP and DRB are non-toxic in rabbit and mouse eyes, and both drugs decrease mouse corneal neovascularization, clinical severity and neutrophil invasion due to HSV-1 infection to levels observed in mice treated with an anti-herpetic control drug, trifluorothymidine. Additionally, FP and DRB also reduce HSV-1 replication in culture and in the eye.

B 7.13- "Rhinovirus Infection Induces Th2-Promoting Innate Cytokines in an *Ex Vivo* Precision Cut Lung Slice Model"

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Introduction: Rhinovirus (RV) infection is associated with asthma exacerbations, but little is known about cellular response to virus leading to exacerbations. We hypothesized that RV39 infection of airways in precision cut lung slices (PCLS) from asthma subjects would induce a pro-allergic cytokine signature, including IL-25, IL-33, and TSLP.
Study Design/Methods: PCLS from subjects with (n=3) and without (n=5) reported asthma were prepared from cadaver lungs and cultured ex vivo. Explants were infected with RV39 at 300$TCID_{50}$/slice, and viral loads were measured using qPCR. mRNA levels for IFN-l, IL-15, IL-33, IL-25, TSLP, and IL-13 were measured over time. Expression of mRNA was normalized to uninfected airways from the same subject and b-actin in both uninfected and infected tissue. Results are expressed as means and standard deviations of these values (DDCT).
Results: qPCR for RV39 confirmed active infection of PCLS at 24 hours (53,330±70,491 virions/mL cDNA). At 24 hours, mRNA expression of IL-15, IL-33, and IFN-l were similarly induced in asthmatics and controls. IL-25 (asthma 3.8±3.78; control -2.4±5.08), TSLP (asthma 4.2 ±3.29; control -2.1±3.69), and IL-13 (asthma 3.8±4.16; control -3.7±4.06) were induced only in PCLS from asthmatics and suppressed in controls.
Conclusions: In PCLS airways from subjects with reported asthma, RV39 infection enhanced mRNA expression of IL-25, TSLP, and IL-13 along with cytokines typically required for innate immune defense against virus. We suggest that IL-25 and TSLP increase IL-13 expression by mast cells, innate lymphoid type 2 cells, and/or T cells in PCLS airways of asthma subjects.
Acknowledgement: This work was funded by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM103625.

B 7.14- "The Contribution of Pyruvate Oxidase to Pneumolysin Release in *Streptococcus pneumonia"*

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Background: *Streptococcus pneumoniae* (pneumococcus) is an important human pathogen causing infections such as pneumonia, meningitis, and otitis media, affecting primarily young children and the elderly worldwide. Pneumococcus produces a pore-forming cytotoxin, pneumolysin (PLY), which is one of its key virulence factors. Despite being a catalase negative organism, the pneumococcus produces up to millimolar concentrations of hydrogen peroxide through the activity of the enzyme pyruvate oxidase (SpxB). SpxB is considered a virulence factor of the organism, as mutants lacking the gene display attenuated virulence *in vivo*. The goal of this study is to investigate the correlation between PLY release and the activity of SpxB.
Methods: We used a colorimetric hydrogen peroxide assay to quantitate the amount of H_2O_2 produced by strains T4R, WU2, AW267, and T4 and isogenic mutants lacking *spxB* (ΔSpxB) at a mid-log phase of growth. ΔSpxB mutants were complemented using a pNE-1 pneumococcal shuttle vector. This analysis was also performed on a panel of 15 clinical isolates along with western blot to determine the relative amount peroxide produced and PLY released between wild-type and ΔSpxB mutants. Dot blotting was used to quantitate PLY in the supernatants of clinical isolates at a mid-log phase of growth. A549 human epithelial cells were exposed to sterile supernatants of wild type, ΔSpxB mutants, and a complemented Δ*spxB* mutant. The cells were assessed for loss of viability by propidium iodide staining quantitated using flow cytometry. A hemolytic assay also quantitated PLY release.
Results: When exposed to sterile wild-type supernatant, a significant loss of cell viability was seen in A549 cells, whereas ΔSpxB supernatant failed to affect viability. Supernatant of the complemented mutant recapitulated the loss of viability observed with the wild-type. Deletion of *spxB* almost entirely eliminated H_2O_2 production and complementation restored H_2O_2 production. We observed a significant reduction in the amount of PLY in the supernatant observed by western blot upon deletion of *spxB* in AW267, WU2, and T4 ($p<0.005$), as well as a significant reduction in PLY release in T4R ($p<0.05$). Furthermore, we observed a significant reduction in the amount of PLY released when WU2 was treated with 10µg of exogenous catalase ($p<0.05$). A significant correlation was observed between H_2O_2 production and PLY released in a panel of clinical isolates ($p<0.05$, $r^2=0.3167$). Finally, complementation of *spxB* to T4RΔ*spxB* and T4Δ*spxB* yielded a significant increase in PLY released into the supernatant versus the mutant alone ($p<0.005$ and $p<0.0005$, respectively).
Conclusion: Based on our findings, an apparent connection exists between the activity of SpxB and the ability of the pneumococcus to release PLY into the extracellular space. We demonstrated that loss of

SpxB reduces PLY release. Furthermore, complementation of the gene restores PLY release. SpxB-induced PLY release correlates with the increased cell toxicity. We hypothesize that this release could be related to the production of H_2O_2 alone, but could also be related to other byproducts of SpxB, such as acetate. It is possible that these products affect membrane potential. Additionally, *sxpB* utilization could be a niche-oriented method of modulating PLY release depending on the oxygen availability of infection site. Finally, it is possible that this mechanism could be generalized for other cryptically secreted proteins in the pneumococcus.
Acknowledgment: The lab (of J.A.T.) is supported by an Institutional Development Award (IDeA) from the NIGMS COBRE grant number (P20GM103646)

B 7.15- "Role of Ttick Antioxidants in *Rickettsia parkeri* Colonization in the Gulf-Coast tick (*Amblyomma maculatum)"

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Background:The Gulf Coast tick (*Amblyomma maculat um*) is a competent vector of *Rickettsia parkeri*, a Spotted Fever Group Rickettsial pathogen of public health significance. The understanding of We tested the hypothesis that *R. parkeri* infection induces the expression of tick's antioxidant and immune systems in order to survive and propagate within the tick before saliva-assisted transmission to the vertebrate host.
Methods: In this study, *R. parkeri* infected, and uninfected *A. maculatum* colonies were maintained to study tick-pathogen interactions. To test our hypothesis a RNA-Seq approach was utilized to determine the differentially regulated gene expression upon *R. parkeri* infection in *A. maculatum* salivary glands. The reads were mapped using *A. maculatum* reference sialotranscriptome (Karim et al., 2011). The expression of tick antioxidant, and immunity related genes were validated by qRT-PCR. RNA interference based approach was used to elucidate the functional role of differentially expressed tick genes in *R. parkeri* colonization within the tick host.
Results: RNA-Seq analysis of the sialomes of uninfected and *R. parkeri* infected salivary glands revealed more than 15,000 CDS. There were 990 transcripts that are 10X overexpressed. Of these, 542 are salivary proteins and 143 are lipocalins, on average 1000 fold overexpressed, while 67 are protease inhibitors, on average 2853X overexpressed. The overexpressed transcripts include several detox/oxidant and immunity related proteins. The expressed of tick's antioxidant machinery, and immunity related genes were validated using a qRT-PCR assay. The RNAi assay showed the silencing of antioxidant target genes significantly decreased the *R. parkeri* infection level in tick tissues.
Conclusions: Our results provide an insight into tick-pathogen interaction and the functional role of reactive oxygen species in vector competence.

B 7.16- "Improved Immune Function and Reduced Chlamydia Genital Infection in a Murine Stress Model Fed with Active Hexose Correlated Compound"

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Background: Previous results in our lab showed that cold-induced stress results in suppression of the immune response and increased intensity of chlamydia genital infection in a mouse model. In this study, we evaluated the potential therapeutic value of active hexose correlated compound (AHCC) against chlamydia genital infection in stressed mice. AHCC is an extract of mushroom commonly used as a dietary supplement known to boost the immune system.
Methods: Mice were infected intravaginally with *Chlamydia trachomatis* after a 24-day cold-stress application. Oral administration of AHCC to stressed or non-stressed mice was carried out seven days before infection and during the course of infection along with cervicovaginal swabbing. Splenic T cells from both animal groups were co-cultured with mouse monocyte J774.2 cell line or cultured by addition of supernatants of AHCC-treated J774.2 cell line for 24 hours. Cytokine production by peritoneal and splenic T cells isolated from mice was measured by ELISA.
Results: Levels of tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6) were significantly increased in stressed mice receiving AHCC compared to stressed mice receiving PBS. Production of interferon gamma (IFN-γ) and interleukin 2 (IL-2) in the AHCC group was significantly high compared to production in PBS-fed group. Splenic T cells from stressed and non-stressed cultured with culture supernatants of AHCC-treated J774.2 cell line resulted in significantly increased TNF-a or IFN-γ production.
Conclusion: Results obtained in this study show that AHCC improves the function of immune cells as indicated by the restoration of levels of cytokines production after AHCC feeding. This is the first report showing that oral administration of AHCC restores the function of the immune system, which could result in increased resistance of the host to chlamydia genital infection.
Acknowledgement: This work was funded by the Center for Natural Product Research (CNPR) Program of WV-INBRE of NIH Grant 5P20RR016477.

B 7.17- "Tracking Antigen-Specific CD4 T Cell Responses to *Chlamydia* Female Reproductive Tract Infection"

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Background: Sexually transmitted *Chlamydia* infections are increasingly prevalent in the US and a major cause of infertility in young women. To date, no *Chlamydia* vaccine is available. Greater understanding of the adaptive immune response to *Chlamydia* female reproductive tract (FRT) infection will be required if an effective vaccine is to be developed.
Methods: We generated novel tools called *Chlamydia*-specific MHC class II tetramers, which for the first time allow direct visualization of the endogenous, antigen-specific CD4 T cell response to *Chlamydia muridarum* FRT infection. Using these tetramers, we quantified and characterized the endogenous CD4 T cell response in wild type and immunocompromised mice.
Results: *Chlamydia*-specific CD4 T cells expanded rapidly and persisted as a stable memory pool for months after systemic infection. The majority of expanded *Chlamydia*-specific CD4 T cells exhibited a Th1 phenotype and produced IFN-γ, TNFα and IL-2, While most lymph node *Chlamydia*-specific CD4 T cells expressed Th1-specific transcription factor T-bet, a small percentage co-expressed regulatory T cell marker Foxp3, and RORγt-expressing Th17 cells were enriched within the FRT.
Conclusion: This study shows that *Chlamydia* infection induces a heterogeneous host CD4 T cell response that persists in vivo and develops into long-term immunological memory.
Acknowledgment: This work was funded by NIAID Grant R21AI117303 and by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM103625.

B 7.18- "Effect of Parasitism on the Distribution of Serotonin in the Nervous Tissues of *Biomphalaria alexandrina*, an Intermediate Host for Intestinal Schistosomiasis"

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Background: Schistosomiasis, or snail fever, is estimated to affect more than 200 million people worldwide. The digenetic trematode worm *Schistosoma mansoni* that causes schistosomiasis employs the freshwater snail genus *Biomphalaria* as its primary intermediate host. It has been proposed that the transition from the free-living *S. mansoni* miracidium to parasitic mother sporocyst depends upon uptake of biogenic amines, e.g. serotonin (5HT), from the snail host. However, little is known about potential sources of serotonin in *Biomphalaria alexandrina* tissues and its potential changes during the course of infection.
Results: Wholemount immunohistochemistry and confocal microscopy were used to localize serotonin-like immunoreactivity (5HTli) in the nervous system of *B. alexandrina* and to examine 5HTli at critical points of the host-parasite interaction. 5HTli fibers were observed innervating the cephalopedal integument, the major site of *S. mansoni* miracidium penetration and transformation. However, no peripheral 5HTli neurons were detected. Clusters of 5HTli neurons were observed in the cerebral, pedal, left parietal, left pleural and visceral ganglia, suggesting that the peripheral serotonergic fibers originate from the CNS (see also Delgado et al. 2012). Specimens infected with *S. mansoni* were examined at 10 days post infection (10 dpi) and during their shedding stage. The total number of central 5HTli neurons decreased from 162.2 ± 40.0 (n = 5) under control conditions to 118.8 ± 11.9 10 dpi and 130.4 ± 6.7 at the shedding stage (one-way ANOVA, $p < 0.05$). Reductions of 5HTli were most evident in the pedal ganglion (control: 33.6 ± 8.8; 10 dpi: 18.0 ± 3.7; shedding: 25.6 ± 5; $p < 0.05$) and the left pleural ganglion (control: 3.2 ± 2.8; 10 dpi: 0 ± 0, 10; shedding: 0 ± 0; $p < 0.05$).
Conclusion: The changes in 5HTli observed following infection by *S. mansoni* indicate that reductions in serotonin levels can occur in specific central neurons in parasitized snails and that these changes might contribute to the modifications in several behaviors that are observed during the course of infection.
Acknowledgment: This work was funded by the National Institutes of Health (USA): RCMI MD007600, NIGMS MBRS: GM-087200; National Science Foundation (USA): DBI-0115825, DBI-0932955, and HRD-1137725; Natural Sciences and Research Council (Canada): Discovery Grant 38863); Ministry of High Education and Scientific Research (Egypt): Partnership and Ownership Initiative (ParOwn 0911).

B 7.19- *"msaA* and *msaC* Plays Role in MsaB Production to Regulates Virulence and Biofilm Development in *Staphylococcus aureus"*

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Backgrounds: *Staphylococcus aureus* causes a wide range of acute and chronic infections including severe invasive biofilm-associated infections in humans. Several global regulators control virulence of these stains. Yet, we do not fully understand the regulation of virulence factors involved in staphylococcal

infections. The *msaABCR* is a four-gene operon that regulates biofilm development, antibiotic resistance and virulence. MsaB is the only protein that is translated from this operon, while the other genes are non-coding. In this study, we investigate the role of 5'-*msaA* and 3'- *msaC* regions of the *msaABCR* operon in the regulation of MsaB production and biofilm developemnt.
Methods: We constructed a series of truncated *msaABCR* operon constructs (TC-1 to TC-12) from both the 5' *msaA* end and the 3' *msaC* end to study the role of *msaA* and *msaC* transcript in the regulation of MsaB production, proteases production, and biofilm development. We also performed mutagenesis experiments to study the interaction between 5' end and 3' ends of the *msaABCR* operon transcript and their role in the stability of the operon transcript.
Results: Full *msaABCR* operon transcript with its complete intergenic region complemented to the wild type level in terms of pigmentation, protease production, biofilm development, and MsaB production. Two constructs, TC-5 and TC-9, complement the *msaABCR* deletion mutant and result in overexpression of MsaB. The constructs TC-1, TC-2, TC-3 and TC-4 did not complement the *msaABCR* deletion mutant and did not produce MsaB. Interestingly, TC-3 and TC-4 complemented biofilm formation suggesting a role for the 3' end in biofilm formation that does not require MsaB. These result also suggest that the 5' *msaA* end and the 3' *msaC* end of the transcript interact and play a role in the production of MsaB and biofilm development.
Conclusion: This study defines the regulatory functions of the *msaA* and *msaC* transcript of the *msaABCR* operon in the production of MsaB and Biofilm development. This study will allow us to eventually identify the environmental and/or host stimuli that control the functions of the *msaABCR* operon.

B 7.20- "Regulation of Respiratory Paramyxovirus-induced Disease by Neutrophils"

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Background: Innate immune cells play a pivotal role in shaping antiviral immune response in the respiratory tract, as they represent a link between innate and adaptive immune response. They are critical for activation of innate immune response and defining disease phenotype during human Metapneumovirus infection.
Methods: In this work, we use a well-established experimental mouse model of paramyxovirus infection to define the role of innate immune cells in the respiratory tract. In brief, BALB/c mice were infected intratrnasally with human Metapneumovirus. Body weight loss, illness score, cellular influx into lungs, cytokine profiling of bronchoalveolar lavage fluid (BALF), and viral PFUs in lung were quantified.
Results: Changes in specific immune cell compartment augmented disease severity and enhances body weight loss in response to hMPV infection. Variation in the recruitment profile of immune cells and BALF cytokine production was observed.
Conclusion: Understanding the aspects of the innate immune cell response that are activated in the respiratory tract upon hMPV, is clinically relevant for a better understanding of the pathogenesis of this respiratory paramyxovirus infection.
Acknowledgement: This work was supported by grants from the National Center for Research Resources (P20RR020159-09) (to A.G-P.) and the National Institute of General Medical Sciences (P20GM103458-09) (to A.G-P.) from the National Institutes of Health.

B 7.21- "Bile Induced Membrane Damage Varies Dependent Upon Oxygen Availability in *Listeria monocytogenes"*

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Background: *Listeria monocytogenes* is a Gram positive, facultative intracellular organism responsible for the foodborne disease listeriosis. To cause disease in humans, *L. monocytogenes* must survive a variety of stressors encountered within the gastrointestinal (GI) tract, including variations in pH, oxygen availability, and bile. Though it is known that the oxidative stress response is expressed following exposure to bile under aerobic conditions, little is known about the response under physiologically relevant anaerobic conditions. The hypothesis for this project was bile-induced damage differs based on oxygen availability.
Methods: *Listeria monocytogenes* strains F2365 (serovar 4b), 10403S (serovar 1/2a), and HCC23 (serovar 4a) were treated with either 0% or 5% porcine bile extract under aerobic or anaerobic conditions. Changes in cell envelope structure were measured by transmission electron microscopy and alterations in membrane lipids were examined by gas chromatography. Redox state of the bacteria was assessed by measuring the intracellular NADH:NAD+.
Results: The average size of all strains decreased following bile exposure, though this difference was greater under aerobic conditions in comparison to anaerobic conditions. However, the thickness of the cell envelope increased for 10403S and F2365 under anaerobic conditions. The fatty acid profiles of cell membranes were altered following exposure to bile: saturated fatty acids palmitic acid and stearic acid and

unsaturated fatty acids linoleic acid and oleic acid increased following exposure. The content of branched chain fatty acids decreased when exposed to bile. These results suggest that modifications to the cell membrane in the proportion of palmitic acid, stearic acid, linoleic acid and oleic acid impacts bile resistance. As bile has previously been found to induce oxidative damage, *L. monocytogenes* strains were cultivated in the presence of the antioxidant esculetin; survival was not improved in the presence of bile, suggesting that bile does not induce oxidative damage. Therefore, to determine if reductive stress could be a possible cause of damage under anaerobic conditions in *L. monocytogenes*, the NADH:NAD+ ratios were analyzed in the presence of bile. Results indicated that ratios of NADH:NAD+ shift under anaerobic conditions, but the effect is limited to bile sensitive strains of *L. monocytogenes.*
Conclusion: These results suggest that the response to bile induced membrane damage varies between strains of *Listeria* and the response is dependent upon alterations at the cell membrane. Alterations to the cell membrane through incorporation of exogenous lipids improve bile survival only under aerobic conditions. Additional research is needed to determine the mechanism by *L. monocytogenes* is resistant to bile induced membrane damage under anaerobic conditions.
Acknowledgement: This work was funded by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM103646.

B 7.22- *"Mycobacterium Tuberculosis* Cell Wall Fractions Induce Inflammatory Cytokines in Primary Mouse Macrophages and Fibroblasts"
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Background: *Mycobacterium tuberculosis* (*Mtb*) infects one third of the world's population resulting in either a latent or active disease. *Mtb* infects and grows within host macrophages, resulting in the recruitment of host immune cells via cytokines and chemokines to form a granuloma. A granuloma is a collection of immune cells that surround and block off an infected area with in a tissue which are formed in order to prevent the spread of the infection and to eradicate the infection. While in the granuloma, some *Mtb* will be degraded within the phagosome. Components of *Mtb*, along with live *Mtb* may be expelled from the host in aerosol form. These fractions of *Mtb* are likely to interact with the host respiratory tract and induce an inflammatory response.
Methods: Here, we test the hypothesis that cell wall fractions taken from *Mtb,* will induce an inflammatory response when exposed to primary mouse macrophages and fibroblasts. Cell wall fractions were derived from the hyper virulent strain HN878 or the lab adapted strain H37Rv. Enzyme Linked Immunosorbent Assays (ELISA) were used to detect the production of inflammatory cytokines from treated cells. A multiplex assay was used to detect the presence of multiple inflammatory cytokines and chemokines in fibroblast samples.
Results: We found that the production of IL-1β in macrophages and in fibroblast is dependent on the strain of *Mtb*. The hyper virulent *Mtb* strain induces a more potent IL-1β response than the lab adapted *Mtb* strain which agrees with what has been published by Gopal et al., 2013, with whole cell lysates.
Conclusions: These findings not only support our hypothesis but also offer novel opportunities for vaccine development against *Mtb* by allowing us to target the delivery of *Mtb* cell wall fractions as vaccine components.

B 7.23- "Molecular Characterization of Glycine Rich Proteins in *Amblyomma americanum*, the Lone Star Tick"
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Introduction: The feeding habits of ticks require extended attachment and extended interaction with the host's immune system requiring the tick to shield itself by modulating the host immune response with the use of multiple families of pharmacologically active compounds. Glycine-rich proteins are secreted from the salivary glands into the host where they may have multiple functions including: attachment, anti-hemostasis, anti-inflammatory, or anti-microbial.
Methods: RNA-seq data was used to select GRPs based on differential expression. Expression was validated using qRT-PCR throughout the bloodmeal. RNA interference was used to determine the function of a single GRP and compensatory mechanisms. SEM and FT-IR were used to determine detailed information regarding cement cone structure and composition.
Results: Transcriptome data revealed nine GRPs differentially expressed throughout the bloodmeal validated by qRT-PCR. No phenotypic change was measurable after RNAi indicating a complex compensatory mechanism system. SEM and FT-IR of the cement cone show the outer surface detail and composition. Data from proteomic analysis of the cement cone will demonstrate the technical difficulties and complexity of these cement cones.
Conclusion: RNAi of a GRP yielded no change in phenotype revealing the intricate network of compensatory mechanisms found in the redundant tick genome. Identification of GRPs which are involved in cement cone formation can be further studied as possible targets for control.

B 7.24- "Administrative Core Functions at the Mississippi State University COBRE"

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Background: The MSU COBRE focus is infectious diseases, and a unifying sub-theme is high throughput data analysis methods such as proteomics, genomics, and bioinformatics. Therefore, collaboration with MSU's High Performance Computing Collaboratory (HPCC) was a critical element of our COBRE. As our COBRE application was being submitted, a new Institute was developed at MSU: the Institute for Genomics, Biotechnology, and Biocomputing (IGBB). This Institute was designed to be the mechanism by which biological researchers at MSU access the Supercomputing capacity here which has consistently been within the top 100 facilities at academic institutions in the U.S. for many years.
Methods: To insure that the existing MSU research cores most relevant to our COBRE benefitted from COBRE work, we partnered with the two most relevant cores to insure they were enhanced by the funding provided by COBRE to our junior investigators who needed these services. This was accomplished by including Dr. Mark Lawrence and Dr Giselle Thibaudeau in the MSU COBRE Leadership Group. Dr. Lawrence is one of the supervisors of the IGBB and Dr. Thibaudeau is the Director of the Institute for Imaging and Analytical Technologies (I^2AT). Therefore, both of these COBRE Leaders have a combined interest in the success of the COBRE and in the success of two institutes that provide key COBRE services (IGBB and I^2AT). In addition, the MSU COBRE has both enhanced and benefitted from training opportunities provided by the MSU Office of Research and Economic Development, such as sponsoring a visit and seminar by the Director of Editorial Services for the American Society of Physiology and encouraging attendance of COBRE investigators at grant writing sessions sponsored by MSU.
Results: All investigators in the MSU COBRE use both IGBB and I2AT in their projects. This enhances their research and also provides ongoing support to institutes that are important to all biomedical researchers at MSU. For example, flow cytometry and high speed cell sorting are techniques provided by COBRE Core C (Cellular Isolation and Analysis), which are supervised by Dr. Thibaudeau as part of the COBRE and I^2AT, but which are also available to other MSU investigators. Similarly, the Luminex multiplexed bead array analysis of protein concentrations is housed within COBRE Core C but is available to non-COBRE investigators as well. Methodologies available through IGBB include RNA sequencing (by next generation methods), proteomics, and bioinformatics analysis expertise. For example, one of our COBRE investigators (Dr. Nanduri) is highly sought by other investigators at MSU and at other institutions in Mississippi for bioinformatics expertise. Dr. Peterson (Director of IGBB) is seeking to enhance our capability in this area, because many investigators are now seeking expertise in analysis of high throughput analysis.
Conclusion: The MSU COBRE leverages existing resources by providing support to two existing institutes and by encouraging those institutes to develop in a way that will enhance long-term biomedical research at MSU.
Acknowledgment: This work was funded by COBRE Grant #P20GM103646 from the National Institute of General Medical Sciences.

B 7.25- "Investigating the Role of Mouse $Sca1^+$ Lung Mesenchymal Stem Cells in Bacterial Pneumonia"

Tirumalai Rangasamy, Shanshan Cai, Sagar Paudel, Laxman I. Ghimire, Samithamby Jeyaseelan
Pathobiological Sciences, Louisiana State University, Baton Rouge, LA

Background: *Klebsiella pneumoniae* is the most common pathogen isolated in intensive care units. Increasing antimicrobial drug resistance, including carbapenem-resistant *K. pneumoniae* (CRKP), accounts for substantial mortality. However, limited antimicrobial options or vaccines available to control infections caused by CRKP. The goal of this study was to determine the antibacterial effects of mouse $Sca1^+$ lung mesenchymal stem cells ($Sca1^+$ LMSC) using *K. pneumoniae*.
Methods: We have sorted the $Sca1^+$ cells from the lung digests of C57BL/6 mice using fluorescence activated cell sorter and analyzed the expression of different cell surface markers using FACScanto II flow cytometer. We determined the colony forming units, differentiation capacity, and inflammatory cytokine secretion of $Sca1^+$ lung cells. Furthermore, we have investigated the antibacterial effects of $Sca1^+$ lung mesenchymal stem cells using the Gram-negative bacterium, *K. pneumoniae.*
Results: Flow sorted $Sca1^+$ lung cells ($Sca1^+$ LC) constituted approximately 20% of the total lung cell population. Flow cytometric analysis revealed the higher level expression of Sca1, different mesenchymal markers (CD44, CD73, CD105, and CD123), as well as CD34, CD54 (CAM1), and TLR4 antigens on the surface of $Sca1^+$ LC. The $Sca1^+$ LC formed colonies and differentiated into osteogenic and adipogenic lineages *in vitro*, suggesting that they are mesenchymal stem/stromal cells. Treatment with the bacterial lipopolysaccharide (LPS, 500 ng/million cells/ml) significantly induced the secretion of IL-6 in $Sca1^+$ LMSC at 24 h. In addition, $Sca1^+$ LMSC significantly inhibited the LPS-induced secretion of

TNFα in bone marrow derived macrophages and neutrophils. Intriguingly, treatment with Sca1+ LMSC [at both low (2X10 5/ml) and high (4X10 5/ml) cell numbers] remarkably inhibited the growth of *K. pneumoniae* than human bone marrow derived MSC (hB-MSC) at 6 h. However, culture supernatants from Sca1+ LMSC showed only mild inhibitory effect on the growth of *K. pneumoniae.*
Conclusions: This study shows that Sca1+ LMSC is potent in inhibiting the growth of *K. pneumoniae* than hB-MSC. Future studies are needed to determine the therapeutic potential of Sca1+ LMSC in a preclinical (mouse) model of *K. pneumoniae* pneumonia.
Acknowledgment: This work was funded by NIH grants 2R01 HL-091958 (to S. J.) and 1R01AI113720-01A1 (to S. J.) and a NIH pilot grant from COBRE P30 GM110760-02 (to T. R.)

B 7.26- "Elucidating the Functional Role of the H_2O_2-Generating Dual Oxidase (Duox) in the Gulf Coast Tick, *Amblyomma maculatum*"

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Background: In the absence of effective targeted control, the increased transmission of disease-causing microbes by the tick vector is of critical concern. At present, little is known about the relationship of tick molecules and their means of controlling their native microbiota on tissue level. Looking to literature of hematophagous arthropods, dual oxidase (Duox) has been implicated to be involved in the defense against pathogen invasion of epithelial tissues facing an external environment through H_2O_2 generation and formation of dityrosine crosslinks between extracellular proteins, such as within midgut lumen.
Methods: For evaluation in the *Rickettsia parkeri* vector, *Ambylomma maculatum*, an RNA interference approach was used to assess the role of Duox in tick blood feeding and modulation of associated total microbiota. Duox-specific dsRNA was injected into female *A. maculatum* and blood fed on sheep. Samples were collected, dissected, and gene expression and total bacterial load was determined using qRT-PCR. Confocal imaging targeting dityrosine was performed to demonstrate Duox role in formation of extracellular dityrosine luminal barriers in target tissues.
Results: Physical weakening in cuticle integrity of partially-fed ds*Duox* ticks was observed when removed, resulting in tearing of the ticks and large sample loss. Replete ticks showed significant increase in engorgement weight compared to controls. Additionally, replete ds*Duox* ticks developed a fungal infection that spread during ovipositioning, affecting both tick and eggs. ds*Duox* injection wound sites also showed an impairment to heal compared to controls. Repeated experiments produced similar results; qRT-PCR data was compared in a gene study with actin used as reference. *Duox* knockdown showed increase in total bacterial load of dissected midgut, salivary gland, and ovaries. Compensatory mechanisms of antioxidant and selenoprotein expression were determined. Confocal imaging confirmed a decrease of dityrosine crosslinks within ds*Duox* cuticle compared to control.
Conclusion: H_2O_2 produced by dual oxidase appears to have a role in the formation of dityrosine crosslinks that strengthen then growing cuticle during tick engorgement as well as a potential role in microbiome homeostasis while blood feeding. Additionally, *Duox* appears to be critical to the maintenance of wound healing and antifungal immunity by ticks during blood meal and preventing susceptibility while ovipositioning.

B 7.27- "Characterization of Host Receptor Profiling and Viral Mutations Determining Influenza Virus Host Tropisms"

Xiu-Feng Wan, Chun-Kai Yang, Minhui Guan, Feng Wen, Lei Zhong
Influenza Systems Biology Laboratory, Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS

Influenza A viruses (IAVs) have been responsible for large losses of life around the world and continue to present a great public health challenge. These **viruses can cause infections in birds, sea mammals, lower mammals (e.g., pigs, dogs, and horses), and ultimately humans.** The birds of wetlands and aquatic environments constitute the major natural reservoirs of IAVs. Avian influenza viruses can infect humans directly or through viral adaptation in pigs. Having both avian-like receptors (α 2,3-linked sialic acid, SA2,3Gal) and human-like receptors (α 2,6-linked sialic acid, SA2,6Gal), the pig serves as an intermediate host "mixing vessel" for generating human-infective influenza pandemic strains. The structures of the carbohydrate receptors were shown to determine influenza host and tissue tropisms; however, the distribution and composition of the endogenous receptors are not well characterized, and the distribution of such structures within and across the host species is not fully understood. The objectives of this study are to characterize and compare the N-linked glycan profiles in chicken and swine trachea, to identify the N-linked glycans responsible for influenza infections, and to characterize mutation patterns determining host receptor binding. Three H1N1 isolates were used, and they were originated from avian, swine, and human, respectively. Through LTQ-Orbitrap mass spectrometry characterization, a total of 56 N-linked glycans were identified in chicken trachea while 150 N-linked glycans in swine trachea by matching with those from glycan databases. A novel pull-down

technique was then developed and applied to determine glycans bound by hemagglutinin of these three H1N1 viruses. Results showed that hemagglutinin from human, swine and avian H1N1 bound to the chicken trachea derived glycans with terminal structures of α2-3 linked sialylated glycan, N-Acetylglucosamine and of galactose on chicken. Avian origin H1N1 prefers to bind the glycans extracted from swine lower trachea than those from swine upper trachea. The bindings between these glycans and IAVs were further validated through solid phase binding assays using synthetic glycans and the whole viral particles. In summary, these results suggest that conserved glcyans be bound by hemagglutinin proteins of those IAVs. Additional experiments will be needed to assess the roles of these glycans in viral infections.

Neuroscience,
Poster Session B
Friday, September 13, 10:45 a.m.-12:15 p.m.

B 8.1- "Developmental Lead (Pb^{+2}) Disrupts Transcriptional Response of Ecdysone-Responsive Genes in the Fruit Fly"

Zoé Márquez, Wilfredo Meléndez, Ángel Rivera Collazo, José Agosto Rivera, Humberto Ortiz-Zuazaga, Adrinel Vázquez-Montes
Universidad del Turabo, Ana G. Méndez System, Gurabo, PR

Background: Metal contaminants such as lead (Pb^{+2}) have been implicated in the etiology of neurodevelopmental disorders showing social dysfunction, such as autism. Hormone-dependent signaling has been proposed as a plausible mechanism of metal-mediated impairment of social behavior. Reports have shown that developmental exposure of *D. melanogaster* to Pb^{+2} retards growth rate and interferes with hormone-dependent behavioral processes such as courtship and locomotion in the adult fly. It is unknown if Pb^{+2}-associated social behavioral disturbances are mediated by hormone-dependent pathways. Data from our research team have shown that developmental exposure of *D. melanogaster* to Pb^{+2} results in global gene expression changes in the larval brain and social behavior impairments later in adult flies. In this study, we present data showing that developmental exposure to Pb^{+2} disrupts developmental transcriptional activation of ecdysone-responsive genes, suggesting hormone-dependent mechanisms mediating Pb^{+2}-induced social impairment in the adult.
Methods: Flies were exposed during development (but not in the adult stage) to control or Pb^{+2}-contaminated corn media. Whole tissue was collected through developmental stages covering two major ecdysone releases regulating metamorphosis processes: third instar larvae, early, middle, and late pupae. Adult flies, which were developmentally exposed to Pb^{+2}, were included to identify dose and sex dependent delay effects. Total RNA was extracted and used to detect mRNA expression of EcR and EcR-responsive genes using qRTPCR and Taqman gene expression assays.
Results: Control flies show two distinct peaks of EiP78C: during early pupae and middle pupae, occurring immediately after theorethical ecdysone releases. Pb^{+2} resulted in sex, dose, and developmental-specific transcriptional disturbances of EcR-responsive early-late gene EiP78C. Pb^{+2}-mediated induction of EiP78C was five fold higher in 500µM exposed larvae as compared with control and other developmental stages, followed by Pb^{+2}–associated suppression during early pupae; suggesting a susceptible developmental time window of Pb^{+2} toxicity occurring at the beginning of the metamorphosis. Noteworthy, EiP78C was significantly induced in adult females, which were developmentally exposed to 100µM Pb^{+2} concentrations as compared with pupae and males, which might be related to delay transcriptional responses in recently emerged flies. Ongoing experiments include the E75C early and FTZ-F1 late genes, belonging to the EcR-dependent signaling pathway.
Conclusion: Pb^{+2} results in transcriptional disturbances of EcR-responsive gene expression during development of the fruit fly, suggesting hormone-dependent signaling pathways mediating Pb^{+2}-induced developmental toxicity. Our model will give insights into the possible contribution of metal contaminants in the etiology of human neurodevelopmental disorders showing social dysfunction.
Acknowledgment: This work was funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20 GM103475.

B 8.2- "Intracellular Mechanisms Modulating Gamma Band Activity in the Pedunculopontine Nucleus"

B. Luster[1], F.J. Urbano[2], E. Garcia-Rill[1]
[1]Center for Translational Neuroscience, University of Arkansas for Medical Sciences, Little Rock, AR
[2]IFIBYNE-CONICET, University of Buenos Aires, Argentina

Background: The Pedunculopontine nucleus (PPN) is a component of the reticular activating system, and is most active during both waking and REM (rapid eye movement) sleep. During waking, our cognitive function is driven by high frequency beta/gamma band activity. However, REM sleep manifests similar high frequency on the cortical EEG. We are interested in determining the differences between gamma activity in waking vs REM sleep. We showed that every cell in the PPN plateaus at beta/gamma band frequencies when depolarized. Moreover, this high frequency activity is mediated by high threshold, voltage-dependent N- and P/Q-type calcium channels. We discovered that the PPN contains cell populations which can manifest gamma band frequencies through only N-type, only P/Q-type, or both N- and P/Q-type calcium channels. Other studies suggest that N-type calcium channels are modulated by the cAMP/PK pathway, which also modulates REM sleep. This study was designed to determine the intracellular mechanisms subserving cells with N-type calcium channels in the PPN.
Methods: Electrical responses were recorded using whole cell patch clamp electrodes on 11-17 day old sagittal rat brain slices. Intrinsic membrane properties

of cells were recorded at 37°C perfused with oxygenated aCSF in an immersion chamber containing the synaptic blockers (SB) gabazine ($GABA_A$ antagonist), strychnine (glycine antagonist), CNQX (AMPA/Kainate receptor antagonist), and APV (NMDA receptor antagonist), and also Tetrodotoxin (TTX) to block sodium channels.
Results: We found that all rat PPN cells (n=13) showed beta/gamma oscillations in the presence of SB+TTX when the membrane potential was depolarized using current ramps. PPN neurons showed beta/gamma oscillations when depolarized above -30 mV, suggesting that their origin may be spatially located beyond voltage-clamp control. In a group of cells tested
(n=7), the cAMP/PK inhibitor H-89 along with the P/Q-type calcium channel blocker **ω**-Agatoxin-IVA combined to inhibit the presence of gamma oscillations (assumed to be N+P/Q cells) ($df=13$, $F=7.04$, $p<0.05$). In another set of cells (n=4), the presence of H-89 had no effect on gamma oscillations while **ω**-Agatoxin-IVA completely blocked them (assumed to be P/Q-only cells) ($df=7$, $F=0.34$, $p>0.05$). In a final set of cells (n=2), H-89 completely blocked the presence of gamma oscillations while **ω**-Agatoxin-IVA had no effect on them (assumed to be N-only cells).
Conclusion: These results suggest that cells in the PPN that manifest gamma band activity through N-type calcium channels are modulated by the cAMP/PK pathway. We hypothesize that N-only cells are equivalent to "REM-on" cells in vivo.
Acknowledgment: Supported by NIH awards R01 NS20246 and P20 GM110702

B 8.3- "The Effects of Aerobic Exercise on Post-stroke Depression, Functional Mobility, and Metabolic Capacity in Individuals with Chronic Stroke"

Catherine Vanderwerker, Aaron Embry, Brian Cence, Chris Gregory
Medical University of South Carolina, Charleston, SC

Background: Common sequelae following stroke include residual functional limitations (e.g. walking) as well as neuropsychological manifestations (e.g. depression) that contribute significantly to disability and compromised quality of life. Aerobic exercise is an established approach for improving depressive symptoms in neurologically healthy individuals and its effects on post-stroke functional mobility have been established. To date, however, the potential interaction between depressive symptomology and post-stroke functional gains has not been documented. This study was designed to investigate the effects of aerobic exercise on post-stroke depressive symptoms, functional mobility, and metabolic capacity as well as the association between changes in these outcomes. Two hypotheses were investigated: after completing an intensive, progressive aerobic exercise program, [1] there will be improvements in post-stroke depressive symptoms, functional mobility, and metabolic capacity and [2] there will be a positive association between changes in post-stroke functional mobility and depressive symptoms.
Methods: Six participants (3 females; 47.3 ± 19.2 yrs old; 33.7 ± 10.4 months post-stroke) completed an 8-week aerobic exercise training program. Exercise was performed 3 times per week (30 min/session) and included both interval and steady state training sessions. Intensity of exercise was progressed weekly beginning at 40-50% of heart rate reserve (HRR) during week 1 up to 70-80% HRR by week 8. Pre and post outcome measures included Patient Health Questionare-9 (PHQ-9) for depressive symptoms, 6-minute walk test (6MWT) for functional mobility and peak oxygen consumption (VO_{2peak}) for metabolic capacity.
Results: In participants with mild to moderate depressive symptoms (PHQ-9 >5), 80% (4/5) demonstrated improved PHQ-9 score and 60% (3/5) demonstrated improvements in 6MWT and VO_{2peak}. Changes in PHQ-9 scores were positively correlated with changes in 6MWT but neither were associated with changes in VO_{2peak}. Of note, the subject who demonstrated the greatest improvement in depressive symptoms also showed the largest gains in 6MWT and VO_{2peak}.
Conclusions: Despite a small average improvement in depressive symptoms, walking endurance, and metabolic capacity following 8-weeks of aerobic cycling exercise training, changes in functional capacity and depressive symptoms were positively correlated in individuals with chronic stroke.
Clinical Relevance: Depression is purported to limit post-stroke functional recovery. This study provides a small step towards furthering our understanding of the association between post-stroke depressive symptoms and functional mobility and serves as the basis for ongoing work assessing the impact of aerobic exercise on post-stroke depression and functional recovery.
Acknowledgement: This work was funded by a pilot award from NIH U54 GM104941 (Binder-Macleod: PI) as well as NIH P20 GM109040 (Kautz: PI) from the NIGMS.

B 8.4- "Consequences of Self-Administered Methamphetamine Throughout Pregnancy on Rat Dams and Their Offspring"

Daniela Rüedi-Bettschen, Simran Chawla, Camille S. Washington, Donna M. Platt
University of Mississippi Medical Center, Jackson, MS

Background: Methamphetamine (METH) abuse in women of childbearing age and those who are pregnant is an urgent health concern. While the harmful effects of METH are well described for adults, there is only limited knowledge of the effects of METH use during pregnancy on the developing child. In the present study, we investigated how daily METH self-administration throughout pregnancy affected rat dams, as well as how the resulting in utero METH exposure affected offspring development through weaning. Yoked saline control dams and their offspring served as controls.
Methods: Female rats (n=6/group) were trained to self-administer METH (0.08 mg/kg/infusion) under a fixed-ratio schedule such that every injection of METH by a dam also resulted in a saline injection to her yoked control dam. When stable levels of self-administration were reached, all females were mated. Daily self-administration sessions continued until litters were born. General health and weight was assessed daily in dams and pups. In addition, pups were evaluated for achievement of age-appropriate developmental milestones.
Results: METH females self-administered 2-3 mg/kg/day prior to mating and throughout gestation. This level of METH self-administration had no effect on dam health, dam behavior or pregnancy outcome. Weight gain throughout pregnancy (and after) did not differ between METH dams and saline controls. All females produced viable litters, and litter size, composition and pup weight at birth did not differ between saline and METH dams. Similarly, maternal pup-directed behavior was not affected by prior METH self-administration experience. In contrast to the dams, pups were negatively affected by in utero METH exposure. Compared to saline-exposed pups, METH exposed pups were delayed in reaching developmental milestones, including righting reflex, eye opening, incisor eruption and negative geotaxis.
Conclusion: These results demonstrate the feasibility of using self-administration of METH by dams as a means to expose developing pups to the drug. More importantly, the results indicate that in utero exposure to low-to-moderate METH doses can profoundly affect offspring development, suggesting that even moderate or recreational METH use during pregnancy adversely affects offspring development.
Acknowledgment: This work was funded by an Institutional Development Award (IDeA) from the NIGMS under grant number P30GM103328.

B 8.5- "Conformational Dynamics in the Propagation of Amyloid-β Oligomers in Alzheimer Disease"

Dexter N. Dean[1], Kayla M. Pate[2], Melissa A. Moss[2], Vijayaraghavan Rangachari[1]
[1]Department of Chemistry and Biochemistry, The University of Southern Mississippi, Hattiesburg, MS
[2]Department of Chemical Engineering, The University of South Carolina, Columbia, SC

Background: Aggregates of the protein Amyloid-β (Aβ) have been implicated in the pathology of Alzheimer disease (AD). Low molecular-weight oligomers of Aβ have been a focal point of research in recent years, as evidence has revealed them to be the primary cause of neuronal dysfunction in the AD brain. We have recently reported the generation and isolation of large fatty acid-derived oligomers (LFAOs) of Aβ, which have a unique property of replication upon interacting with Aβ monomers. Details presented here reveal biophysical insight into LFAO assembly and dynamics, which manifests in LFAO replication efficiency and cellular activity.
Methods: Freshly purified LFAOs were incubated at varying concentrations (0.5-14 μM) with 50 μM Aβ monomer in quiescent conditions for 72 hours (25°C). LFAO replication efficiency was assessed using SDS-PAGE with immunoblotting and size exclusion chromatography. LFAO assembly and dynamics were investigated using fluorescence spectroscopy, fourier transform infrared spectroscopy (FTIR), and circular dichroism (CD). Cellular apoptosis in human neuroblastoma SH-SY5Y cells was assessed using DAPI and TUNEL staining.
Results: FTIR revealed LFAOs are composed of parallel β-sheets, similar to previously reported Aβ fibrils. As revealed by fluorescence spectroscopy, LFAOs have increased solvent-exposed hydrophobic surfaces compared to Aβ fibrils. Upon dilution, it was shown LFAOs undergo alterations in tertiary structure (oligomer assembly) which resulted in an increase in replication efficiency and cellular apoptosis.
Conclusions: The results of this investigation reveal conformational dynamics in the propagation and cellular activity of LFAOs. At low concentrations, LFAOs replicate more efficiently and induce maximum cellular apoptosis. As the concentration of LFAOs increases, both replication efficiency and cellular apoptosis are decreased. Overall, this work reveals insight into the propagation mechanism of toxic oligomeric species implicated in AD pathology.
Acknowledgements: This work has been partly funded by an Institutional Development Award (IDeA) from NIH NIGMS (P20GM103476) to the MS IDeA Network of Biomedical Research Excellence (MS INBRE) at USM, by the NSF GK-12 at USM "Connections in the Classroom: Molecules to Muscles" (0947944), and the NSF Graduate Research Fellowship Program (1445151).

B 8.6- "Design, Synthesis and Biological Evaluation of Novel Benzofuran Cannabinoid Ligands"

Eric W. Bow, John M. Rimoldi
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Background: The cannabinoid receptors, members of the G-protein coupled receptors (GPCRs) superfamily, have been implicated in numerous human physiological functions and diseases. The receptors, CB1 and CB2, are most concentrated in the central nervous system and immune cells, respectively, and have each become a target of interest. Dual CB1/CB2 agonists such as ∆9-tetrahydrocannbinol (THC) have demonstrated efficacy in the treatment of nausea, pain, and glaucoma, but suffer from CB1 mediated psychotropic effects, motivating the search for CB2 selective therapeutic agents. Selective modulation of the CB2 receptor has therapeutic potential in many human health issues such as pain, inflammation, and cancer.
Methods: In our efforts to develop CB2 selective ligands, we preliminarily examined structure activity relationships of synthetic and natural terpenoid cannabinoids to design a benzofuran aurone-like scaffold. In this work, we describe the synthesis of a lead compound, which showed promising activity and selectivity in computational docking studies. A homolog series exploring a variety of heteroatom substitutions was synthesized and tested for CB1 and CB2 receptor affinity as well further probe the potential of this scaffold.
Results: Our lead compound was found to be a low-micromolar CB2 ligand, with modest selectivity for CB2. Further synthesis of additional analogs led to the discovery of several compounds with nanomolar activity at CB2, with increased selectivity.
Conclusion: This work may lead to the development of a new class of cannabinoid ligands with a novel scaffold. Several compounds tested thus far have shown a modest preference for the CB2 receptor over CB1. Further synthesis and testing of new analogs will be synthesized to fully explore the potential of this benzofuran scaffold.
Acknowledgement: This research was funded by Grant Number P20GM104931 from the National Institute of General Medical Sciences (NIGMS), COBRE-NPN.

B 8.7- "Protein Succination is Increased on Select Proteins in the Brainstem of the Ndufs4 Knockout Mouse"

Gerardo G. Piroli, Allison M. Manuel, Anna C. Capps, Michael D. Walla, John E. Baatz, Richard D. Palmiter, Albert Quintana, Norma Frizzell
University of South Carolina School of Medicine, Columbia, SC

Background: We have previously identified the irreversible protein modification S-(2-succino)cysteine (2SC), also termed protein succination in adipocytes under diabetic conditions. 2SC is formed when the Krebs cycle metabolite fumarate reacts with protein cysteine residues. The increase in 2SC in the adipocyte occurs as a direct result of glucotoxicity and increased mitochondrial stress, resulting in the accumulation of NADH, and feedback inhibition of the Krebs cycle. Considering that glucotoxicity driven mitochondrial stress leads to fumarate accumulation, we hypothesized that oxidative phosphorylation deficiencies, such as those observed in some mitochondrial diseases, would also lead to increased protein succination.
Methods: In this work, we used the Ndufs4 knockout (Ndufs4 KO) mouse, a model of Leigh syndrome. We studied protein succination by immunoblotting, and we identified targets of succination and the sites of this modification by liquid chromatography-mass spectrometry (LC-MS/MS). The effects of succination on tubulin functionality were studied by tubulin polymerization assays.
Results: We demonstrate for the first time that protein succination is increased in the brainstem of Ndufs4 KO mice versus controls. We have also demonstrated that succination is most significantly increased in a specific region, the vestibular nucleus, which is the most pathologically affected region exhibiting neurodegeneration in this model. In contrast, protein succination is unchanged in other tissues such as muscle and liver. We have also identified the voltage-dependent anion channel (VDAC) 2 as a specific target of succination in this model versus control brain. We further confirmed the protein identity by immunoblotting. Using targeted LC-MS/MS mass spectrometry, Cys77 was identified as a site of endogenous succination in VDAC2. We also confirm that tubulin is the most abundant succinated protein in both control and Ndufs4 KO mouse brains, although the 2SC levels do not increase with disease state. Tubulin succination does not alter tubulin polymerization; however, the succination of tubulin alters anti-tubulin antibody affinity, suggesting that microtubule dynamics may be affected if tubulin interactions with other proteins are modulated by this.
Conclusion: Given the important role of VDAC isoforms in the exchange of ADP/ATP between the cytosol and the mitochondria, and the already decreased capacity for ATP synthesis in the Ndufs4

KO mice, we propose that the increased protein succination observed in the BS of these animals would further decrease the already compromised mitochondrial brain function. Continued investigations will confirm if fumarate is a novel biochemical link that may contribute to the progression of the neuropathology in this mitochondrial disease model.
Acknowledgment: This work was supported by grants from the National Institutes of Health (P20 GM-10909, R03 HD-077187 and R37 DK-19971) and a University of South Carolina Research Foundation ASPIRE-I award.

B 8.8- "Glutamatergic Output from the Medial Prefrontal Cortex Modulates the Daily Rhythm in Amphetamine Reward"

Nedah N. Nemati, Ron Cassada, Ian C. Webb
The University of Mississippi Medical Center, Jackson, MS

Background: Psychostimulant reward, as assessed via the conditioned place preference (CPP) paradigm, exhibits a daily rhythm with peaks in the late dark and early light periods, and a nadir near the light-to-dark transition. We have previously shown that medial prefrontal cortex (mPFC) lesions, or pharmacological inactivation at the acquisition or expression phases of CPP testing, increases drug reward at the nadir time, thereby attenuating the daily rhythm. Here, we used a chemogenetic approach to test the hypothesis that glutamatergic output from the mPFC attenuates amphetamine reward during the late light period.
Methods: To specifically inhibit mPFC glutamate neurons, male Sprague-Dawley rats received bilateral injections of an adeno-associated virus that induces the expression of an inhibitory DREADD receptor (AAV5-CAMKIIa-hM4D(Gi)-mCherry) in these cells. Three weeks following the surgery, rats were systemically administered vehicle or clozapine-N-oxide (CNO), and the influence on amphetamine CPP at previously observed peak (zeitgeber time [ZT] 23) and nadir times (ZT11) was examined.
Results: CNO administration significantly increased drug-paired chamber dwell time at ZT11, thereby eliminating the daily rhythm in amphetamine reward. In contrast, CNO administration at ZT23 did not influence amphetamine CPP as compared to vehicle.
Conclusion: These results indicate that glutamatergic output from mPFC modulates the diurnal rhythm in amphetamine CPP during the expression of learned reward-context associations. Moreover, as the loss of rhythmicity occurs via an increase at the nadir point, these results suggest that glutamatergic output from the mPFC normally inhibits context-elicited reward seeking prior to the light-to-dark transition.
Acknowledgement: This work was funded by a COBRE pilot project grant (I.C.W.) from the NIGMS under grant number P30GM103328.

B 8.9- "Allosteric Modulation of the Cannabinoid Receptor Type 1 (CB_1) by Org 27569"

Carolina Deifelt Streese, Sara A. Pettaway, Tracy A. Brooks, Janet A. Lambert, Samuel H. Hans, Meredith E. Stocks, Stephen J. Cutler
The University of Mississippi, University, MS

Background: Org 27569 (5-Chloro-3-ethyl-*N*-[2-[4-(1-piperidinyl)phenyl]ethyl-1*H*-indole-2-carboxamide) is a commercially available allosteric modulator of the CB_1 receptor that can enhance the binding of CB_1 agonist CP 55,940 ((-)-*cis*-3-[2-Hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol). Previous investigators have used mouse brain membrane preparations in studies of this compound (e.g., Price et al., 2005). This study examined the allosteric effects of Org 27569 on cloned human CB_1 receptors stably overexpressed in HEK293 cells.
Methods: We measured the effects that Org 27569 had on radioligand equilibrium binding assays and saturation assays with the agonist [^{3}H]-CP 55,940 and inverse agonist [^{3}H]-SR 141716A (N-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboximide hydrochloride).
Results: In equilibrium binding assays, Org 27569 increased the binding affinity of [^{3}H]-CP 55,940 to the CB_1 receptor, indicating a positive cooperative allosteric effect; however, the data could not be fitted into the allosteric ternary complex model (TCM) due to unexpectedly high levels of binding. Subsequent saturation binding assays in the presence and absence of 1 μM Org 27569 showed no change in the equilibrium dissociation constant (K_d) or the total concentration of available CB_1 receptors when CB_1 inverse agonist [^{3}H]-SR 141716A was used as the radioactive probe; however, when [^{3}H]-CP 55,940 was used as a probe, a 4.0-fold decrease in the K_d and a 1.8-fold increase in B_{max} were observed, indicating an increase in the number of available CB_1 receptors.
Conclusion: This increase in B_{max} is not accounted for by the current allosteric TCM. Additional investigation is needed for a thorough analysis of the allosteric properties of Org 27569.
Acknowledgment: This work was funded by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM104932.
Reference: Price MR, Baillie GL, Thomas A, Stevenson LA, Easson M, Goodwin R, McLean A, McIntosh L, Goodwin G, Walker G, Westwood P, Marrs J, Thomson F, Cowley P, Christopoulos A, Pertwee RG, & Ross RA. (2005). Allosteric modulation of the cannabinoid CB1 receptor. *Mol Pharmacol*., 68(5):1484-95.

B 8.10- "The COBRE *In Vitro* Core of the University of Mississippi"

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Background: The *In Vitro* Pharmacology Core enables investigators to use *in vitro* assays to serve as a guide in the isolation and identification of biologically-active natural products and to evaluate pure compounds predicted to have activities in the endocannabinoid, opioid, and allied neuropharmacological systems through rational drug design. These receptors represent drug targets affecting areas as diverse as drug addiction, pain, and obesity.
Methods: Screening assays measure the ability of an extract, fraction, or purified compound to bind to target receptors at a single fixed concentration. Dose-response competitive binding assays determine a pure compound's binding affinity for the receptor. Dose-response GTPγS functional assays determine a compound's efficacy and potency. Additional molecular neuropharmacological assays available are monoamine oxidase A and B (MAO-A and MAO-B), acetylcholinesterase (AChE), and butyrylcholinesterase (BCHE) inhibition assays. Additional cell-based assays include neuritogenesis and neurotrophic assays. Assays under development include screening, binding, and functional assays for NPFF and sigma receptors, cyclic AMP (cAMP) functional assays, and allosteric modulation assays. The core is also open to developing and optimizing new assays if sufficient demand exists.
Results: For screening assays, the sample's ability to displace the radioligand (i.e., percent displacement) for each receptor is reported. For competitive binding assays, we determine the compound's K_i (equilibrium dissociation constant). For GTPγS functional assays, we report the compound's efficacy [i.e., full agonist, partial agonist, (neutral) antagonist, or inverse agonist] and potency (i.e., the EC_{50} or IC_{50} value, the concentration of a drug that gives half-maximal response). Neutral antagonists are further evaluated in fixed agonist and fixed antagonist assays. For MAO-A and MAO-B enzyme inhibition, we report the IC_{50} and IC_{90} (from dose-response analysis), as well as K_m, V_{max}, K_i , and the nature of the inhibition (competitive, non-competitive, uncompetitive or mixed) for enzyme inhibition.
Conclusion: The University of Mississippi *In Vitro* Pharmacology Core provides valuable services for investigators who want to identify and evaluate extracts, fractions, or pure compounds for their biological activity in the endocannabinoid and opioid systems. Investigations regarding the site(s) as well as mechanism(s) of action can provide researchers direction for their follow-up investigations

Acknowledgment: This work was funded by an Institutional Development Award (IDeA) from the NIGMS under grant number P20GM104932.

B 8.11- "The Role of Acid Sensing Ion Channel 2 in Modulating Seizure Activity and Cerebral Edema"

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Background: Acid Sensing Ion Channels (ASICs) are part of the Degenerin family of proteins and function as chemosensors. While ASIC1a has been shown to be important for seizure progression/ termination, the role of ASIC2 in seizures is not known. This study tested the hypothesis that loss of ASIC2 increases sensitivity to seizures and cerebral edema formation.
Methods: Age-matched ASIC2+/+ and ASIC2-/- mice were randomly assigned to receive 20, 40, or 60mg/kg pentylenetetrazol (PTZ, i.p). Seizure activity was video monitored for 30 minutes after which, mice were euthanized under isoflurane anesthesia, and brains collected. Brains were hemisected and further dissected into frontal brain, hippocampus, striatum, and cortex. Brain regions were weighed then dried for 48h at 60°C. Brain water content was then calculated.
Results: There was a main effect of genotype on brain water content in the frontal brain and hippocampus ($p<0.05$). Loss of ASIC2 resulted in a significant increase in brain water content at 60mg/kg PTZ in the frontal brain and hippocampus ($p<0.05$). Mice lacking ASIC2 were more sensitive to the highest dose of PTZ resulting in decreased survival (75% (3/4) of ASIC2+/+ and 33.3% (1/3) of ASIC2-/- mice).
Conclusion: These results support the hypothesis that ASIC2 offers protection against seizure-induced mortality and cerebral edema formation. Further analysis will determine whether loss of ASIC2 increases severity or prolongs the duration of PTZ-induced seizures.
Acknowledgements: This work was funded by IDeA grants P20GM104357 and P30GM103328. Dr. Heather Drummond provided the mice used in this study.

B 8.12- "Excitatory and Inhibitory rTMS as Mechanistic Contributors to Walking Recovery"

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Background: Repetitive transcranial magnetic stimulation (rTMS) has been used to successfully alter upper extremity motor control after stroke, and preliminary findings demonstrate promise in rehabilitation of the lower extremities. However, we currently lack the foundational work necessary to utilize rTMS as a potential adjunctive therapy for post-

stroke walking rehabilitation. The purpose of this proposal is to investigate the effects of high frequency (excitatory) rTMS, low frequency (inhibitory) rTMS, and sham rTMS on neurophysiological, neuromuscular, and neuromechanical outcomes in individuals with post-stroke hemiparesis.
Methods: Twenty individuals with chronic stroke will receive ipsilesional rTMS at 10 Hz (excitatory), contralesional 1 Hz rTMS (inhibitory), and sham rTMS, each on a separate day, randomly sequenced. Each rTMS treatment session will be preceded and followed by biomechanical gait assessments, TMS-based neurophysiologic assessments (using a Magstim double cone coil and neuro-navigation), and strength assessments of the paretic tibialis anterior (TA) and soleus (SOL). To date, four individuals have completed the assessments: 3 female, 1 with right hemisphere lesion, aged 62±21.6 years, 46.5±43 months since stroke.
Results: After excitatory rTMS, resting motor threshold (rMT) decreased in both TA (11.7%) and SOL (9.2%), paretic propulsion (Pp, a walking-specific measure of coordinated force output) improved by 9.8%, and isometric strength decreased by 16.7% (plantarflexion) and 6.2% (dorsiflexion). After inhibitory rTMS, Pp decreased by 9.7% and dorsiflexion strength increased by 14.3%.
Conclusion: Results are preliminary, with only 4 participants out of 20 having completed testing. Following excitatory rTMS, rMT and Pp results are consistent with the hemispheric imbalance model for cortical excitability. However, this model does not predict decreases in Pp or paretic isometric strength (as seen with ipsilesional excitation) with either mode of rTMS. Completed data sets will be compared to sham stimulation. Future work plans to assess different stimulation models in combination with a locomotor rehabilitation protocol.
Acknowledgment: Research reported in this publication was supported by a Career Development Award-2 RR&D N0787-W (MGB) and an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20-GM109040.

B 8.13- *"In vitro* Phenotypic Cell-Based and Molecular Target-Based Neuropharmacological Assays for Determining Neuroactive Properties of Natural Products"

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The central and peripheral nervous systems function through the complex networks of chemical messengers. Based on the specific response pathways and functions, these messengers are commonly referred as neurotransmitters, neuromodulators, neuro regulators, neuromediators and neurotropic factors. Molecular neuropharmacology involves the study of neurons and their neurochemical interactions, with the overall goal of developing new lead drugs that have therapeutic effects on neurological functions. The studies involving interactions of neurotransmitters, neuropeptides, neurohormones, neuromodulators, enzymes, second messengers, co-transporters, ion channels, and receptor proteins in the central and peripheral nervous systems are important for developing drugs to treat different neurological disorders, including pain, neurodegenerative diseases such as Parkinson's and Alzheimer's disease, psychological disorders, addiction, etc.
The *in vitro* neuropharmacological evaluations with phenotypic cell-based and molecular target-based assays are important tools for screening compound libraries (natural products or its derivatives) and discovery of new leads drugs. The enzyme-based assays with recombinant human monoamine oxidase-A and B, acetyl and butyrylcholine esterases have been established for screens. The potent inhibitors are advanced further for analysis of inhibition mechanism, kinetics and enzyme-inhibitor binding. A combined *in vitro* neurotrophin assay that measures and quantifies neurite outgrowth along with the cell viability in a single assay method has been established. Neurotrophins repair damaged neurons through stimulation of neuritic outgrowth. These compounds may be important for restoration and readjustment of normal neuronal functions of the damaged neurons. Bioactive small molecules with neurotrophic and neuritogenic activities hold great promise as therapeutic agents for treatment of neurodegenerative diseases and neuronal injuries by virtue of their ability to stimulate neuritic outgrowth. The lead molecules are progressed further for interrogation of neurotrophin signaling pathways (p-Trk-A and p-Erk) and understanding the mechanisms for neurotrophic actions.
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B 8.14- "Sleep Disturbance and Inflammation: A Role for Stress Hormones?"

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Background: Primary sleep abnormalities (insomnia, shift work, and obstructive sleep apnea (OSA)) promote an inflammatory environment and are associated with the development of cardiovascular and metabolic disease. Our lab has pinpointed specific tissues that are susceptible to increased pro-inflammatory cytokine gene expression following either sleep deprivation or fragmentation and they include: liver, spleen, adipose tissue, heart, and several brain regions. In addition, the hypothalamic-pituitary-adrenal axis can also be activated from sleep loss, and triggers release of glucocorticoids from the adrenals. These hormones generally suppress inflammation, but it is unclear how they mediate inflammatory pathways from sleep loss.
Methods: Adult male C57BL/6j mice were subjected to several different methods to disturb sleep: (1) multiple platform method, which induces paradoxical sleep deprivation, or (2) sleep fragmentation using a sweeping bar to periodically awaken mice. After 24 h, tissues were collected to assess cytokine gene expression using RTPCR or serum corticosterone titers using ELISA. To assess effect of glucocorticoid elevation, further experiments were conducted that involved adrenalectomy or pharmacological blockade of glucocorticoid receptors (mifepristone).
Results: Both methods of inducing sleep loss produced variable patterns of pro-inflammatory gene upregulation in liver, spleen, fat, heart, and brain (pre-frontal cortex, hypothalamus, hippocampus). In addition, 24 h of sleep deprivation or highly fragmented sleep (every 20 s) induced an elevation in baseline corticosterone titers compared with controls (no sleep loss), whereas a lower degree of sleep fragmentation (every 2 min) did not. Adrenalectomy or pharmacological blockade suppressed inflammatory responses to sleep loss in some tissues.
Conclusion: We provide evidence that stress hormones may mediate inflammatory pathways associated with sleep loss. Future research should investigate the molecular mechanisms that underlie this endocrine-immune interaction.
Acknowledgment: This work was funded by an Institutional Development Award (IDeA) from the NIGMS under grant number #2P20GM103436-14.

B 8.15- "Lithium Decreases the Effects of Neuronal Calcium Sensor Protein 1 in Pedunculopontine Neurons"

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Background: Human postmortem studies reported increased expression of neuronal calcium sensor protein 1 (NCS-1) in the brains of some bipolar disorder patients. Furthermore, reduced or aberrant gamma band activity has been reported in this same disorder. This disorder is also characterized by sleep dysregulation, suggesting a role for the reticular activating system (RAS). In a previous study examining the pedunculopontine nucleus (PPN), we found that NCS-1 at 1 mM significantly increased the frequency and amplitude of gamma oscillations, whereas very high concentrations of NCS-1 at 10 mM concentration reduced or blocked gamma band oscillations within these cells. This suggest that while 1 mM NCS-1 seems to be the most critical concentration of gamma oscillation modulation, 10 mM NCS-1 is an excessive level in keeping with significant over expression and may be responsible for the decreased gamma band activity present in some bipolar disorder patients. Lithium has also been proposed as a neuroprotective agent and has been shown to effectively treat the mood disturbances seen in bipolar disorder patients, although limited by side effects. Lithium was proposed to act by inhibiting the interaction between NCS-1 and inositol 1,4,5-triphosphate receptor protein (InsP). NCS-1 is known to enhance the activity of InsP, which is present in the PPN. Since Lithium has been shown to act by inhibiting the enhancing effects of NCS-1, we hypothesis that lithium will be able to reduce the effects of over expression of NCS-1 and prevent the down regulation of gamma band activity and restore normal levels of gamma oscillations.
Methods: Whole-cell patch-clamp responses were recorded on 9-13 day old adult timed-pregnant Sprague-Dawley rat brainstem slices. Slices were recorded at 37^0C perfused with oxygenated aCSF in an immersion chamber containing the synaptic blockers gabazine ($GABA_A$ antagonist), strychnine (glycine antagonist), 6-cyano-7-nitroquinoxaline-2,3-dione (AMPA/kainate receptor antagonist), APV (NMDA receptor antagonist), and mecamylamine (nicotinic receptor blocker), and also Tetrodotoxin to block sodium channels.
Results: Lower concentrations of lithium have a more significant effect on both oscillation frequency and amplitude. Lower concentrations (1 mM) of NCS-1, enhanced oscillation frequency and amplitude after 20 minutes. Lithium at the 1 mM concentration was able to reduce the enhancing effects of NCS-1 on gamma band oscillations, potentially preventing the down regulation of gamma band activity.
Lithium at the 1 mM concentration is able to reduce the enhancing effects of NCS-1 on gamma band oscillations, potentially preventing the down regulation of gamma band activity.
Conclusion: Our study shows lithium's ability to help maintain gamma band oscillations dependent on P/Q-

type calcium channels in the presence of excessive NCS-1.These findings taken together resolve the 60 year mystery of the physiology of lithium action in bipolar disorder and suggest that lithium may reduce the effects of over expressed NCS-1 in bipolar disorder, thereby normalizing gamma band oscillations mediated by P/Q-type calcium channels modulated by NCS-1. In summary, these recent discoveries provide novel therapeutic targets for alleviating some of the arousal and sleep/wake disturbances in this devastating disease.
Acknowledgment: This work was supported by NIH award R01 NS020246, and by core facilities of the Center for Translational Neuroscience supported by NIH award P20 GM103425 and P30 GM110702 to Dr. Garcia-Rill. In addition, this work was supported by grants from FONCYT-Agencia Nacional de Promoción Científica y Tecnológica; BID 1728 OC.AR. PICT 2008-2019 and PICT-2012-1769 (to Dr. Urbano).

B 8.16- "Alterations in Kinesin Catalytic Activity Resulting from Mutations that Cause Hereditary Spastic Paraplegia"

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Background: Hereditary Spastic Paraplegia (HSP) is a neurodegenerative disease that involves progressive loss of axons in the corticospinal tract, manifesting in lower limb spasticity. In complicated forms of HSP, patients have additional neurological deficits, including ataxia, retinopathy, and cognitive deficit. 10% of all complicated forms of HSP are due to mutation in the neuronally-enriched kinesin transport motor, Kif5A. Of particular interest to this study, 20 of the 22 mapped disease-causing mutations are in the kinesin catalytic domain, responsible for nucleotide binding and hydrolysis, microtubule binding, and force generation. We are testing the biochemical and biophysical properties of recombinantly-expressed mutant kinesin motors to determine the functional deficit at the heart of this human disease.
Methods: We cloned the wild type human Kif5A gene into a bacterial expression vector and performed site-directed mutagenesis to insert each HSP-causing mutation. We then expressed and purified each construct for use in a series of well-characterized *in vitro* assays, including basal and microtubule-stimulated ATPase rates, microtubule affinity in a variety of nucleotide intermediate states, stopped flow analysis of nucleotide binding, microtubule gliding assays, and single molecule motility assays. In addition, we performed molecular dynamics simulations to determine any likely protein conformation changes as a result of each mutation.
Results: Since each mutation causes the same disease, our hypothesis was that we would see a similar functional deficit in kinesin catalytic activity as a result of each mutation. However, we found a variety of altered enzymatic properties, from lower rates of nucleotide hydrolysis to decreased microtubule affinity. In those rare instances where core catalytic activity (ATPase and microtubule binding) was intact, we saw a significant decrease in the rate of kinesin motility. The most intriguing mutations are those that appear to form a mechanotransduction network between the microtubule interface and the nucleotide hydrolysis site. In these mutants, there is a disconnect in the normal nucleotide state-dependent microtubule affinity that is the basis for kinesin's hand-over-hand stepping behavior at the heart of this cellular motor's processive motility.
Conclusion: Mutations in the neuronal kinesin, Kif5A, that cause HSP have a variety of detrimental effects on the enzymatic activity of the motor. The severity of functional deficit – on a continuum from complete inability to hydrolyze ATP on one end, to subtly reduced motility on the other – does not seem to have any impact on the large-scale manifestation of the human disease. This suggests that the disease is due to altered transport kinetics of cellular cargo(es) that are exquisitely tuned to cellular physiology. Any deficit in transport function is ultimately deleterious to the neuron over time. We are now focusing on identifying the cellular cargoes transported by Kif5A in order to test this hypothesis by examining the dynamics of cargo transport in neurons expressing Kif5A with disease-causing mutations.
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