42\textsuperscript{nd} Summer Medical Student Research Day

ABSTRACTS

July 31, 2015
ABSTRACT

LIPOPOLYSACHHARIDE INDUCED PULMONARY ENDOTHELIAL BARRIER DISRUPTION: CRITICAL ROLE FOR BICARBONATE STIMULATION OF ADENYLYL CYCLASE ISOFORM 10. Jordan Nickols, Sponsored by Sarah Sayner, Ph.D., Department of Physiology and Cell Biology, University of South Alabama College of Medicine, Mobile, Alabama.

Lipopolysaccharide (LPS) induced sepsis is a common cause of endothelial barrier disruption in lung vasculature and can lead to pulmonary edema or acute respiratory distress syndrome (ARDS). Patients with ARDS often require low pressure, ventilatory support and as a consequence of this strategy patients can develop hypercapnic acidosis. ARDSNet recommends bicarbonate infusion in order to correct the acidosis; however, this treatment is controversial and in some cases has been shown to worsen the patient's current condition. In controlled experiments using rat pulmonary microvascular endothelial cells (PMVECs), increasing bicarbonate levels have been shown to correlate with an increase in endothelial permeability. This bicarbonate dependent response is thought to be due, in part, to stimulation of adenylyl cyclase (AC) isoform 10 (AC10). AC isoforms 1-9 are trans-membrane isoforms that can be stimulated by forskolin or G_{ss} and, when stimulated, result in tightening of the cell-cell barrier; however, soluble AC10 is a cytosolic isoform that, when stimulated, results in a destructive effect on the cell-cell barrier and leads to an increase in monothelial layer permeability. This role of compartmentalization of adenylyl cyclases has begun to unfold in recent studies. Our study looks at AC10’s role in a rat PMVEC model and how bicarbonate stimulation may lead to increased edema in lung injury patients. Our method of inducing lung injury is the addition of LPS, an endotoxin from E. coli that will initiate disruption of the cell barrier. We intend to show that increases in bicarbonate concentration correspond with an increase in endothelial damage (stimulated by LPS) as measured by ECIS studies and Dextran permeability assays. This bicarbonate induced increase will be observed with the addition of KH7, an AC10 inhibitor, and in AC10 overexpressing cells in order to implicate AC10 as the mediator for this response.
ABSTRACT

INHIBITION OF TRIPLE NEGATIVE BREAST CANCER METASTASIS THROUGH THE UPREGULATION OF QKI – 6 USING SULINDAC SULFIDE. Stephen Ambrose. Sponsored by Yaguang Xi M.D., Ph.D., Department of Oncologic Sciences, Mitchell Cancer Institute, Mobile, AL.

Breast cancer is the most common cancer diagnosed in women and the second most common cause of death in women each year. Approximately 10-20% of women with breast cancer are diagnosed with Triple Negative Breast Cancer (TNBC). TNBC cells lack the estrogen, progesterone and human epidermal growth factor 2 receptors making these cells resistant to receptor targeted therapies. This aggressive subset of breast cancer has a high recurrence rate and readily metastasizes; therefore, patients diagnosed with TNBC have a poor prognosis. Dr. Xi’s lab proposes novel methods of inhibiting the metastasis of TNBC. Using BALB/c lab mice, we demonstrated the effects of sulindac sulfide (SS) on tumor metastasis. SS is a non-steroidal anti-inflammatory drug that works by inhibiting the cyclooxygenase pathways. In previous studies, SS also exhibited the ability to upregulate the tumor suppressor gene Quaking – 6 (QKI-6). We propose that SS will induce the upregulation of QKI – 6; the higher concentrations of QKI – 6 will then inhibit tumor cell metastasis by causing the membrane localization of both beta-catenin and E-cadherin aiding in cell-to-cell attachment. In a small trial experiment, two groups of mice were given 4T1 mouse breast cancer cells. In one group of mice, QKI – 6 was endogenously overexpressed compared to the control group. In each group the mice received SS at differing concentrations of 10, 25 and 50 mg/kg. The mice receiving the highest concentration of SS all died prior to the completion of the experiment. We concluded that the 50 mg/kg dose is too toxic for the mice. Each week we observed the cancer cells via a luciferase gene only expressed in the cancer cells. Twice a week for 6 weeks, we imaged the mice using the in vivo imaging system. Preliminary results suggest QKI – 6 inhibits tumor metastasis. At the end of the experiment none of the mice with QKI – 6 overexpressed exhibited metastasis. Only the mice in the control group that did not receive the SS showed tumor metastasis. As expected, QKI – 6 overexpression inhibited tumor metastasis. Also, the mice in the control group receiving the SS did not show metastasis supporting our hypothesis. Currently, more in vivo studies are ongoing to demonstrate conclusively the role SS and QKI – 6 play inhibiting TNBC metastasis.
ABSTRACT
THE EFFECT OF ELECTRONIC TOYS ON GESTURAL AND SPOKEN COMMUNICATION BY CHILDREN ON THE AUTISM SPECTRUM. Adam Powell and Dare Hicks. Sponsored by Amy Mitchell, MS, Brenda Beverly, PhD, Hanes Swingle, MD, Departments of Speech Pathology and Audiology and Pediatrics.

Impairments in social communication, e.g., reduced sharing of interests, failure to initiate or respond to social interactions, and deficits in verbal and nonverbal communication (abnormalities in eye contact, facial expression and use of gestures), are core features of Autism Spectrum Disorder (ASD). Language development is impacted by the linguistic environment to which children are exposed, e.g., language skills are enhanced when young children are read to daily and impeded in a TV-rich environment. Toys impact the child’s linguistic environment. Over the past decade, there has been an exponential rise in the use of handheld electronic games and toys by young children, yet the impact of these electronic devices on language development is unknown. Twenty children, 9 typical and 11 on the autism spectrum, were video-recorded playing with their parents with a familiar book, their favorite non-electronic toy, and a handheld electronic device. Parent and child verbalizations were transcribed and analyzed using Systematic Analysis of Language Transcripts (SALT) to determine the number of different words (NDW), number of total words (NTW), and mean length of utterances (MLU). Statistical analysis was conducted using SPSS. The mean age of the children was 45.6 months, 14 were male, 8 were Caucasian, 5 African-American, and 3 other.

<table>
<thead>
<tr>
<th></th>
<th>MLU</th>
<th>NDW</th>
<th>NTW</th>
<th>Gestures†</th>
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<tr>
<td>All three toys</td>
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<tr>
<td>Children with ASD</td>
<td>2.0</td>
<td>30.9 (30.7)*</td>
<td>67.3 (86.2)</td>
<td>4.9 (4.4)</td>
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<tr>
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<td>3.3</td>
<td>69.8 (29.2)*</td>
<td>144.3 (66.5)</td>
<td>10.5 (9.1)</td>
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<tr>
<td>Book</td>
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<tr>
<td>Children with ASD</td>
<td>1.9</td>
<td>29.5 (28.4)</td>
<td>58.3 (60.5)</td>
<td>6.4 (5.2)</td>
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<tr>
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<td>72.6 (19.1)</td>
<td>144.4 (47.3)</td>
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<td>Non-electronic toy</td>
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<tr>
<td>Children with ASD</td>
<td>1.9</td>
<td>34.5 (32.9)</td>
<td>73.2 (85.6)</td>
<td>4.8 (4.3)</td>
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<tr>
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<td>79.0 (41.9)</td>
<td>165.8 (88.5)</td>
<td>7.0 (5.0)</td>
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<tr>
<td>Handheld electronic device</td>
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<tr>
<td>Children with ASD</td>
<td>2.3</td>
<td>28.7 (33.4)</td>
<td>70.5 (112.4)</td>
<td>3.3 (3.5)</td>
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<td>57.8 (23.7)</td>
<td>122.8 (65.1)</td>
<td>5.5 (3.0)</td>
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</table>

Mean values with Standard Deviation in ().

*p=0.029.
† Statistical analysis pending.

Conclusion: As expected, the NDW was lower in children with ASD than in the controls. We did not find statistical differences in the NDW, MLU, and NTW with the three toys tested, although there may be a trend toward higher expressive language with the child’s preferred non-electronic toy. This pilot study is limited by the number of subjects tested.
Cystic fibrosis (CF) is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), a critical chloride channel in pulmonary, gastrointestinal, and genitourinary epithelia. Lung disease in CF is characterized by a vicious cycle of airway obstruction, chronic infection and hyper-inflammation. Inhibitors of Type 4 cyclic nucleotide phosphodiesterases (PDE4s), a group of isoenzymes that hydrolyze and inactivate the second messenger cAMP, have well-established anti-inflammatory properties and are used for this reason to treat Chronic Obstructive Pulmonary Disease (COPD). CF and COPD share a number of clinical manifestations (i.e. CFTR hypofunction, mucoviscidosis and hyper-inflammation) implying a potential use of PDE4 inhibitors for CF. However, CF is associated with altered, generally more exaggerated immune responses in the airways. Thus, it is unclear whether PDE4 inhibition would remain equally effective in CF. To this end we explored the effects of PDE4 inhibition on inflammatory responses in primary airway epithelial cells from CF patients and non-CF controls. We found that PDE4 inhibition suppresses production of the pro-inflammatory cytokine TNFα to similar extents in non-CF and CF cells, suggesting that PDE4 inhibition retains its anti-inflammatory properties in the hyper-inflammatory state of CF and may, thus, represent a valid therapeutic approach for the disease. We are now exploring the effect of PDE4 inhibition on additional pro-inflammatory cytokines produced by bronchial epithelial cells. Furthermore, we aim to identify the specific PDE4 isoforms controlling inflammatory responses as potential targets for the development of isoform-selective PDE4 inhibitors with an improved safety profile compared to the non-selective PDE4 inhibitors available to date.
ABSTRACT

UNDERSTANDING CIRCUMSTANCES AND PURCHASING PRACTICES OF FOOD ASSISTANCE BENEFICIARIES. Michelle Ghandhi. Sponsored by Martha Arrieta, Ph.D., Centers for Healthy Communities, University of South Alabama College of Medicine, Mobile, AL.

The food insecurity-obesity paradox has become increasingly significant in the United States, as obesity rates continue to rise. Low-income individuals are more likely to use food assistance, may suffer from lack of food, and have the highest rates of obesity. The educational programs regarding nutrition offered to beneficiaries vary from state to state and are limited in the Mobile area. The current study aims to understand the barriers to healthy eating for beneficiaries and determine if an educational program is deemed useful. Fifteen community members, recruited through convenience sample were interviewed about their food purchasing practices, the barriers they experience to healthy eating, and what type of curriculum would be useful to them, if any. Data was coded and analyzed using Atlas Ti 6.28. The main barriers to preparing healthy foods were time, money, convenience, and knowledge of how foods tasted. Some minor barriers included lack of transportation, lack of appliances, and children’s preferences. Most sited multiple reasons, suggesting complexity of the problem. Researchers found that people in the area varied on their level of preparation of foods, from cooking meals at home to purchasing exclusively pre-packaged foods, but diets were consistent and lacked variety within each household. The overall perceptions of health were foods that are conventionally considered healthy, such as fruits, vegetables, and lean meats. Areas in which people generally needed clarification or help included the following: identifying whole grains, recipes for quick and healthy snacks, and knowing appropriate values on nutrition labels. We concluded that though the former three might be areas in which education might be useful, it is apparent that more than classes will be required to alleviate the food insecurity-obesity paradox. Changes at the policy and industry level are necessary, too.
HEALTH INSURANCE AND KNOWING YOUR DOCTOR’S NAME- A PATH MODEL FOR HEALTH CARE UTILIZATION. Alan Akira. Sponsored by Dr. Kenneth Hudson, PhD and Dr. Errol Crook, MD, USA Center for Healthy Communities

Health insurance (HI) and having a steady primary health care provider have been strongly related to better health outcomes through increased health care utilization (HCU). Disparities in HI exist in impoverished and African American populations, and lack of HI is believed to be a factor in driving increased mortality from cardiovascular disease (CVD) and Type 2 Diabetes Mellitus (T2DM), which are more prevalent in these populations. We hypothesized that HCU, which we defined as having blood pressure, blood sugar, and cholesterol measured at a physician’s office within the past year of the most current interview date, was the result of a complex mechanism between HI and knowing your primary health care provider’s name. Since 2007, we report that 22% of participants that live in the most impoverished census tracts in Mobile, AL had HI at the time of multiple interviews, 58% had a regular doctor/regular provider, and 41% knew their doctor’s name. HI was a positive predictor of HCU with p=0.002, and knowing your doctor’s name was a positive predictor of HCU with p=0.004. Elucidating the causes of HCU in a population that has increased mortality via CVD and T2DM will lead to recommendations that physicians prioritize establishing relationships with patients, and patients maintain a single primary health care provider in order to increase overall health outcomes.
COMPARING DDAVP CLINICAL RESPONSE TO DDAVP CHALLENGE TEST IN PATIENTS WITH VON WILLEBRAND DISEASE. Divya Nadella. Mentored by Abdul Hafeez Siddiqui, M.D., and Dr. Hamayun Imran, M.D., Division of Pediatric Hematology/Oncology, Department of Pediatrics, University of South Alabama, Mobile, AL.

Introduction: Von Willebrand Disease (vWD) results in mucosal bleeds in 1% of the population and is characterized by a qualitative or quantitative defect in the vW protein. DDAVP increases the release of vW protein from the endothelium and is one of the main treatments. Currently, a day long challenge test is performed to determine a patient’s response. We proposed that a therapeutic trial of Stimate, an intranasal form of DDAVP, is clinically more useful than a DDAVP challenge test.

Methods: With IRB approval, we reviewed the medical records at the USA inpatient and outpatient facilities and identified 82 pediatric patients between 2005 and 2015. We collected bleeding symptoms and vWD lab profiles (vWF:antigen, vWF:Ristocetin cofactor activity, and Factor VIII levels). A complete response was defined as a 2-fold increase in the lab profile at 1-3 hours or at least a 1.5 fold increase at 4-6 hours post-IV DDAVP administration. SPSS version 23.0 was used to analyze demographic and lab data. Where appropriate, frequencies and paired t-tests were performed for comparison.

Results: Of 82 patients, 62 (75.6%) were females. Of 61 patients with a vWD diagnosis listed, 34 (55.7%) were type 1, 6 (9.9%) type 2, 1 (1.6%) type 3, and for 20 (32.8%) type was not specified. Challenge results were documented for 48 (58%) patients. Most patients (73%) had a complete response. Post-DDAVP levels were significantly higher than baseline (p = <0.001) for vW lab profile for both times (1-3 and 4-6 hours).

Thirty-seven patients had both challenge results and clinical symptoms reported with 29 (78%) considered responders and 8 (22%) considered non-responders. Twenty-five (86.2%) responders were initially put on Stimate alone while the other 4 received combined therapy with addition of a plasma derived product. All non-responders still received DDAVP therapy (either IV or Stimate) with some improvement in their clinical symptoms. Four (50%) non-responders and 11 (38%) responders switched to plasma derived products, most for better symptom control.

Conclusion: Due to most non-responders demonstrating some clinical improvement and a high proportion of the responders switching to a plasma derived product, a therapeutic trial of Stimate may have been more effective in our patient population than a costly challenge test at determining a patient’s response to therapy. More studies addressing the challenge test in vWD management would be needed before anything conclusive can be claimed.

Limitations: Retrospective design, small sample size, single institution and missing data.
Disruption of laminar flow within the carotid vasculature predisposes vessels to harmful remodeling and atherosclerosis. We recently found that inducing low flow alone in a partial carotid ligation model leads to vascular remodeling and formation of neointima. We examined if this remodeling was associated with endothelial dysfunction and whether differences in dysfunction between males and females might delineate a difference in cardiovascular risk between the genders. For these studies, we performed isometric force measurements on isolated carotid rings from mice undergoing partial-ligation (2-weeks) of carotid branches; left carotid ligated (PL) and right carotid remaining open (control). We assessed endothelium-dependent vasodilation in response to acetylcholine (10^{-8} – 10^{-5} M). ACh-induced vasorelaxation (1 µM) was significantly impaired in partially ligated vessels vs. controls in both males (22 ± 6% vs. 65 ± 15%, P<0.05, n=5) and females (22 ± 10% vs. 74 ± 16%, P<0.05, n=5). In addition, relative to control arteries, phenylephrine-induced (1 µM) contractions were larger in injured vessels (male, 1.55 ± 0.66 mN vs. 0.75 ± 0.08 mN; female, 1.15 ± 0.23 mN vs. 0.43 ± 0.12 mN) while contractions in response to KCl (60 mM) were lower (male, 0.55 ± 0.17 mN vs. 1.01 ± 0.23 mN; female, 0.31 ± 0.05 mN vs. 0.68 ± 0.16 mN) Our findings suggest that chronic (2-weeks) low flow in the carotid artery leads to substantial endothelial dysfunction and the degree of impairment is similar between male and female mice.
ACCUMULATION OF PRO-INFLAMMATORY MITOCHONDRIAL DNA DAMAGE ASSOCIATED MOLECULAR PATTERNS (mtDNA DAMPs) IN STORED PLATELET RICH PLASMA. Michael Marshall. Sponsor: Jon Simmons, M.D., Department of Surgery, University of South Alabama College of Medicine, Mobile, AL. Mark Gillespie, Ph.D., Department of Pharmacology, University of South Alabama College of Medicine, Mobile, AL.

Introduction: Evidence that endogenously-formed Mitochondrial DNA (mtDNA) DAMPs contribute to human disease is compelling. We have previously shown that all stored blood components contain varying quantities of mtDNA DAMPs and that exogenous mtDNA DAMPs administered to isolated rat lungs cause vascular endothelial injury. Because transfusion of blood components imposes a significant risk for acute lung injury, the goal of this study was to determine if mtDNA DAMPs accumulated in platelet rich plasma (PRP) in a time dependent-manner and if exogenous DNase degrades extracellular mtDNA in stored PRP.

Methods: We employed qPCR to quantify 200 bp sequences from the ND6 region of the mitochondrial genome in DNA extracted from samples of PRP over the span of the product shelf-life. To determine if DNase degraded the mtDNA in PRP, we separated the PRP into an experimental and control group. The experimental group received 10-fold the typical dose for the anticipated quantity of mtDNA. MtDNA was quantified at multiple defined time points to determine the half-life of the activity of DNase and to ensure the total mtDNA was removed from the experimental group.

Results: The quantity of mtDNA DAMPs accumulated over time in PRP. Exogenous DNase degrades mtDNA that is isolated from PRP. However, DNase is not active when added directly to the PRP.

Conclusion: We conclude that mtDNA DAMPs accumulate over time in stored PRP. Although DNase degrades mtDNA after its isolation from stored platelet rich plasma, the enzyme is inactivated by constituents present in normal platelet storage conditions.
ABSTRACT

Restructuring the Cardiovascular Card Constructs Library.
Austin Brown. Sponsored by Jeffery Sosnowski, Ph.D., M.D., Lynn Batten, M.D., and Mary Townsley, Ph.D. Division of Medical Education, Departments of Pediatrics and Physiology & Cell Biology, University of South Alabama College of Medicine, Mobile, AL.

Recently, medical education has experienced a push towards team based learning (TBL), which is a self-directed learning modality designed to increase students' cognitive understanding according to Bloom’s taxonomy. However, TBL exercises often focus on a single pathology. Further, the tools required to present multiple clinical cases while simultaneously challenging pre-clerkship students' interpretation and analysis skills are often not available. We searched MedEdPORTAL, and other resources for preclinical medical education, finding no published datasets. Thus, to meet this need we have designed five cardiovascular case construct exercises that each combines eight different clinical cases involving a common physiological process. The exercises focus on 1) congenital and acquired cardiac defects, 2) arrhythmias, 3) heart failure, 4) systemic hypertension, and 5) vasculitides. Each case is separated into history, physical exam, labs and imaging, differential diagnoses, and treatments, and then the entire set is randomized and given to small teams of students to reorganize. Early versions of these case sets had been utilized in the Cardiovascular Module in 2014-2015. The work goals for this project were to refine existing case sets based on feedback from students and faculty, standardize case sets for terminology, develop additional cases to bring the number to eight for each exercise, and optimize use of relevant diagnostic images, such as ECGs, echocardiograms, x-rays, and MRIs. Further, we developed a complete reference database for images and other multimedia materials incorporated into the case sets. Finally, we have prepared each exercise as a module to submit for publication through MedEdPORTAL. As a requirement of that submission process, we developed objectives and a detailed instructor’s guide for each exercise. In addition, each module was critically reviewed in light of MedEdPORTAL submission standards for scholarship. In our view, these exercises will provide an important resource that could be utilized not only for integrated systems-based preclinical medical education, such as ours, but also as mechanism for review in clerkships or in residency training. The use of such case constructs provides a tool for developing and assessing higher levels of cognitive understanding, such as analysis of clinical presentations, interpretation of lab results, using clinical reasoning to correlate differential diagnoses and therapies for cardiovascular diseases, and synthesize knowledge of pathophysiology with problems in clinical disease.
ARTIFACTS AFFECTING FRET AND FLUORESCENCE MEASUREMENTS. Jordan Lowery. Sponsored by Thomas Rich, Ph.D., Silas Leavesley, Ph.D., Department of Pharmacology, Center for Lung Biology, University of South Alabama College of Medicine, Mobile, AL.

Many diverse cellular functions are regulated by cAMP. The majority of studies monitoring cAMP signals in single cells have relied upon Förster resonance energy transfer (FRET)-based probes. FRET is a common method for measuring protein-protein interactions as well as detecting intracellular signals. FRET involves transfer of energy from a fluorescent donor (CFP) to a fluorescent acceptor (YFP). The distance between these two molecules is inversely proportional to the amount of energy transferred. Energy transfer can be measured as the ratio of the fluorescence emitted by acceptor fluorophore to that of the donor fluorophore. Proteins can be tagged with these fluorophores to monitor interactions in live cells. Unfortunately, changes in FRET signals are often difficult to interpret. This is in part because FRET is exquisitely sensitive to changes in the intracellular environment (e.g., temperature, pH, or ROS). We propose to overcome this limitation by carefully monitoring environmentally-induced changes in emission intensity from cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) as well as CFP-YFP FRET probes. We also propose an approach to correct for effects of environmental variables on FRET measurements. The cAMP probes used in these studies are comprised of a cAMP binding protein (EPAC2) sandwiched between a fluorescent donor (CFP) and acceptor (YFP). FRET-based probes or single fluorophore controls were expressed in HEK-293 cells. Cells were lysed and both excitation and emission spectra were taken at different cAMP concentrations or at different temperatures using a spectrofluorometer. We demonstrated a decrease in fluorescence of both CFP and YFP, with the magnitude of the decrease being greater for CFP. Additionally, we found a decrease in FRET efficiency with increasing temperature. These results indicate that single fluorophore control experiments can be used to compensate for artifacts associated with changes in the intracellular environment. (P01HL066299)
Abstract

The mitochondrial genome is a target for reactive oxygen species generated in ischemia-reperfusion injury.

Winston Crute. Sponsored by Mark Gillespie, Ph.D., Department of Pharmacology, Center for Lung Biology, University of South Alabama College of Medicine, Mobile, AL.

Ischemia-Reperfusion (IR) injury occurs in many organs and is characterized by cell death and dysfunction accompanied by local and/or systemic inflammation. One of the key signaling events in IR injury is increased mitochondrial generation of reactive oxygen species (ROS). Along with serving as a trigger for cell death and dysfunction, mitochondrial ROS production also activates the innate immune system. Very recently, it has been demonstrated that a key inciting event in IR injury is mitochondrial accumulation of succinate, which leads to reversal of the electron transport chain (ETC) and attendant mitochondrial ROS generation from Complex I. Importantly, neither the molecular target of succinate-induced ROS nor the mechanism by which mitochondrial ROS initiates inflammation are known. Because mitochondrial DNA (mtDNA) is highly sensitive to ROS damage and since oxidative mtDNA damage is both cytotoxic and leads to formation of pro-inflammatory mtDNA Damage Associated Molecular Patterns (DAMPs), here we tested the hypothesis that the succinate-induced rise in mitochondrial ROS leads to mtDNA damage and initiates mtDNA DAMP release. Using cultured rat pulmonary arterial endothelial cells (PAECs) as a model system, we measured mitochondrial superoxide anion production by microscopic analysis of CH2XRos fluorescence, mtDNA damage by quantitative southern blot analysis, and mtDNA DAMPs release into culture medium using qRT-PCR. Cells were incubated in culture medium alone or challenged with 4 mM succinate in the absence or presence of the inhibitor of succinate-mediated ETC reversal, malonate. We found that succinate increased the ROS accumulation in the mitochondrial matrix. Malonate failed to influence baseline mitochondrial ROS production but suppressed the increase evoked by succinate. The succinate-induced mitochondrial ROS generation was accompanied by oxidative mtDNA damage and release of mtDNA DAMPS into the culture medium. Collectively, these findings demonstrate in PAECs that a biochemical trigger of IR injury – succinate accumulation - increases mitochondrial ROS generation which targets the mitochondrial genome for oxidative damage and initiates release of pro-inflammatory mtDNA DAMPs. This simple cell culture system may be useful in future studies to delineate mechanisms of IR injury and pathways regulating mtDNA DAMP trafficking.
Corticosteroid insensitivity creates a continual challenge for patients with severe asthma, and thus represents a significant unmet medical need. Because of this, understanding the biology of glucocorticoid actions at a cellular level is crucial to the development new treatment options. Serine/threonine protein phosphatase 5 (PP5) is known to alter glucocorticoid receptor (GR) actions, and more recently PP5 overexpression has been implicated in the promotion of corticosteroid insensitivity in patients with severe asthma¹. These studies suggest that changes in PP5 expression or activity within airway cells could affect corticosteroid responsiveness in patients with corticosteroid-insensitive asthma. The biology surrounding the association of PP5 with HSP90 and the GR is well known. However, the exact action of PP5 on the GR within the airways, or any cell type, is presently unclear. Currently there are no small molecules that specifically inhibit the activity of PP5. However, our co-crystal structures of PP5 in complex with novel 7-oxabicyclo[2.2.1]heptane-based inhibitors revealed that their inability to inhibit PP4 is due to a single amino acid difference (F to W). The structures show close contacts between the inhibitor bridgehead oxygen and both a catalytic metal ion and a non-catalytic phenylalanine residue, which is substituted by tryptophan in PP4. Steric clashes with the bulkier tryptophan side chain force 7-oxabicyclo[2.2.1] heptane-based inhibitors into an unfavorable binding mode, disrupting the strong coordination of active site metal ions observed in PP5 co-crystal structures and thereby rendering PP4 insensitive to this class of inhibitors. With this knowledge we decided to use clustered regularly interspersed short palindromic repeats-Cas9 nuclease (CRISPR-Cas9) system to genetically edit the genes encoding PP2Aα and PP2Aβ, with the goal of generating a cell line in which our novel inhibitors only acted on PP5. To accomplish this we developed a repair template in which the genes encoding PP2Aα and PP2Aβ contain the desired F to W substitution. To test the Cas-9 system, which is dependent upon homology directed repair, first a repair template containing a fluorescent marker was inserted into exon 1 of PP5. This produces a fluorescent cell when PP5 expression is disrupted. After validating the HDR-dependent Cas 9 system, we next employed a similar technique, altering the inhibitor binding site of PP2Aα and PP2Aβ using single stranded oligonucleotides (SSODNs) for repair. One key obstacle in creating these novel cell lines is the isolation of cells with homozygous alterations from a mixed population after transfection with gene altering nucleases and repair templates. In order to isolate and verify the cells of interest, we are developing a PCR based strategy to screen individually sorted cells for homologous recombination in the correct region and orientation.

Poly-(ADP-ribose) polymerase 1 (PARP1) is an enzyme involved in the Base Excision Repair (BER) mechanism that is crucial for the repair of single-stranded DNA breaks. Activated PARP1 synthesizes poly-(ADP-ribose) (PAR) and recruits other BER proteins to the site of DNA lesions to facilitate damage repair. While successful DNA repair attenuates PARP1 activity, unrepaired DNA damage causes hyperactivation of PARP1 followed by NAD⁺ and ATP depletion that will ultimately lead to cell death.

Recent findings from this lab show that the molecular mechanism underlying PARP1 activation is at least partially NAD⁺ independent and PAR synthesis is responsible for the inhibition of hexokinase 1 (HK1), the first enzyme in the glycolysis pathway that converts glucose to glucose-6-phosphate. In this study, we looked at the metabolic consequences of PARP1 activation following exposure to an alkylating agent (MNNG) using two cellular models. We exposed two specific cell types to MNNG; cells overexpressing methyl-specific DNA glycosylase (MPG), which causes an acute response to alkylating damage, and cells lacking expression of poly-(ADP-ribose) glycohydrolase. PARG (PARG-KD). The loss of PARG results in the stabilization of the increased PAR signal. We compared real-time metabolic measurements using an XF24 flux analyzer to evaluate the levels of glycolysis and oxidative phosphorylation and also measured total cellular ATP and NAD⁺ levels. We found that activation of PARP1 in cells over-expressing MPG causes a dramatic decrease in glycolysis measured as ECAR (extra-cellular acidification rate), and was accompanied by decreases in both NAD⁺ and ATP levels. Conversely, activation of PARP1 in cells depleted of PARG, even when expressing a strong PAR signal, neither attenuate glycolysis nor the ATP level. At the same time, we observed a dramatic decrease in NAD⁺ levels in PARG-KD cells. In this study, we employed a metabolic approach to unravel the molecular mechanisms used by cancer cells to respond to genotoxic stress. These findings are consistent with previous studies and support the hypothesis that hyper-activation of PARP1 inhibits glycolytic pathways within the cell in an NAD⁺ independent manner.
ABSTRACT

PREVENTION OF ICU DELIRIUM THROUGH NON-PHARMACOLOGIC SLEEP INTERVENTION. Beth Terry. Sponsored by Sidney Brevard, M.D.; Kaitlin McGinn, Pharm.D.; and Noelle Davis, C.R.N.P., Department of Surgery and Critical Care

Delirium is characterized by disorientation coupled with an acute change in mental status. Delirium develops in up to 80% of mechanically ventilated patients and up to 70% of trauma critical care patients. The development of delirium in the ICU is associated with an increased risk of negative outcomes including increased duration of mechanical ventilation, increased hospital length of stay, and increased mortality. Multi-trauma may cause agitation and altered mental status, and has been shown to predispose patients to developing delirium in the ICU. Management of agitation and delirium can be challenging in multi-trauma patients, but is imperative to facilitate patient recovery and improve functional outcomes. Normalization of sleep-wake cycles has been shown to prevent ICU delirium in a diverse ICU patient population, although there are no high quality studies in patients with multi-trauma. The goal of this study is to develop a protocolized approach to the prevention of ICU delirium, particularly in multi-trauma patients. With the hope of enhancing sleep quality, preventing delirium, and improving patient outcomes. Implementing this protocol should improve sleep quality and this should lead to decreased development of delirium. The prevention of delirium should also lead to better outcomes including decreased length of stay and decreased mortality. This study is a single center, observational, pre-post study. All of the participants were selected because they were patients in the Surgery Trauma ICU and fit the following criteria: over the age of 19, were a multi-trauma case, and remained in the ICU for >72 hours. Once a sufficient number of participants have been included in the study, the primary outcome of development of delirium based on CAM-ICU score and the secondary outcomes of duration of delirium, ICU length of stay, hospital length of stay, duration of mechanical ventilation, in hospital mortality, and benzodiazepine usage will be assessed for both the pre and the post groups.
Sialic acid (N-acetylneuraminic acid) is commonly found as a terminal residue on the glycocalyx of different cell types. In pulmonary endothelial cells, sialic acids are important for barrier integrity, as removal of sialic acids increased endothelial permeability. In addition, chemically modifying this residue has been shown to have many different effects. For example, it has been shown in neuronal cells that modified sialic acid residues can induce cell proliferation. We hypothesized that chemically modifying N-acetylneuraminic acid on the nitrogen in endothelial cells would have effects regarding the properties of the cell—specifically proliferation, migration, and barrier integrity. In order to synthesize modified sialic acids, we took advantage of the biosynthetic pathway of sialic acid in the cell. We prepared derivatives of the mannosamine precursor (N-butyl, N-propyl, and N-acetylmannosamine). The mannosamine derivatives were then added to endothelial cell lines derived from the pulmonary artery and the microvasculature. In order to test the effects of these modified sialic acids on endothelial barrier integrity, we performed electrical cell substrate impedance sensing (ECIS). To determine the effects on cell migration, we performed wound assay experiments. To determine the effects on vasculogenesis, we performed a Matrigel assay. Finally, to see if the modified precursors had any effect on proliferation, a growth curve was measured. Synthesis of the modified mannosamine precursors was successful. However, technical difficulties extended the time needed for the synthesis. Thus, evaluation of the effects of modified sialic acids on endothelial cells is still in progress.
CO-LOCALIZATION OF SK3 AND TRPV4 IN CAVEOLAE. Justin Jong.
Sponsored by Mike Lin, Ph.D., Mark Taylor, Ph.D., David Weber, Ph.D., and Xiangming Zha, Ph.D. Department of Physiology and Cell Biology, University of South Alabama College of Medicine, Mobile, AL.

Small-conductance Ca^{2+}-activated K^+ (SK3) channels and Ca^{2+}-permeable transient receptor potential (TRPV4) channels are expressed in vascular endothelium. Their activities play an important role in modulating endothelial permeability, especially in microvasculature. Previous studies have shown that in pulmonary circulation, TRPV4-induced Ca^{2+} influx, which activates SK3 channels, leads to pulmonary edema. Currently, this interaction is poorly understood, but there is evidence that it may occur within caveolae, invaginations of the plasma membrane. Caveolae play an important role in signal transduction and are predominantly formed by and lined with caveolin1 (Cav1) proteins. Previous studies have shown that SK3 channels interact directly with Cav1; however, it is currently unknown how TRPV4 channels are localized in caveolae, and whether they form interactions with SK3 channels. To investigate this interaction, we have successfully identified the molecular Cav1 interaction domain on SK3 required for SK3-Cav1 interaction, and developed an SK3 channel mutant that does not bind Cav1. To examine SK3 and TRPV4 channel co-localization in caveolae, I performed transfection and immunostaining techniques using human embryonic kidney (HEK) cells. The results showed that compared to control cells expressing wild-type SK3, cells expressing mutant SK3 channels had less co-localization of SK3 and TRPV4; the areas that did show co-localization were much less uniform across the cell. Therefore, SK3 channel expression within caveolae may play an important role in regulating caveolar expression of TRPV4 channels.
ABSTRACT

HSP27 AS A NOVEL BIOMARKER FOR CAD AND ASSESSING THE RISK OF ACUTE CORONARY SYNDROME – C. Blair Gaines. Sponsored by William Gerthoffer, Ph.D., Department of Biochemistry and Molecular Biology, University of South Alabama College of Medicine, and Clara Massey, M.D., Alexandria Hellmich, Dr. med., Division of Cardiology, Department of Internal Medicine., University of South Alabama Medical Center, Mobile AL.

CAD is the leading cause of death for Americans, with much higher rates seen in African Americans and southeast US. Recent studies have shown that HSP27 and naturally produced HSP27 autoantibodies play a role in the progression of CAD. HSP27 is an anti-atherogenic protein that is important in preventing inflammation and cholesterol uptake. HSP27 autoantibodies form immune complexes with HSP27 and are involved in extracellular signaling. This study focused on comparing HSP27 and HSP27 autoantibody levels between healthy subjects and CAD patients, and how these levels increased in CAD patients following an acute coronary event. Our goal is to find a more affordable, non-invasive method of identifying the risk of acute coronary event in underserved/high risk populations, the vast majority of whom present at later stages of the disease and with multiple co-morbidities that negatively impact their clinical outcomes. As part of this five-year longitudinal cohort study, we obtained blood samples from 272 CAD patients seen at the USA Medical Center Cardiology Clinic every six months. In collaboration with Dr. Edward O’Brien, serum HSP27 and HSP27 autoantibodies were measured in our study patients and in a smaller 79 patient cohort whose subjects were similar in age, gender, and race to the patients seen in the clinic. We found that these protein and autoantibody levels were lower in patients with CAD than in the healthy controls, consistent with our hypothesis that lower levels of HSP27 and HSP27 autoantibody correlate to increasing plaque instability. The results to this point are promising, as we are now in year 3 of the study. Moving forward we hope to gain more data on HSP27 and HSP27 autoantibody levels following an acute coronary event that may ultimately provide significant prognostic value for cardiologists in predicting a patient’s clinical outcome following treatment.
ABSTRACT

ELECTRONIC HEALTH RECORD AND DELAYS IN CHART COMPLETION. Amy Traylor. Sponsored by Carol Motley, M.D., Department of Family Medicine, University of South Alabama College of Medicine, Mobile, AL.

As more incentives are created for physicians to use electronic health records, it is becoming increasingly important to ensure that documentation is completed efficiently. Previous studies have shown that physicians may spend as much time documenting a patient encounter as they spend face-to-face with the patient. This is partially due to the construction of the electronic health records (EHR) system, which facilitates billing and other concerns rather than clinically relevant information. A survey was constructed to determine what providers in family medicine and pediatrics in USA Health Systems perceived delayed or improved documentation time. A retrospective chart analysis was also performed to determine the current lag times in chart completion and what factors contribute to delinquent charts. Delinquent charts were considered to be those that took longer than two days to complete. A risk assessment score developed by the Department of Family Medicine was used to estimate complexity for each patient; patients per half day, number of days in clinic, and the number of acute and chronic problems were also looked at for each encounter by provider and level of training. The average lag time overall was 10.6 days and 25% of charts were found to be delinquent. Although every provider surveyed agreed that greater complexity of the patient/number of acute problems increases lag time, this was not substantiated by the chart review. Lag time increased as the number of patients seen per half day increased and as the providers level of training increased, with attending physicians having the highest average lag time at 22 days. No significant differences in documentation time were noted between the family medicine and pediatrics departments, although the family medicine department saw patients with higher risk assessment scores and more chronic problems on average. These findings suggest that scheduling fewer patients per session for attending physicians, third year residents, and nurse practitioners may decrease the average chart completion time.
ABSTRACT

Breast Cancer (BC) is the most common type of cancer in U.S and the second most frequent cause of death from cancer in women. African American (AA) women are more likely to have aggressive breast cancer and experience greater mortality rates as compared to Caucasian (CA) women. It is being increasingly appreciated that tumor microenvironment (TME) plays an important role in BC racial disparity likely due to prevalence of obesity-associated inflammation in AA women. Findings suggest the existence of a TME-tumor-cell interaction driven regulatory loop, which is predominantly active in AA patients. Resistin and IL-6 have been shown to be a part of this regulatory loop. We hypothesize that an intrinsic increase in these particular cytokines occurs in AA BC patients. To examine our hypothesis, blood was taken from both AA and CA patients with varying forms of breast cancer, and at different stages of treatment. The serum was extracted from the blood of these patients, frozen, and ELISA assay was performed to measure the presence and amount of resistin and IL-6. We found that serum levels of resistin and IL-6 are significantly elevated in AA BC patients compared to their CA counterparts. These findings suggest that intrinsic differences in tumor biology contribute to BC racial disparity and these cytokines are a part of an important regulatory loop controlling the aggressive and therapy-resistant phenotypes of BC cells.
ABSTRACT

Cloning and expression of recombinant PARP4 and ALDH1A3 in mesenchymal glioma stem cells. Ben Bush. Sponsored by Robert W. Sobol, Ph.D., Mitchell Cancer Institute, University of South Alabama, Mobile, AL.

Previous findings from this lab have shown that mesenchymal glioma stem cells (Mes GSC) and proneural glioma stem cells (PN GSC) contain genetically distinct mRNA profiles. Mes GSCs are more often found in high-grade glioma (according to WHO grading scales). The highly unique mRNA profiles present the possibility of specific drug targets. We sought to investigate two possible drug targets: PARP4 and ALDH1A3. Each have been found to have elevated and unique expression profiles in Mes GSCs. Given the information available as to the importance of ADP-ribosylation on DNA repair and gene expression, the poly-ADP-ribose polymerase family (PARP) was analyzed for expression in Mes GSC. It was found that PARP4 was expressed at a considerably higher level than any of the 17 other PARPs. Additionally, high levels of only one aldehyde dehydrogenase (ALDH) isotype (1A3) were found in Mes GSC when compared to PN GSC. Although much is known about the chemical function of both of these classes of proteins, little is known about the biological function of these proteins in Mes GSC. We suggest that both proteins could be potential targets for drug therapies of high-grade, mesenchymal-derived glioma. To better understand the role that these proteins play in Mes GSC phenotype, our next step was to clone each cDNA for expression in Mes GSCs so as to provide the tools needed to evaluate the function of each. First, we cloned both PARP4 and ALDH1A3 as a fusion protein with green florescent protein (GFP) for expression in Mes GSCs cells. These GFP-fusion constructs were then sub-cloned into pLENTI-NEO vectors for lentivirus production and subsequent transduction of Mes GSC, which was confirmed by imaging of GFP. These constructs will aid in future investigation of the function and significance of PARP4 and ALDH1A3 in Mes GSC.
CHARACTERIZATION OF CYTOTOXIN INDUCED ENDOTHELIAL INCLUSION BODIES. Richard Huettemann. Sponsored by K Adam Morrow, Ph.D., Ron Balczon Ph.D., Troy Stevens, Ph.D., Department of Physiology, Center for Lung Biology, University of South Alabama College of Medicine, Mobile, AL.

*Pseudomonas aeruginosa* infection of pulmonary endothelium results in prolonged permeability defects due to microtubule de-stabilization. *P. aeruginosa* possesses a type III secretion system (T3SS), through which it injects effector exoenzymes, including ExoS, ExoT, ExoU, and ExoY. ExoU, a cytotoxic phospholipase, and ExoY, a soluble purine and pyrimidine cyclase, are more recognized because of their pathogenicity and ability to induce inter-endothelial gap formation through de-stabilization of microtubules. Infection promotes cellular release of microtubule-stabilizing protein(s), such as hyperphosphorylated tau. Transfer of these proteins onto naïve cells induces cytotoxicity, intracellular tau aggregates, and the formation of cytosolic inclusion bodies, characteristic of a transmissible proteinopathy. At present, the composition and formation of these structures is unknown. We therefore sought to characterize the prevalence and molecular anatomy of these cytosolic bodies generated by the cytotoxic supernatant. To address this, we infected pulmonary microvascular endothelial cells (PMVECs) with four separate *P. aeruginosa* strains: ExoY⁺ (introduces a catalytically active ExoY), ExoY\textsuperscript{K81M} (introduces a catalytically inactive ExoY), PA103 (possesses ExoU and ExoT), and ΔUΔT (has a functional T3SS but no exoenzymes). After infection, supernatant was collected, filter-sterilized, and applied to naïve PMVECs for 20-22 hours. We found that supernatant collected from PA103 generated a higher number of these cytosolic bodies when compared to ΔUΔT. We found no significant difference between ExoY⁺ and ExoY\textsuperscript{K81M} in their ability to generate cytosolic inclusion bodies. In addition to infection, to test the specific role of ExoU in generation of the cytotoxic supernatant, an ExoU-inducible system in PMVECs was utilized where we induced ExoU expression for either 1 hour or 6 hours. At 1 hour post-ExoU induction, there was no apparent cellular damage or detectable extracellular tau. However, by 6 hours, there was significant cellular damage and abundant extracellular tau. At each time point, supernatant was collected, filter-sterilized, and applied to naïve PMVECs. Supernatant from 6 hours of ExoU induction produced significantly more cytosolic inclusion bodies when compared to supernatant from 1 hour ExoU induction. Because PA103 consistently generated a high number of these inclusion bodies, we applied this supernatant to naïve PMVECs for ~16 hours, at which point the cells were fixed, and stained using the LC3B antibody as an autophagy marker. Although prominent LC3B staining was observed, it did not co-localize with the inclusion bodies, suggesting that the bodies do not have an auto-phagocytic role. Together, these data suggest that these cytosolic inclusion bodies can be formed following supernatant application from multiple *P. aeruginosa* strains to naïve cells and that the structures are not consistent with auto-phagosome function.
Colorectal cancer is the second most common cancer and the second leading cause of cancer related deaths among men and women in the United States\(^1\). In 2014, there were approximately 2,350 individuals newly diagnosed with colorectal cancer and 950 colorectal cancer deaths in Alabama\(^2\). Current screening tests for colorectal cancer have the ability to both detect the disease at an early stage when the 5-year survival rate is over 90 percent, as well as prevent the disease altogether through the removal of precancerous polyps\(^1\). Screening tests along with the defined up-to-date status includes: a colonoscopy every 10 years, a flexible sigmoidoscopy every 5 years or a fecal occult blood test within the past year. However, despite the overall effectiveness of colorectal cancer screening, more than 40 percent of colorectal cancer diagnoses in Alabama are made at a late stage (stage 3 or 4) and only 66 percent of current Alabamians (age 50 and older) have been appropriately screened\(^3,4\). The Center of Disease Control (CDC) and the American Cancer Society are working at a national level to increase the CRC screening rate to 80% by 2018. The CDC along with the Alabama Department of Public Health (ADPH) funded a study to gain pilot data and qualitative information about innovative ways to improve colorectal cancer screening. Working with Margaret Sullivan, Kristen Van Buren, Austin Cadden and the rest of the team at the Mitchell Cancer Institute we sought to improve colorectal cancer screening rates though a worksite wellness initiative involving the three employer groups: Evonik, the University of South Alabama, and Austal. Our study is based on the premise that targeted messaging and educational outreach improves colorectal cancer screenings rates. Baseline colorectal cancer screening rates would be determined and compared to screening rates obtained through the intervention to determine the validity and efficiency of our interventions. The University of South Alabama was the first site to complete this intervention, allowing us to analyze that data and gather results. The baseline up-to-date screening rate for the population (n=146) was 51.9%, well short of the 80% target. After our interventions at this site, up-to-date colorectal cancer screening rates were raised to 86.3%, which is above the target set by CDC and American Cancer Society. These findings suggest that the cost-effective worksite interventions do have a successful impact on a percentage of the population to become up-to-date on their colorectal screening. Along with this finding, we educated hundreds of USA employees and dependents, aged 50 or older about the benefits of screening for colorectal cancer.
ABSTRACT

C4d EXPRESSION IN RENAL ALLOGRAFT BIOPSIES USING IMMUNOHISTOCHEMISTRY. Evan Davidson. Sponsored by Andrea G. Kahn, M.D., Javier A. Laurini, M.D. Department of Pathology, University of South Alabama Medical Center, Mobile, AL.

The role of classical complement cleavage product, C4d, has been well established in kidney allograft failure, and its detection is considered a key part of transplant failure workup. The Banff working classification has a scoring system based on the percentage of peritubular capillary staining. Literature regards the C4d detection by immunofluorescence (IF) as a highly specific and sensitive method; however, there are caveats associated with this technique, namely, fading of IF signal over time, increased turnaround time, and associated laboratory technician labor. Commercial immunohistochemical (IHC) antibodies for C4d have evolved as an alternative to IF and have demonstrated variable detection results in comparative studies. The benefits of C4d by IHC over IF include: superior turnaround time, decreased technician labor, and indefinite storage of slide preparations with good quality signal.

The goal of the study is to determine the analytical concordance between archived pathology renal allograft biopsies that utilized IF technique for C4d scoring with an IHC C4d stain protocol.

We evaluated renal allograft biopsy specimens obtained from the archives of the USA Department of Pathology. The database was searched for ‘renal allograft biopsy’ and ‘C4d.’ Cases with previously reported C4d immunofluorescence, both positive and negative, with available slides for review were used for the study, and the slides along with the paraffin blocks were retrieved from the archives. The C4d IHC stain (SP91, Rabbit Monoclonal, Cell Marque, Rocklin, CA) was optimized on the Dako Link Autostainer with appropriate positive controls. New sections of the paraffin blocks were cut at 4 micrometers and subjected to the newly optimized C4d IHC stain. The original case IF slides were evaluated according to the 2007 Banff criteria for C4d scoring and established to be positive (C4d2 or C4d3) or negative (C4d0 and C4d1). The newly cut and stained IHC slides were evaluated with the same criteria. This evaluation was double blinded. The original IF slides were scored to be considered the gold standard, since IF has well documented specificity and sensitivity. The scores were placed into a spreadsheet to determine analytical specificity, sensitivity, and overall concordance.

A total of 42 cases were included in this study dating back to 2003. The expected results from IF slide evaluation scored 22 C4d positive cases and 20 negative cases, and, comparatively, the IHC C4d preparations scored 21 positive cases and 21 negative cases, which is accounted for by one false negative case on IHC. The quality of the archived IF slides was optimal and the comparative IHC slides had a similar pattern of staining, in most cases. Overall the analytical sensitivity, specificity, and overall concordance were 95.4%, 100%, and 97.6%, respectively.

We conclude that the C4d IHC stain has satisfactory concordance with the IF technique, and that the protocol could be beneficial in future renal allograft kidney biopsy workups and clinical use.
ABSTRACT

PHOSPHODIESTERASE10A EXPRESSION IN COLORECTAL CANCER. Alisa Trinh. Sponsored by Gary Piazza, Ph.D., Drug Discovery Lab, Mitchell Cancer Institute, University of South Alabama College of Medicine, Mobile, AL.

Phosphodiesterase 10A (PDE10) has been studied as a therapeutic target in neurological conditions, but emerging evidence indicate that overexpression of this enzyme may also play a role in colon adenomas and adenocarcinomas as previously reported by this lab. To determine the clinical relevance of PDE10 as a human biomarker for colorectal cancer, we analyzed a collection of colon tumors with matching uninvolved colon collected from 12 patients at Mitchell Cancer Institute for PDE10 mRNA expression using real-time polymerase chain reaction (RT-PCR). We found that 6 patients had significantly increased expression of PDE10 in the tumors compared to uninvolved tissue counterpart. These results suggest that PDE10 could potentially serve as a diagnostic marker for cancer as well as provide further support for PDE10 as a novel cancer target.
ABSTRACT

THE EFFECT OF HEAT SHOCK PROTEIN 90 INHIBITOR 17-AAG ON TRIPLE NEGATIVE BREAST CANCER CELLS  Thomas Lunsford. Sponsored by Ming Tan, M.D., Ph.D., Shruti Desai, Ph.D., and Susan LeDoux, Ph.D. Mitchell Cancer Institute, University of South Alabama, Mobile, AL.

The heat shock response is an evolutionarily conserved pathway that cells use to combat a wide variety of environmental stresses. The master regulator of this pathway is the transcription factor Heat Shock Factor 1 (HSF1) which controls the expression of numerous chaperone proteins known collectively as heat shock proteins (HSPs). Recently, researchers have sought to understand the roles HSF1 and HSPs play in the survival of cancer cells. It is now well established that breast cancer cells express increased levels of HSF1 and that this is associated with poor prognoses. This knowledge has encouraged researchers to develop new drugs that specifically target the heat shock pathway. These drugs will be of particular importance to patients diagnosed with triple-negative breast cancer (TNBC) which lacks genetic expression of the estrogen receptor, progesterone receptor, and Her2 receptor. Many antineoplastic drugs commonly used to treat breast cancer target one of these receptors and are thus ineffective against TNBC. One drug which researchers hope may prove to be useful against TNBC is 17-N-allylamino-17-demethoxygeldanamycin (17-AAG). Rather than targeting the estrogen receptor, progesterone receptor, or Her2 receptor, 17-AAG targets HSP90, one of the many chaperone proteins regulated by HSF1. We proposed that TNBC cells with diminished expression of HSF1 would experience higher rates of apoptosis when treated with 17-AAG than TNBC cells with normal expression of HSF1. We incubated two sets of TNBC cells with 17-AAG over a twenty-four hour period, one set with normal expression of HSF1 and one with HSF1 expression stably knocked down by shRNA. Apoptosis significantly increased directly with dose level of 17-AAG in both sets of cells. However, we saw greater rates of apoptosis in HSF1 knockdown cells as compared to the control. These findings suggest that 17-AAG may be most effective in patients whose cancer cells naturally express low levels of HSF1 or in patients on an HSF1 inhibitor. This study provides important information for the development of urgently needed treatment strategy for TNBC.
FAK REGULATES EMT-ASSOCIATED GENE EXPRESSION VIA CHANGES IN DNA METHYLATION. Alex Koichi Sponsored by Steve Lim, Ph.D., Department of Biochemistry & Molecular Biology, University of South Alabama College of Medicine, Mobile, AL.

Focal Adhesion Kinase (FAK) is a non-receptor protein tyrosine kinase that plays a major role in regulating cellular adhesion, motility, and proliferation. FAK overexpression in cancer has been correlated with a poor prognosis, and FAK inhibitors in human clinical trials have demonstrated a potential in tumor therapeutics. FAK can function in a kinase-dependent as well as in a kinase-independent manner. FAK inhibition reduces FAK activity, and at the same time, also promotes FAK nuclear localization. Increasing evidence suggests that nuclear FAK functions contribute to a wide array of gene regulation mechanisms. Recently, we found that nuclear FAK reduces the expression of DNA methyltransferase 3A (DNMT3A) via enhanced ubiquitination-proteasome degradation in primary smooth muscle cells, and the reduced DNA methylation prevented hyperplasia by promoting anti-hyperplasia gene expression. We have attempted to determine if FAK regulates DNMT3A in human breast cancer, MCF-7 and affects cancer phenotypes such as epithelial-to-mesenchymal transition (EMT) by modulation of gene expression.

In order to test if FAK regulates DNMT3A in MCF-7, FAK inhibitor (VS-4718) was treated for varying periods of time. We found that upon FAK inhibition for 3h, DNMT3A expression was decreased, and addition of a proteasome inhibitor, MG132, recovered DNMT3A expression suggesting that FAK is promoting DNMT3A degradation. We then investigated if decreased DNMT3A levels are correlated with changes in the DNA methylation. Anti-5mC (5-methylcytosine) dot blotting indicated that FAK inhibition significantly decreased DNA methylation levels in MCF-7. To test if reduced DNA methylation is associated with altered gene expression in MCF-7, among many methylation-sensitive genes, Forkhead Box F2 (FoxF2), an EMT suppressor, was chosen. Interestingly, FAK inhibition increased levels of FoxF2 protein, which in turn increased an epithelial cell marker, E-cadherin confirmed by western blotting. Cell staining also verified a much higher E-cadherin expression in FAK inhibitor-treated cells.

We conclude that FAK inhibition reduces DNMT3A expression in MCF-7 breast cancer, and loss of DNMT3A further regulates EMT-associated gene expression to prevent EMT. These findings uncover a potential kinase-independent role of FAK in cancer therapeutics.
Recent research has shown patients who are more actively engaged in their health care experience have better health outcomes and incur lower costs. Patient engagement includes educating them about their condition and involving them more fully in making decisions about their care. An example of patient engagement is shared decision-making in a preference-sensitive condition in which patients and providers together consider the patient's condition, treatment options, the medical evidence behind the options, the benefits and risks of treatment, and patient's preferences, and then arrive at and execute a treatment plan. Shared decision-making is patient-specific and influenced by many factors such as culture, language, sex, age, and education. However, it is uncertain how patients perceive shared decision-making. Our goal this summer was to determine if the patients at USA Family Medicine understand the concept of shared decision-making. In order to measure the level of understanding, we administered a questionnaire to all of the patients 19 years and older that was made up of certain scenarios to test their knowledge of the subject.
ABSTRACT

THE EFFECTS OF MITOCHONDRIAL DNA ON B-CELL TOLERANCE AND PROLIFERATION. Greg Van Wagner. Sponsored by Robert A. Barrington, Ph.D., Department of Microbiology and Immunology and Lyudmila Rachek, Ph.D., Department of Pharmacology, University of South Alabama College of Medicine, Mobile, AL.

Systemic Lupus Erythematosus and Rheumatoid Arthritis are both autoimmune disorders with complex etiologies whereby self-reactive B and T cells break immune tolerance. B cells play an important role in the adaptive immune system and are responsible for antibody production. In healthy individuals, B cells that bind self-antigen undergo immune tolerance by either clonal deletion or by rendering them non-responsive (called anergy). Interestingly, engagement of both B cell receptor and toll-like receptor 9 (TLR-9), an endogenous pattern recognition receptor that responds to double-stranded DNA, induces breaks in B cell tolerance, leading to autoantibody production and autoimmune disease. We hypothesized that oxidatively damaged mitochondrial DNA (mtDNA) is a TLR-9 ligand responsible for mediating breaks in B cell tolerance. Two major approaches tested this hypothesis: 1) First, we examined whether disrupting TLR signaling only in B cells (referred to as B cell-MyD88) reduced the frequency and development of autoreactive B cells in autoimmune-prone mice; and 2) to directly test whether oxidatively damaged mtDNA was a potent TLR-9 agonist in B cells, we oxidatively damaged mtDNA PCR products using H$_2$O$_2$ and tested whether these products could induce proliferation of splenic mouse B cells. Our in vivo studies using autoimmune-prone mice revealed that B cell-MyD88 mice generated fewer germinal centers, structures required for antibody production, compared to autoimmune-prone mice with intact TLR signaling. Moreover, using mice harboring an autoreactive B cell receptor transgene (called 564Igi) to identify known autoreactive B cells, we observed that the frequency and number of 564Igi B cells was reduced in B cell-MyD88 mice compared to autoimmune-prone mice with MyD88. In vitro, we determined that, whereas undamaged mtDNA PCR products induced modest B cell proliferation in the presence of B cell receptor crosslinking, the combination of oxidatively damaged mtDNA and B cell receptor crosslinking induced proliferation in a larger fraction of mouse B cells. These preliminary data provide a proof-of-concept supporting that oxidatively damaged mtDNA can have a role in mediating autoimmunity in mice.
Pulmonary Arterial Hypertension (PAH) is a devastating disease in which mean survival from diagnosis is only 2.8 years and for which, despite efforts, there is currently no effective treatment. Although the mechanism of PAH is largely unknown, inflammation appears to play a role in the vascular remodeling that is typical of this disease. Recent findings indicate that microparticles isolated from the blood of PAH rat models induce increased intracellular adhesion molecule (ICAM-1) expression in pulmonary endothelial cells when compared to treatment with microparticles from control animals. It has also been found that the protein alpha-pix, a guanine exchange factor responsible for downstream ICAM-1 expression, is found in the microparticles of PAH patients at concentrations seven times that of microparticles isolated from control patients- causing speculation that this protein may be the source of increased ICAM-1 expression in the PAH microparticle-treated endothelial cells. Before it can be determined whether the microparticles are delivering or stimulating increased alpha-pix expression, we first had to determine the constitutive expression of alpha-pix in pulmonary endothelial cells. We used several methods to confirm protein expression in both pulmonary microvascular endothelial cells (PMVECs) and pulmonary artery endothelial cells (PAECs) including western blotting, immunocytochemistry, and flow cytometry. These methods proved that there was alpha-pix expression in both PMVECs and PAECs, but that expression was considerably higher in PAECs (25% vs 2%) and that localization of the protein was perinuclear. These results suggest that alpha-pix is expressed in both endothelial cell types but more abundantly and with clear nuclear localization in the pulmonary artery endothelial cells. These findings may contribute to our knowledge of the function of alpha-pix in PAH and help us determine the endothelial response to circulating microparticles in the setting of pulmonary arterial hypertension.
ABSTRACT

USING LIFE EXPECTANCY TO INFORM CANCER SCREENING DECISION-MAKING. Danielle Akira-Keyes. Sponsored by Dr. Gerald Liu, MD, USA Family Medicine and Dr. Allen Perkins, MD, MPH, USA Family Medicine

Screening elderly patients for cancer is challenging because it is unclear how the screening impacts quality of life. A proposed framework for decision making in the context of cancer screening in older patients includes considering quantitative estimates of life expectancy, risk of death from cancer, screening outcomes, and qualitative consideration of the estimated benefits and harms based on a patient’s unique values and preferences. Physicians believe that having a tool to estimate life expectancy when care is established could improve decision-making around the patients’ care. Using a prediction model for screening could lead to a decrease in over-screening and under-screening, by clarifying the risks and benefits for each individual patient based on their health status. The purpose of this study is to determine if patients are willing to use estimated life expectancy to inform decisions about whether a patient should be screened for cancer. Adults over the age of 19 were surveyed in clinic and they were given four patient scenarios. The patient scenarios consisted of two patients who were expected to be alive in ten years and two who were not expected to be alive in ten years. Determining if those surveyed think these patients should be screened differently or the same will give physicians insight into patients’ willingness to allow a prognostic index be used in determining appropriate screening care.
ABSTRACT

MORPHOMETRY OF ALVEOLAR CAPILLARY ENDOTHELIUM: RESOURCE DEVELOPMENT FOR MODELING OF CALCIUM SIGNALING C. Alex Wiles. Sponsored by Mary Townsley, Ph.D., Department of Physiology & Cell Biology, and Thomas Rich, Ph.D., Department of Pharmacology. University of South Alabama College of Medicine, Mobile, AL.

Calcium (Ca\(^{2+}\)) serves as an intracellular signaling molecule in several diverse pathways. The effect that Ca\(^{2+}\) entry has on a cell varies greatly depending upon the cell type, the channel of entry, or the set of effectors with which it interacts. An example of this diversity has been observed in pulmonary microvascular endothelial cells in the alveolar septal wall (PMVECs). PMVECs express both the voltage-gated T-type Ca\(^{2+}\) channel and the non-selective cation channel TRPV4. Interestingly, Ca\(^{2+}\) entry via the T-type channel leads to increased expression of P-selectin on the endothelial surface, while Ca\(^{2+}\) entry via TRPV4 leads to increased endothelial permeability. Observing these discrete outcomes despite similar Ca\(^{2+}\) entry raises the question of how specificity in the cell’s functional response is organized. In an effort to gain insight into this problem, a mathematical model of a PMVEC with realistic geometry will be constructed to predict Ca\(^{2+}\) concentrations at various locations within the cell after activation of the T-type channel or TRPV4. The basis of this project has been to collect the quantitative data required to populate the model. First, we conducted a thorough literature review to identify existing data on the biophysical properties of these two Ca\(^{2+}\) channels of interest, septal endothelial cell morphometry, and properties of Ca\(^{2+}\) diffusion in cells. We found that the T-type channel has a conductance of 7 pS and a channel density estimated at 48 channels per cell, while the TRPV4 channel conductance and density is much greater at 60 pS and 150 channels per cell. Similar data was gathered for calcium-gated potassium channels that are coupled with TRPV4 and the T-type channel (conductances: BK: 200 pS; IK: 40 pS; SK: 10 pS; K_\(c\): 25 pS). Together with membrane potential, gating of these latter channels sets the electrochemical gradient for Ca\(^{2+}\) entry. Second, we performed stereological morphometric analysis of septal capillary endothelial cells (n=105) to assess the density and location of possible Ca\(^{2+}\) buffering organelles. Using electron micrographs (25,000x) from mouse lung, we have determined volume densities, relative to total cell volume, for the nucleus (22.8%), mitochondria (2.7%), and endoplasmic reticulum (ER, 1.7%). Further, we identified proportions of mitochondria (28.2% near junctions; 12.9% near the nucleus) and ER (27.4% junctional; 25.7% nuclear). The large proportion of organelles located near cell junctions, areas of PMVECs that are often no more than 200 nm thick, was surprising. Third, as a starting point, we have constructed a theoretical three-compartment model of a PMVEC expressing TRPV4. Subsequent model development will incorporate the data catalogued from this project. The fully functioning model will assess the impact that various factors of Ca\(^{2+}\) spread and constraint by buffers play in determining the cell’s outcome from an initial Ca\(^{2+}\) signal.
ABSTRACT

ENDOTHELIAL CALCIUM SIGNALING IN A PARTIAL LIGATION LOW-FLOW VASCULAR INJURY MODEL. Katie Glosemeyer. Sponsored by Mark S. Taylor, Ph.D. and David Weber, Ph.D., Department of Physiology and Cell Biology, University of South Alabama College of Medicine, Mobile, AL.

Endothelial dysfunction disrupts physiological signaling cascades in a wide variety of cardiovascular pathologies including coronary artery and peripheral artery disease. These pathologies are associated with flow disturbance, vascular remodeling, and progressive loss of vasodilator signaling due to decreased nitric oxide (NO) production, potentially a result of altered endothelial cell Ca2+ dynamics. In our study, the role of disturbed flow and low shear was investigated in mice carotid arteries for overt remodeling and alterations in endothelial Ca2+-dependent NO signaling. To examine the effects of solely disturbed flow, three of superior branches of the left common carotid were ligated while the superior thyroid artery remained patent, introducing the endothelium to low-flow. After two-weeks recovery from surgical ligation, the left and right common carotid arteries of mice were harvested to characterize the degree of injury and examine Ca2+-dynamics and NO signaling patterns through imaging. We found vascular remodeling indicative of significant neointima formation with an average intima to media ratio in ligated vessels being .42 ± .13, while minimal change noted in the medial area (~10% increase). No remodeling occurred in the non-ligated contralateral control carotids. Using Fluo-4AM as a fluorescence indicator, positive calcium signaling was observed in both the injured and control carotid arteries after the administration of acetylcholine. Ligated arteries demonstrated a decrease in the number of sites and the duration of Ca2+ events. To detect and characterize NO production DAR-AM was used. Our current observations indicated minimal involvement of NO in either control or ligated carotid arteries in the response to acetylcholine and immunostaining confirmed similar expression patterns of eNOS in both the injured and control arteries. At this time, the role of calcium-dependent NO signaling in the carotid arteries warrants further investigation.
ELECTRON TRANSPORT CHAIN MODULATION OF MITOCHONDRIAL DISTRIBUTION IN PULMONARY ARTERY ENDOTHELIAL CELLS. Kyle Duncan. Sponsored by Abu-Bakr Al-Mehdi, M.D., Ph.D., Department of Pharmacology, University of South Alabama College of Medicine, Mobile, AL.

Redistribution of mitochondria within the cytoplasm of cells stressed by hypoxia or other stimuli plays an important role in mitochondrial signaling pathways. Although the mechanism of mitochondrial translocation is known, the second messengers that initiate and terminate their movement have not been defined. Here we tested the hypothesis that modulation of two second messengers elaborated in hypoxia - ATP and reactive oxygen species (ROS) - are determinants of mitochondrial translocation. Rat pulmonary artery endothelial cells were labeled with MitoTracker Red and subjected to hypoxia. ATP and ROS levels were modulated with succinate and oligomycin. Cytoplasmic mitochondrial disposition was evaluated by fluorescence microscopy and quantitative image analysis. As anticipated, hypoxia engendered perinuclear mitochondrial clustering. Under normoxic conditions, succinate also led perinuclear clustering, and when combined with hypoxia, enhanced translocation of mitochondria to the perinuclear region. These data suggest that ATP and/or ROS generated by complex I promotes mitochondrial perinuclear clustering in endothelial cells and exaggerate signaling events activated by hypoxia to direct mitochondrial translocation.
ABSTRACT

INVESTIGATING THE SECRETION AND ROLE OF B7-H3 (CD276) IN OVARIAN CANCER.  Imran Mohiuddin. Ileana Aragon. Sponsored by Jennifer Scalici, M.D., Rodney Rocconi, M.D., and Luciana Madeira da Silva, Ph.D., Department of Gynecologic Oncology, University of South Alabama Mitchell Cancer Institute, Mobile, AL.

Ovarian cancer is currently the leading cause of death from gynecological malignancies, with approximately 70% of patients diagnosed in the advanced stage of the disease, and a five year survival rate of only 20%. However, survival rates climb to 90% when patients are diagnosed in the early stages, highlighting the effectiveness of biomarker development and other early detection methods in combating the disease. B7-H3 (CD-276), is a recently discovered cell surface glycoprotein biomarker found on immune cells, that is overexpressed in a variety of cancers. Moreover, B7-H3 overexpression has been associated with tumor progression, metastasis and poor patient outcome. In this study, we analyzed tumor samples from the Mitchell Cancer Biobank, in order to determine if B7-H3 is differentially expressed in ovarian tumors when compared to normal ovarian tissues obtained from the same patients. Analysis of the B7-H3 mRNA levels by qPCR showed no consistent pattern of overexpression between tumors and normal tissue, but preliminary western blots showed that B7-H3 is overexpressed at the protein level in 8 ovarian solid tumors when compared to normal matched tissue. Likewise, we performed an ELISA assay on malignant ascites samples obtained from ovarian cancer patients, which showed that B7-H3 is not limited to solid tumors but is also secreted into the peritoneal fluid. In the future, we plan on finishing the analysis of our solid tumor samples by western blot and qPCR, while also examining whether B7-H3 is secreted in a soluble form or in extracellular microvesicles. Lastly, we will investigate B7-H3 levels in serum to determine whether detectable differences exist between ovarian cancer patients and healthy controls.
ABSTRACT

EXTRACELLULAR MITOCHONDRIAL AND NUCLEAR DNA IN NORMAL AND NECROTIC CONDITIONS. Christopher Musselwhite. Sponsored by Mikhail Alexeyev, Ph.D., Department of Physiology & Cell Biology, University of South Alabama College of Medicine, Mobile, AL.

Increased mitochondrial (mt) DNA levels have been thought to play a role in a number of diseases, but little research has been done on quantitatively measuring mtDNA under different conditions. We incubated mouse embryonic fibroblasts, 3T3 wildtype (WT) and 3287 #6 with mutated uracil glycosylase, in Dulbecco’s Modified Eagle’s Medium, which included glucose, L-glutamine, uridine, pyruvate, gentamicin, and fetal bovine serum (termed DMEM). We created necrotic conditions by incubating cells in DMEM complete made without glucose, or without glucose, L-glutamine, uridine, and pyruvate (termed DMEM –nutrients). We took samples of the extracellular medium after plating overnight (prior to substituting necrotic media), after washing the cells and replacing the media, and at 1, 3, 6, 8 and 24 hour intervals after media replacement. We analyzed the samples with real time quantitative polymerase chain reaction using mitochondria or nuclear primers. 3T3 WT cells had the highest extracellular mt and nuclear (nuc) DNA levels (in copy number) of 1,665 and 1,754 at T=3 and T=6 hours respectively in DMEM, 11,088 and 4370 at T=1 and T=3 hours in DMEM –glucose, and 62,296 and 30,430 at T=24 for both in DMEM –nutrients. 3287#6 cells had the highest extracellular mt and nuc DNA levels (in copy number) of 1,748 and 4,579 at T=24 for both in DMEM, 3,477 and 3,371 at T=24 for both in DMEM –glucose, 51,337 and 19,735 at T=24 for both in DMEM –nutrients. Mt and nuc DNA levels for inside the cell were calculated and compared to mt/nuc ratio in the extracellular media. For 3T3 cells, the internal DNA ratio calculated was 61.12 while the maximum ratio in DMEM, DMEM –glucose, and DMEM –nutrients was 1.59, 13.96, and 2.06. For 3287#6 cells, the internal DNA ratio calculated was 10.67 while the maximum ratio in DMEM, DMEM –glucose, and DMEM –nutrients was 0.91, 0.94, and 3.56 respectively. 3T3 WT and 3287#6 varied in their extracellular mt and nuc DNA released. 3T3 WT peaked in extracellular mt and nuc DNA levels in between 1 – 6 hours after changing media with the exception 3T3 in DMEM –nutrients when all the cells died from starvation (the max mt and nuc DNA levels were reached at T=24). 3287#6’s mt and nuc DNA continued to accumulate in the extracellular media giving maximum values at T=24. This might be because of low levels of expression of the mUNG causing mt DNA damage. Another difference between the two cell lines is the ratio of mt/nuc DNA. 3T3 had higher ratios indicating more mt than nuc DNA in the extracellular environment, but 3287#6 had lower ratio’s, sometimes below 1 indicating more nuc than mt DNA in the extracellular environment. While 3T3 WT and 3287#6 did vary in mt and nuc DNA levels, both released the most DNA in DMEM -nutrients.
ABSTRACT

IMPROVED METHODS FOR DETECTION OF HIGH RISK BREAST CANCER POPULATIONS IN WOMEN PRESENTING FOR IMAGING. Francie O’Hea. Sponsored by Dr. Joel Lightner, MD, Radiology, USA Children and Women’s Hospital and Dr. Lynn Dyess, MD, Breast/Endocrine Surgery, Mitchell Cancer Institute

Breast cancer represents 14% of all newly diagnosed cancer cases in the United States. It is the most common type of cancer in females and it is the second leading cause of death in American women. The American Cancer Society estimates that there will be 231,840 newly diagnosed breast cancer cases and 40,290 deaths due to breast cancer in 2015 alone. These numbers translate into 1 in every 8 women developing breast cancer at some point during their life—a 12.4% lifetime risk. While these numbers are intimidating, the positive news is that the 5-year survival rate of patients with breast cancer is almost 90%. This encouraging statistic emphasizes the importance of early detection—the earlier breast cancer is detected, the earlier the treatment and remission processes can begin. Our goal this summer was to accurately identify which women who present for routine mammography are at an increased risk of developing breast cancer during their lifetime. Women that are identified as high risk merit additional surveillance or are candidates to consider means to reduce their risk of developing breast cancer. In order to identify the sub-population of high risk patients, we formulated a patient questionnaire focusing on major risk factors predisposing women to the development of breast cancer—age, height, weight, family history of breast and/or ovarian cancer, endogenous and exogenous hormone exposures and number of previous benign breast disease. After the patient answers the questionnaire herself, it was then reviewed with the patient to ensure that valid data has been collected. The information was then compiled into the Tyrer-Cusick risk assessment calculator. This risk assessment model is currently the only one to incorporate family history, estrogen exposures, and benign breast disease—it includes the most inclusive set of variables of all the breast cancer risk assessment models. Patients who have a greater than 20% risk as calculated by the Tyrer-Cusick model are identified as high risk and merit additional breast cancer surveillance methods. This typically is done by a mammogram followed by an additional screening method, such as MRI or ultrasound, 6 months later. We conducted risk assessment on a total of 277 patients and found that we were able to identify 23 of these as high risk, meaning their lifetime risk of developing breast cancer was calculated to be greater than 20%. These women will be followed more closely and frequently in the future in order to identify the development of breast cancer at an earlier time, if it is to occur. We also found that the majority of patients were able to successfully fill out the questionnaire themselves. Therefore, we plan for the continued use of the questionnaire and for continued calculation of each patients’ lifetime risk of developing breast cancer to ensure early detection can be made.
Cancer is potentially curable by surgical removal of the tumor from the patient; however, cancer cells may become motile, travel throughout the body, invade healthy tissues, and thus metastasize to secondary sites. This cancer cell motility reduces the effectiveness of surgical removal as a treatment. Therefore, identifying cellular signals and proteins that regulate and enable cancer cell migration and metastasis is an important target of anti-cancer therapies. It is known that the Arp2/3 complex is required for chemotaxis of cancer cells across a substrate, but less is known about the role of the Arp2/3 complex in cancer cell invasion. We therefore hypothesized that disrupting the Arp2 subunit protein of the Arp2/3 complex may inhibit breast cancer cell invasion, and thus be a viable option for inhibition of breast cancer metastasis as an anti-cancer therapy. Our goal was to use the Crispr/Cas9 nuclease system as a gene editing tool to induce a mutation in exon 2 of the Arp2 gene, leading to production of a non-functional Arp2 protein. After identifying cells with the disrupted Arp2 gene, cell sorting allowed isolation of single mutant cells. In the future, cell motility assays should be performed on the identified mutant cells in order to assess the impact of Arp2/3 complex disruption on the cell’s ability to migrate and invade a gel matrix that simulates human tissue.
ABSTRACT

ANALYSIS OF THE ROLE OF PRION PROTEIN IN RAT PULMONARY MICROVASCULAR ENDOTHELIAL CELLS. J. Chandler Van Dyke. Sponsored by R. Balczon, Ph.D., Center for Lung Biology and Department of Molecular Biology, University of South Alabama, Mobile AL, 36688

The prion protein (PrP) has been studied extensively as the causative agent in various human and animal diseases including Creutzfeldt-Jakob disease, Kuru, and bovine spongiform encephalopathy. Despite intensive investigation into its role as an infectious agent, the cellular role of PrP is poorly defined. To analyze the role of PrP Crispr technology was used to delete the gene in rat pulmonary microvascular endothelial cells (PMVECs). The deletion of PrP was verified by DNA sequencing, RT-PCR, and western blot analyses. Observation of PrP-/- PMVECs identified two obvious phenotypes: rapid acidification of cell culture media and poor cell substrate adhesion. Studies were performed to analyze cell substrate adhesion in the knockout cells. Initial studies using fluorescence microscopy identified similar actin stress fiber patterns and integrin α5 labeling in wild type (wt) and PrP-/- PMVECs. Functional studies identified obvious differences between wt and knockout cells. Analysis of cell-matrix tethering using 4-aPDD, a calcium channel activator that disrupts cell-matrix adhesion, determined that PrP-/- cells lost substrate attachment at concentrations of 4-aPDD that had no effect on wt PMVECs. In scratch wound healing assays, wt PMVECs migrated into the wound region by 6 hours post-injury and had nearly repaired the injury by 24 hours. In contrast, PrP-/- cells retracted following the scratch wound forming a large gash that filled the entire viewing field and never began to repair the wound over the course of the experiment. In Matrigel network forming assays the PrP-/- cells also performed poorly. Whereas wt type cells formed an elaborate vessel network that coursed throughout the well within 24 hours, PrP-/- cells attempted to form vessels but were unable to maintain organized vessels in this assay. Instead, the immature vessels retracted and formed balls of cells in the matrigel network. Collectively, these data demonstrate that the PrP protein is intimately involved in cell matrix adhesion by PMVECs and suggest that the activity of this protein is important for vessel growth and wound healing process. Additional studies are in process to further characterize the role of PrP in PMVECs.
ABSTRACT

ExoY IMPAIRS THE RAPID Growth OF PULMONARY MICROVASCULAR ENDOTHELIAL CELLS. Reece P. Stevens. Sponsored by K. Adam Morrow, Lauren Hartman, Ron Balczon, and Troy Stevens, Ph.D., Department of Physiology and Cell Biology, Center for Lung Biology, University of South Alabama College of Medicine, Mobile, AL.

*Pseudomonas aeruginosa* is a common cause of ventilator associated pneumonia that can progress to sepsis and acute lung injury. Virulence of this bacterium is determined by the presence of a type 3 secretion system, which introduces four exoenzymes, or exotoxins, into host cells. Exoenzyme Y (ExoY), which is the most recently discovered exoenzyme, is a promiscuous cyclic nucleotidyl cyclase. During the course of infection, *P. aeruginosa* intoxicates lung endothelium with ExoY, and the resulting increase in cyclic nucleotides cause microtubule breakdown leading to loss of cell-to-cell junctions, cell rounding, and increased permeability. In addition to this increase in permeability, ExoY reduces endothelial cell proliferation and consequently impairs vascular recovery following infection. However, single endothelial cells possess a hierarchy of growth potentials, ranging from low to intermediate to high. Here, we tested the hypothesis that ExoY inhibits the growth of high proliferative potential endothelial cells. To test this idea, pulmonary microvascular endothelial cells were grown to confluence, and infected with a bacterial strain capable of introducing functional (ExoY+) or inactive (ExoYK81M) ExoY through a type 3 secretion system (MOI 20). Four to six hours after infection, media was removed and replaced with media that contained a mixture of antibiotics. Twenty four hours later, cells were trypsinized, single cells were seeded into wells within a 96 well plate, and colony size was measured after two weeks. In uninfected control experiments, approximately 34% of cells grew to fewer than 500 cells (low proliferative potential), 20% grew to between 500-2000 cells (intermediate proliferative potential), and 49% grew to more than 2000 cells (high proliferative potential). A similar hierarchy of growth potentials was seen in cells infected with ExoY+ and ExoYK81M. We noted a wide range of growth potentials within the high proliferative potential cells, where some wells had colonies comprising nearly 400,000 cells. This subpopulation of ultra-high proliferative potential cells utilized aerobic glycolysis to sustain their growth, generating a lactic acidosis that was discernible by a yellow medium color. Whereas this subpopulation of cells was relatively common in uninfected control cells, it was rare in ExoY+ infected cells. Thus, ExoY+ decreases the number of ultra-rapidly growing colonies, but it does not impact the relative distribution of low, intermediate, or high proliferative potential endothelial cells.
ABSTRACT

Pulmonary morphology and vascular calcium dynamics in pulmonary arterial hypertension using the gel-infused lung preparation. Patrick James Huettemann. Sponsored by Michael Francis, Kaori Oshima, and Troy Stevens. Department of Physiology and Cell Biology, Center for Lung Biology, University of South Alabama College of Medicine, Mobile, AL.

Pulmonary arterial hypertension (PAH) is a disease characterized by high pulmonary artery pressure due to vasoconstriction and vascular remodeling. Endothelial dysfunction and hyperproliferation is a prominent feature of the vascular remodeling apparent in PAH. There is mounting evidence that abnormal intracellular calcium signaling underlies this endothelial dysfunction. Here, we test the hypothesis that endothelial cell calcium transients can be resolved in the gelatin-agarose lung slice preparation, in both normotensive and hypertensive circulations. To test this idea, pulmonary arterial hypertension was induced in Fischer 344 rats using a single inoculation of Semaxinib (20 mg/kg) followed by 3 weeks exposure to hypoxia (10%) and a 2 week return to normoxia. Pulmonary arterial pressure in these animals was >70 cm H2O and the Fulton Index was ≈ 0.74 ± 0.04. Normotensive and PAH animals were anesthetized, ventilated, and pulmonary arteries catheterized. Gelatin (6%) was filled into the pulmonary circulation and then agarose (4%) was instilled into the airways. Heart and lungs were removed en bloc, and lungs were sectioned into 300-500 µm sections using a vibrotome. Lung slices were loaded with a Fluo-4 calcium dye, and imaged and z stack images generated. PAH vessels displayed endothelial hyperproliferation, which in some instances occluded the lumen, and significant smooth muscle hypertrophy, when compared to normotensive controls. Fluo-4 fluorescence could be detected in vascular segments, although it was not homogeneous throughout the tissue. A baseline calcium transient was detected in PAH endothelium, although typical spontaneous calcium transients were not resolved. Altogether, these data demonstrate the feasibility of evaluating complex calcium dynamics in living endothelium obtained from normotensive and hypertensive pulmonary arterioles.
ABSTRACT

Technology or Human Contact coupled with practical support? Insights from an intervention study of tele-monitoring of glucose status among type 2 diabetic patients in primary care. Geri Langham. Sponsored by Martha Arrieta MD, MPH, PhD, Department of Internal Medicine and Center for Healthy Communities, University of South Alabama College of Medicine, Mobile, AL and by the J.L. Bedsole Foundation, Scholars Program.

Background: Long term glycemic control can prevent complications of Type II Diabetes Mellitus. The use of telemedicine has opened new disease management pathways. In a recent study, we found that the intervention (use of tele-monitoring system) resulted in improved glycemic control. However, patients in the control arm of the trial also experienced improvement, and there was not a statistically significant difference in the percent improvement on HbA1c levels across the two arms of the trial. Conversely, both our intervention and control groups did significantly better than the Internal Retrospective Control Group (assembled to represent usual care).

Hypotheses: We hypothesized that two aspects of the trial procedure: a) periodic contact with health care providers, and b) provision of subsidized SMBG supplies as well as other practical support to patients would explain the significant difference in glycemic control observed within the trial as compared to patients managed under usual care conditions. Methods: To evaluate the role of periodic contact with health care providers, we classified records of interactions between the Nurse Coordinator for the study and trial participants into eight categories. We then compared the number of interactions in each category among the concurrent study groups. To evaluate the role of the provision of subsidized SMBG supplies, we analyzed the transcripts of focus groups for the intervention arm and the comparison arm, as well as transcripts of personnel focus groups and one interview with the Nurse Coordinator. To reconstruct the experience of the retrospective control group, we reviewed the electronic charts of eligible T2DM patients seen at the same primary care facility as the patients in the trial.

Results: For the intervention and comparison groups, there was a correlation between participants who had greater intensity of contact with the Nurse Coordinator and reduction in HbA1c. Members of the intervention and control groups, Nurse Coordinator, and other health providers had favorable perceptions of the provision of SMBG supplies and practical support. Discussion: Participants in both the intervention and comparison arms showed significant reduction in the levels of HbA1c when compared with patients managed in usual care. Provisions of SMBG supplies and practical support to patients were the difference between diabetes management components within the trial as compared to the management of the retrospective control group. Conclusion: Close contact with health providers, subsidized SMBG supplies, and practical support foster long-term Diabetes control in primary care.
ABSTRACT

Pediatric Obesity Prevention in African American Children. Kelcy Walker, Mentor, Sharon Fruh, PhD, RN, FNP-BC. Sponsored by University of Alabama at Birmingham, Birmingham, Alabama and University of South Alabama, College of Nursing, Associate Professor Undergraduate Research Program-Center for Healthy Communities

This review of literature provided helpful information and resources to guide African American families on reducing the incidence and prevalence of obesity with their children, ranging in age from 2-5 years. The best way to avoid obesity is to prevent young children from becoming overweight or obese. Once obesity is present, it is a disease that is difficult to manage and has a high treatment failure rate which is why prevention early in life is critical. Findings suggest that BMI at age 6 correlates with BMI at 20 years of age.

Research has consistently shown that African American children are at a higher risk for obesity because of: 1) unhealthy food and beverages in the home 2) lack of education 3) low income and socioeconomic status and 4) high screen time and physical inactivity.

It is important that family based pediatric obesity prevention programs teach parents strategies on creating a healthy home environment. Parents, especially mothers are role models and are key to promoting healthy eating and activity in young children. Parents select and purchase foods and beverages in the home. Healthy home food consumption is correlated with home availability.

Frequent healthy family meals is associated with healthy eating and lower obesity rates. When families share three or more meals per week, their children and adolescents are more likely to be in a normal weight range. They also have healthier diet and eating patterns than those who share fewer than three family meals per week.

Helping mothers achieve a healthy weight status can be an effective measure in preventing and treating childhood overweight and obesity. It is important to encourage parents as change agents, related to children’s diets and physical activity levels. One of the best ways to prevent young children from becoming overweight or obese is by establishing a healthy home environment through informed and engaged parents. The home is the most important environment for establishing healthy eating and exercise habits.
ABSTRACT


Background: Poor sleep quality, daytime sleepiness, long and short sleep duration are sleep characteristics associated with increased risk for stroke. The data on racial differences in such sleep characteristics are inconclusive. Previous reports have shown that allostatic load (AL) predicts premature cardiovascular events, however the predictive value of AL on stroke has been inconclusive. Allostatic load, a cumulative measure of dysregulation across biological systems, has been found to be associated with certain sleep characteristics. In a recent issue of Sleep Medicine, Clark et al. (2014) found that sleep disturbances was associated with higher allostatic load scores when controlling for age, sex, menopausal state, socioeconomic status, alcohol consumption, smoking, physical activity, stress, and depressive symptoms. According to Salazar et al. (2014), in a birth cohort of older Danish adults, allostatic load was found to be a useful prognostic tool for stroke mortality risk. Purpose: The purpose of this work is to examine the geographic and racial differences in the sleep characteristics of long and short sleep duration, and daytime sleepiness among participants of the REGARDS study and determine the associations between these sleep characteristics and, allostatic load and stroke symptoms. In addition, determine whether the associations differ by sex groups. These results will then be used in further analyses evaluating the associations between daytime sleepiness, sleep duration, sleep quality, and allostatic load as a multisystem risk index in predicting stroke events. Methods: The REGARDS study used data collected by telephone, mail questionnaires, and in-home examinations. REGARDS recruited 30,239 US blacks and whites, aged 45+ years in 2003 to 2007 who are being followed every 6 months for events. All stroke events are physician-verified; those with prior diagnosed stroke or transient ischemic attack are excluded from this analysis. At baseline, participants were asked 6 questions regarding stroke symptoms. Implications: There has been evidence that suggests sleep disturbances are more prevalent among African Americans than Whites. Hypertension is well documented to be higher in blacks, and more uncontrolled, or higher in persons with resistant hypertension. African Americans with hypertension, confounded by sleep disturbances, are more likely to experience stroke events. Allostatic load cumulative risk scores as a prognostic tool to estimate stroke risk may be able to discern racial and geographic differences by using traditional and non-traditional markers. Even though stroke deaths have declined, Blacks still have a higher risk of being a victim of stroke. Therefore, highlighting the racial and regional disparities will be an incremental gain toward identifying unique differences such that interventions can be developed to prevent adverse outcomes for African American populations.
ABSTRACT

INFLAMMATION IS A MAJOR RISK FACTOR ASSOCIATED WITH INCREASES IN OBESITY RELATED CO-MORBIDITIES IN LOWER ALABAMA. Kyle Reiss. Sponsored by Diego F. Alvarez, M.D., Ph.D., Departments of Internal Medicine, Pharmacology, Center of Lung Biology, University of South Alabama College of Medicine, Mobile, AL.

Obesity elicits inflammation and impairs mitochondrial function leading to a condition known as a chronic-low grade inflammation or meta-inflammation. It is well recognized that obesity is a major risk factor for the development of cardiovascular diseases and metabolic disorders both of which represent a significant cause of death and health care problems in the U.S. The Southeast population of the U.S. is particularly vulnerable to different cardiovascular diseases and metabolic disorders. Indeed, mortality and health care cost from those conditions are the highest among all regions in the country. However, whether meta-inflammation is a risk factor for the obesity-related cardiovascular diseases and metabolic disorders is unknown. Therefore, we aimed at determining whether meta-inflammation is associated with obesity and their different related co-morbidities. Therefore, we sought to assess whether the Mobile region is more susceptible to obesity and related co-morbidities compared to the Black belt and the Northern region of the state of Alabama. To address our goals we selected specific counties based on zip code data to generate our three regions of interest. We analyzed data collected from 42,000 individuals between 19 to 60 years old enrolled in Medicaid from the three different regions. ICD codes containing information about obesity and ten common different associated clinical conditions were analyzed from 2009-2012. We count the number of individuals diagnosed with obesity or the ten selected co-morbidities during that period. Meta-inflammation was defined by the diagnosed of elevated c-reactive protein. A p<0.05 was considered statistically significant (Fisher’s exact test). The figure displays proportion of individuals at risk of developing cardiovascular diseases or metabolic disorders.

In addition we found that individuals in the Mobile region who are obese are at higher risk of being diagnosed with Metabolic syndrome, Hyperlipidemia and Stroke compared to the other regions. We conclude that inflammation is an independent risk factor for the development of cardiovascular diseases and metabolic disorders. Moreover, the association of inflammation and obesity increases the risk for developing those conditions. The population of Mobile, Alabama is sensitive to develop some of the most common obesity-related co-morbidities.
THE CHC (CENTER FOR HEALTHY COMMUNITIES) PIPELINE – A TEN YEAR REVIEW OF SUCCESS. ALEXANDRIA BROADNAX, AND BREANNA HEARD-PINHO. SPONSORED BY ERROL D. CROOK, MD, HATTIE M. MYLES, PHD, MARY C. WILLIAMS, UNIVERSITY OF SOUTH ALABAMA COLLEGE OF MEDICINE (M2), MOBILE, ALABAMA AND THE UNIVERSITY OF SOUTH ALABAMA CENTER OF EXCELLENCE, CENTER FOR HEALTHY COMMUNITIES, MOBILE, ALABAMA

Synopsis: The University of South Alabama Center for Healthy Communities, Center of Excellence (COE), sponsors a pipeline program to encourage minority students from underserved communities to enter career paths committed to reduction of health disparities. The current structure of the program includes S.T.A.R.S. (Student Training for Academic Reinforcement in the Sciences) and S.T.R.I.P.E.S. (Special Training to Raise Interest and Prepare for Entry into the Sciences), Shadows, and UGRP (Undergraduate Research Program). The S.T.A.R.S. program aims to assess and increase the interest level and focus of high school students in science and math. The S.T.R.I.P.E.S program has as its ultimate aim to increase the number of minority students who enter and graduate from college in the sciences. Both programs require students to participate in a series of Community Volunteer Services and Health Advocacy. The USA CHC delete utilizes its service area as a vehicle to increase the pool of minority students who can successfully complete professional level education. Such students will constitute the next generation of minority scientists.

Methodology: Fifteen capable High School rising juniors for S.T.A.R.S. and the successful rising seniors who complete S.T.A.R.S. and are promoted to S.T.R.I.P.E.S. are enrolled into four and six-week, respectively, Academic Pipeline for Minority Scientists Program. These first two academic enrichment sessions conducted over consecutive summers are designated PipeLinks I and II. The objective the two summer sessions is to solidify the students’ necessary academic foundation and skills for competitive performance on their college entrance exams and for success in their college education. Students who successfully complete PipeLink I must “earn their STRIPES” through community service in order to be invited back for PipeLink II. Upon successful graduation from high school, former participants are invited to complete an eight-week internship in a health-care provision site or a medical research facility during the third summer (PipeLink III); an opportunity to “Shadow” care givers. The objective of this Link is to expose students to practical experiences in health-care provision or medical research.
ABSTRACT


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The second messenger cAMP exerts a negative constraint on the activation of the innate immune response by limiting production of pro-inflammatory cytokines such as TNFα. Activation of toll-like receptor 4 (TLR4) by bacterial lipo-polysaccharides (LPS) induces the expression of the cAMP-phosphodiesterase variant PDE4B2, which hydrolyzes and inactivates cAMP, thus allowing for full activation of the inflammatory response. Counteracting the LPS-induced expression of PDE4B2 by inhibiting its enzymatic activity is a critical mechanism by which inhibitors of Type 4 phosphodiesterases (PDE4s) exert anti-inflammatory properties. Probing the potential of PDE4 inhibition for the treatment of cystic fibrosis, we found that LPS-induced TNFα production in airway epithelial cells is reduced by treatment with the archetypal PDE4 inhibitor Rolipram. We also observed the established LPS-induced surge in PDE4B2 expression. However, PDE4B2 levels were not only induced by LPS, but also by treatment with PDE4 inhibitor per se; and PDE4 inhibition further amplified the LPS-mediated PDE4B2 induction. This induction is unique to PDE4B2, as other PDE4B splicing variants were not induced and the expression levels of PDE4 subtypes PDE4A, PDE4C and PDE4D were not altered by LPS and/or PDE4 inhibitor treatment. We observed the PDE4 inhibitor-induced surge in PDE4B2 expression not only in airway epithelial cells, but also in mouse macrophages (Raw246.7) or mouse embryonic fibroblasts (MEFs), suggesting it is a wide-spread phenomenon. PDE4B2 induction is not unique to Rolipram treatment, but was observed upon treatment with a number of structurally distinct compounds, suggesting it is a class effect of non-selective PDE4 inhibitors. Mechanistically, PDE4 inhibitor-induced PDE4B2 induction appears to result from excess activation of cAMP and PKA activity levels, because it is ablated by treatment with the PKA inhibitor H89 or, vice versa, can be induced by stimulation of cAMP production with saturating concentrations of the adenylyl cyclase activator Forskolin. PDE4B2 protein levels remain elevated for several hours upon washout of PDE4 inhibitor. Thus, PDE4B2 induction may limit the efficacy of PDE4 inhibitors to elevate cAMP and reduce inflammation at clinically relevant, submaximal concentrations. Inactivation of individual PDE4 subtypes using isoform-selective inhibitors did not induce PDE4B2 expression, likely because they trigger smaller, locally restricted increases in cAMP/PKA compared to the non-selective inhibition of total cellular PDE4. Our lab has been interested in targeting individual PDE4 subtypes as a way to separate the side effects from the therapeutic benefits of the non-selective PDE4 inhibitors available to date. Our present results indicate that subtype-selective PDE4 inhibitors might have the added benefit of not inducing the pro-inflammatory variant PDE4B2.
ABSTRACT

Microparticles are submicron vesicles that carry molecules that participate in intercellular communication. Increased expression of intercellular adhesion molecules (ICAM) is required for recruitment of inflammatory cells and the arteriopathy that occurs in pulmonary arterial hypertension (PAH) is surrounded by infiltrating inflammatory cells. ICAM expression is mediated by NFkB transcription factor activation. Previous results indicated that microparticles from the circulation of PAH rats increase ICAM expression in pulmonary artery endothelial cells (PAECs). We hypothesize that inhibition of NFkB will inhibit microparticle-stimulated ICAM expression in PAECs. Adult male rats were injected with SU5416, a vascular endothelial growth factor blocker, and exposed to hypoxia for 3 weeks and then to normoxia for an additional 5 weeks (total 8 weeks). Microparticles were isolated from platelet free plasma. Following NFkB inhibitor treatment and microparticle treatments, whole cell and membrane ICAM expression were measured using labeled anti-ICAM and flow cytometry. As a positive control for ICAM expression, PAECs were stimulated with alveolar macrophage supernatant (cytomix). Our preliminary results indicate that NFkB inhibition did not influence ICAM expression in either MP or cytomix exposed PAECs. Treatment with NF-kB inhibitor did not inhibit the membrane or whole cell expression of ICAM in PAECs following exposure to PAH MPs or cytomix. However, the effectiveness of NFkB inhibition using the current pharmacologic tool in PAECs has not been examined. Thus, more molecular approaches may be necessary.