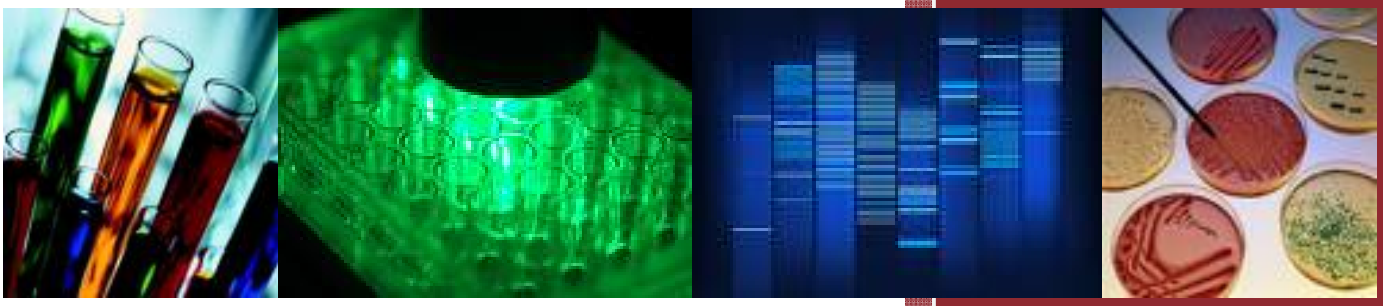
Poster Session and Reception

2016

Summer Research Program



July 29, 2016
9:00 AM - 3:00 PM
Health Professions Education Building
Bryan, TX

HEALTH SCIENCE CENTER
TEXAS A&M UNIVERSITY

Schedule of Events

July 29, 2016

8:30am	**Registration Table Opens** HPEB LL Lobby
9:00am-12:00pm	**Poster Viewing** HPEB LL43 A&B
12:00pm-1:00pm	**Lunch** HPEB LL11 A&B
1:00pm-2:00pm	**SRP Featured Speaker** **Dr. Robert Alaniz** *"Metabolite-mediated Mechanisms of Microbiota Regulation of the Mucosal Immune System"* HPEB LL46
2:00pm-3:00pm	**Presentation of Certificates** Dr. Brett Mitchell, Summer Research Program Director HPEB LL46 **Presentation of Dean's Recognition Awards** HPEB LL46
3:00pm	**Group Picture & Adjourn**

Acknowledgements

The Texas A&M Health Science Center College of Medicine's Summer Research Program continues to attract the top students from universities all across the country. This year we had 34 participants who completed the 10-week program. These students were selected from a large pool of applicants based on their research experience, desire to attend graduate and/or medical school, grades, exam scores, and letters of recommendation. I would like to thank the selection committee who dedicated their time to read through each application.

- Kayla Bayless, Ph.D.
- Sharon DeMorrow, Ph.D.
- David Huston, M.D.
- Xu Peng, Ph.D.
- Samba Reddy, Ph.D.
- Mendell Rimer, Ph.D.
- Emily Wilson, Ph.D.
- Warren Zimmer, Ph.D.

I would also like to thank the faculty that gave their time as mentors. You have provided each of these students with a valuable experience that will undoubtedly help them achieve their career goals. The program would not be able to sustain its quality without you.

The program was made possible by the following people who provided the funding and sponsorships.

- Dean Paul Ogden, M.D. – Texas A&M Health Science Center
- Van Wilson, Ph.D. – Texas A&M Health Science Center
- Jack Hart, Ph.D. – Temple Health and Bioscience District
- Robert Dearth, Ph.D. – University of Texas Rio Grande Valley
- Dennis Daniels, MPH, Dr.PH – Prairie View A&M University
- Adebayo Oyekan, DVM, Ph.D. – Texas Southern University
- Filomeno Maldonado, Ph.D. – Texas A&M Health Science Center
- Warren Zimmer, Ph.D. – Texas A&M Health Science Center

Each week we had Roundtable Discussions in which students got to individually engage with faculty mentors at their respective locations regarding numerous "behind the scenes" aspects of science. This was made possible by the time and dedication of the following Site Coordinators:

- Temple – Drs. Xu Peng and David Dostal
- Houston – Drs. David Huston and Margie Moczygemba

Finally, I would like to thank the SRP Coordinator **Mary Ann Wolff** who did a ton of work arranging the arrival, housing, registration, processing, and weekly feeding of the students. Thank you as well to **Monica Flores** in Bryan/College Station, **Tammy Kocurek** in Houston, and **Manuela Smith** in Temple who kept each of the sites running smoothly. Thank you to our poster judges who had an extremely difficult task of picking the best out of the best. Thank you students for your hard work and everyone for a memorable summer – Gig 'em!

Brett Mitchell, Ph.D., F.A.H.A.
Director, Summer Research Program

Abstracts

TAMHSC 2016 SUMMER RESEARCH PROGRAM

ISOBOLOGRAPHIC ANALYSIS OF ANTICONVULSANT INTERACTIONS BETWEEN NEUROSTEROIDS AND THE SELECTIVE GABA REUPTAKE INHIBITOR TIAGABINE IN THE 6-HZ SEIZURE MODEL: CORRELATION WITH TONIC CURRENTS

Hayden Anz, Bryan Clossen, Shu-Hui Chuang, D. Samba Reddy
Department of Neuroscience and Experimental Therapeutics
Texas A&M Health Science Center College of Medicine
Bryan, TX

Epilepsy is a chronic neurological condition characterized by recurrent seizures. Neurosteroids are powerful anticonvulsants with therapeutic potential for epilepsy, which is often treated by multidrug therapy. However, little is known regarding neurosteroid interactions with other antiepileptic drugs. The objective of this study was to assess the characteristics of interaction between the synthetic neurosteroid ganaxolone (GX) and the selective GABA reuptake inhibitor tiagabine (TG) in 6-Hz-induced seizures in mice. Additionally we tested their effect on extrasynaptic tonic currents in dentate gyrus granule cells (DGGCs). Isobolographic analysis protocols were used to ascertain additive or synergic interactions. Using log-probit analysis, we determined the ED50 of GX and TG to be 1.459 mg/kg and 0.198 mg/kg, respectively. Then, the theoretical additive ED50 was calculated for 3 fixed geometric ratios of combined doses (1:1, 1:3, and 3:1 – GX:TG). Results indicated that GX combined with TG at 3 fixed ratios of 1:3, 1:1, and 3:1 exerted additive interaction in seizure protection in mice; the combination of GX and TG at the fixed ratio of 1:1 displayed a clear tendency towards synergism. The combination index (CI), a measure of pharmacological synergism, for GX:TG 1:1, 3:1 and 1:3 combinations were 0.53, 0.94 and 0.96, respectively. Moreover, all GX+TG ratios displayed a striking synergism for potentiation of tonic currents in DGGCs, likely due to a greater allosteric agonism of ganaxolone at extrasynaptic GABA-A receptors from tiagabine-induced elevated levels of extracellular GABA in the hippocampus. These results provide strong mechanistic rationale for synergistic potential of ganaxolone with tiagabine for epilepsy therapy, possibly with reduced side effects.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

INTERACTIONS OF BUTYRIC ACID (BA) WITH ARYL HYDROCARBON RECEPTOR (AHR) ACTIVE MICROBIOTA METABOLITES

Andrew Asante, Yating Cheng, Stephen Safe
Department of Veterinary Physiology & Pharmacology
Texas A&M Health Science Center College of Medicine
College Station, TX

The aryl hydrocarbon receptor (AhR) is a transcription factor that is activated by both exogenous and endogenous ligands. The activation of AhR by these ligands induces the expression of different downstream genes such as cytochrome P450 family 1 subfamily A member 1 (CYP1A1) and cytochrome P450 family 1 subfamily B member 1 (CYP1B1). AhR plays a major role in the regulation of inflammation, immune disease and tumor growth. The most potent exogenous compound that activates AhR is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which is an environmental toxicant, and belongs to the family of halogenated aromatic compounds.

This project is part of an extensive study focused on studying the AhR agonist and antagonist activities of microbiota metabolites and their functions in gut health. Butyric acid (BA) is a short-chain fatty acid which is synthesized by bacteria in the gut from foods rich in fiber and starch. BA promotes gut health and plays a vital role in the regulation of anti-inflammation, immunoregulation, apoptosis and colonocyte proliferation, and this may be due in part to the activities of BA as a histone deacetylase (HDAC) inhibitor. In this study, we investigated the interaction of butyric acid with AhR active tryptophan metabolites in young adult mouse cells (YAMC) and human Caco2 cancer cells using CYP1A1 as marker gene. We observed that BA enhances CYP1A1 expression induced by AhR active compounds and the underlying mechanisms are currently under study.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

DETERMINING PROBABILITY DISTRIBUTION FUNCTIONS TO FACILITATE MACHINE LEARNING IN INTEGRATED OUTCOMES

Preston Baker, Seth Polsley, Mohammad Atif Tahir, Muppala N Prasanth Raju, Akintayo A Akinleye, Amber Schulz, Duane Steward
Department of Biomedical Informatics
Texas A&M Health Science Center College of Medicine
College Station, TX

Since the Health Information Technology for Economic and Clinical Health act was passed in 2008, there has been a proliferation of Electronic Medical Records (EMR's). These EMR's are being used for clinical research however, most research has been focused on populations rather than treatment on an individual level. Our goal is to use past EMR's to determine common interventions and correlated outcomes of medical practices to provide doctors with more feedback on the status of their patients in the form of an Information Technology (IT) System. This will require collection of data on interventions prescribed by health care providers, anticipated outcomes to a specific intervention, and a list of all common outcomes after an intervention. Data is being collected from multiple health care domains in order to broaden the scope of this study for better measurement of the capability of the system. Focus groups were held in each health care domain to gain knowledge from health care experts on the applicability of this type of system in their domain as well as information about their EMR's structure and security. After EMR's were collected and de-identified analysis was done in the form of Probability Distribution Function's (PDF's). PDF's will be used to train our IT System and determine what data is best for machine learning. After PDF's are formed, individual interventions will be chosen for study and our IT System will be developed. Following development errors and shortfalls of the system will be quantified for study in development of future products.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

OPTIMIZATION OF ULTRABITHORAX FUSION PROTEIN MATERIALS FOR USE IN COATING STENTS

Lauren Beck, David Howell, Kayla Bayless, Sarah Bondos
Department of Molecular and Cellular Medicine
Texas A&M Health Science Center College of Medicine
College Station, TX

Cardiovascular disease, which affects over 80 million Americans, is primarily caused by atherosclerosis, a disease in which plaque accumulates on artery walls. The presence of plaque narrows the vessel, reduces blood flow, and increases the formation of blood clots. To reopen the vessel, angioplasty is performed by deploying a stent into the vessel. Drug-eluting stents are used to help prevent the occurrence of restenosis, but they also slow the time it takes for the endothelial cells (ECs) to regrow, increasing the occurrence of thrombosis. Providing a new stent coating that allows the endothelium to grow more quickly will reduce closure due to thrombosis. We aim to genetically fuse the protein Ultrabithorax (Ubx) to proteins that modulate EC behavior to coat the stent. Ubx is a *Drosophila melanogaster* Hox protein capable of self-assembling into fibers and films. Ubx fused to proteins that aid in re-endothelialization are still able to self-assemble into fibers and films. Cell migration assays have proven that the proteins still remain active in these fibers by attracting ECs. Several methods were attempted to coat the stent with these materials. Wrapping the stent with fibers or film provided an uneven distribution of material that would inadequately promote regrowth of ECs. Previous studies have also created sheets of Ubx that exhibit self-adhesion and flexibility. We experimented with different conditions to optimize the formation of these sheets to give a more uniform coating of the stent. Sheets were never successfully formed under any conditions tested and will require further optimization.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

PREECLAMPSIA IS ASSOCIATED WITH INCREASED GAMMA-DELTA T CELLS AND PLACENTAL LYMPHANGIOGENESIS

Katharine Beckman, Kayla Hudson, Catalina Lopez Gelston, Lauren Phillips, Brett Mitchell
Department of Medical Physiology
Texas A&M Health Science Center College of Medicine
College Station, TX

Preeclampsia (PE), hypertension and proteinuria during pregnancy, is characterized by immune system activation and placental inflammation. We reported that activation of Toll-like receptors (TLR) 3 and 7, viral RNA receptors, during pregnancy promotes PE in mice and women. We tested whether activation of TLR4, a bacterial lipopolysaccharide (LPS) receptor, also causes PE in mice. Gamma-delta T cells (gdT cells) become pro-inflammatory upon TLR activation however how they contribute to PE has not been examined. Lymphatic vessels play a major role in interstitial fluid homeostasis and immune cell transport; however, how placental lymphatic vessels are altered in PE is also unknown. We tested the following hypotheses: 1) TLR4 activation by LPS will cause hypertension in pregnant mice, 2) gdT cell inhibition will ameliorate TLR4-induced PE in mice, and 3) placental lymphangiogenesis will be increased in placentas from mice and women with PE. LPS treatment caused a significant increase in systolic blood pressure and splenic gdT cells. Depletion of gdT cells with a neutralizing antibody prevented the hypertension induced by LPS. Additionally, pregnant gdT cell deficient mice did not exhibit hypertension in response to LPS. Lastly, to test whether placental inflammation in PE is associated with lymphangiogenesis, we examined immunostaining of podoplanin and LYVE-1 in placentas from TLR3, TLR4, and TLR7 PE mice as well as from women with PE. Mice and women with PE exhibited a marked increase in the number of placental lymphatic vessels. These data suggest TLR4 activation of gdT cells and placental lymphangiogenesis play a role in PE.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

THE IMPACT OF INTERSTITIAL FLOW ON ADIPOCYTE DIFFERENTIATION AND FUNCTION

Benjamin Boyett, Naveen Menon, Evon Looper, Joseph Rutkowski
Dept. of Medical Physiology
Texas A&M Health Science Center College of Medicine
College Station, TX

The obesity epidemic in America is characterized in part by pathological expansion of adipose tissue. This leads to hypoxia and inflammation in the adipose tissue, as well as a more fibrotic interstitial space, because the expanded fat tissue does not have a corresponding growth in angio and lymphangiogenesis as adipocytes expand. We hypothesize that this inflammation and fibrosis is due to the lack of interstitial flow between blood vasculature and lymphatic vasculature in expanded adipose tissue. Goals of the study were to achieve adipocyte differentiation in 2 and 3 dimensions, to design 3 dimensional flow systems compatible with interstitial flow, and to quantify changes in adipocyte biology through gene expression and function through metabolic flux analysis. It was found that we could differentiate adipocytes, both in 2 and 3 dimensions using both type I collagen or a thiol-crosslinked hyaluronic acid (HA) matrices. We were also able to develop an initial system representing physiologically-relevant levels of interstitial flow by using transwell plates and micro-fluidics chambers. It was found that adipocytes tolerate both matrices well, but preferentially perform better in the HA matrix regarding expression of adipocyte differentiation markers. With interstitial flow, adipocytes exhibited decreased markers of inflammation in the HA matrix. These results provide the basis for future studies that will involve a more customizable flow chamber with regards to geometry, flow rates, and matrix composition, as well as the application of primary isolated cells, which will allow us to identify mechanical mediators of adipocyte biology in obesity.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

MORPHOLOGICAL INDICES OF POST-TRAUMATIC EPILEPSY AFTER TRAUMATIC BRAIN INJURY (TBI) IN MICE

Julia Bruggemann, Victoria Golub, Bryan Clossen, D. Samba Reddy
Department of Neuroscience and Experimental Therapeutics
Texas A&M Health Science Center College of Medicine
Bryan, TX

Traumatic brain injury (TBI) is a highly complex disorder that includes varying degrees of contusion, brain injury, hemorrhage and hypoxia, as well as long-term neurological deficits such as epileptic seizures and cognitive dysfunction. Post-traumatic epilepsy (PTE) is characterized by spontaneous recurrent seizures that occur within a few months or years after TBI. Presently, there are very few valid animal models of PTE with spontaneous seizures. In this study, we sought to characterize a mouse PTE model using chronic EEG and morphological approaches. We used a controlled cortical impact (CCI) paradigm, which simulates aspects of human TBI, to investigate the incidence of epileptic seizures and the morphological outcomes of TBI. Mice were perfused 4 months after TBI for evaluation of multiple key morphological indices of brain trauma/epileptogenesis, including: (i) brain lesion volume, (ii) NeuN+ principal cell degeneration, (iii) GFAP+ astrocyte activated neuroinflammation, and (iv) Timm staining for aberrant mossy fiber sprouting in the hippocampus. Our results show a massive lesion, both in the entire hemisphere and within the hippocampus. There was strong evidence for neurodegeneration and neuroinflammation in the contralateral hippocampus. Finally, the extent of mossy fiber sprouting, a valid morphological index of epileptogenesis, was greater in the hippocampus of animals with PTE. A subgroup of TBI mice treated with the HDAC inhibitor sodium butyrate showed amelioration in lesion volume and neurodegeneration. These pilot studies show the viability of using the CCI model of PTE to document critical neuropathological outcomes, which are consistent with human TBI.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

EFFECTS OF MIR363 ON POST-STROKE OUTCOMES IN THE MCAO ENDOVASCULAR SUTURE MODEL

Delfina Bur, Aditya Panta, Farida Sohrabji
Dept. of Neuroscience
Texas A&M Health Science Center College of Medicine
Bryan, TX

Stroke is the fifth leading cause of death and a major cause of disability in the US. Nearly 75% of all strokes occur in people over the age of 65. Young women are at a lower risk for stroke compared to age-matched men, but after menopause, their risk equals, and later exceeds, that of men. Few therapeutic options are available for stroke and preclinical studies have tested drugs in clinically relevant groups such as aging females. MicroRNAs (small noncoding RNAs) are emerging as novel treatments in fields ranging from cancer to cardiovascular disease. Our previous published data show that availability of mir363 is inversely correlated to infarct volume in female rats. Thus, in this study we aim to investigate if mir363 has neuro-protective effects in reproductively senescent (postmenopausal model) female rats using the intraluminal suture-induced MCAo stroke model. Middle-aged female rats were pre-tested for sensory-motor function and randomly assigned to two treatment groups. Ischemic stroke was induced using a silicon-coated nylon filament suture to occlude the Middle Cerebral Artery (MCA) and removed 75-min after to allow for reperfusion. Mir363 mimetic or control-oligo were injected into the tail vein 4h later. Histological staining for infarction and behavioral tests revealed no significant differences between groups. The cytokine analysis, however, revealed significant peripheral suppression of MCAo-induced pro-inflammatory cytokines by mir363. This work shows that despite peripheral effects, 75-min occlusion is not ideal to test neuro-protective effects of mir363 centrally, and indicates that subsequent studies should focus on a longer occlusion time.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

BILE ACID-MEDIATED CHOLESTEROL BUILDUP IN THE BRAIN CONTRIBUTES TO HEPATIC ENCEPHALOPATHY

Lauren Canady, Matthew McMillin, Stephanie Grant, Gabriel Frampton, Sharon DeMorrow
Dept. of Internal Medicine
Texas A&M Health Science Center College of Medicine
Temple, TX

Type A hepatic encephalopathy (HE), a neuropsychiatric disorder caused by acute liver failure (ALF), greatly decreases the chance of recovery for ALF patients. During ALF, serum and brain bile acid levels are elevated. Bile acids are synthesized from cholesterol in a pathway that is highly regulated through feedback inhibition to ensure cholesterol homeostasis. Bile acids bind farnesoid X receptor (FXR) which interacts with small heterodimer partner (SHP) to influence liver X receptor beta (LXRβ) and Cyp46A1. This signaling pathway subsequently impacts brain bile acid synthesis and cholesterol clearance. We hypothesize that these interactions between bile acid signaling and cholesterol metabolism in the brain contribute to HE pathology. Bile acid signaling was assessed using an *in vivo* mouse ALF-induced HE model (using azoxymethane injections) and cell culture studies employing primary mouse neurons. Genetic and pharmacological strategies were undertaken to manipulate bile acid signaling and cholesterol homeostasis in both models. During AOM-induced HE, brain cholesterol levels were increased. Cortical Cyp46A1 and LXRβ expression was decreased while FXR and SHP expression was increased during AOM-induced HE. Reducing bile acid levels or FXR signaling in AOM-treated mice restored Cyp46A1, LXRβ, and SHP expression to levels comparable to control mice and reduced the elevated brain cholesterol levels. In conclusion, high brain cholesterol levels found during AOM-induced HE are dependent on bile acid-mediated FXR signaling and contribute to HE. Our next steps include investigating effects of clinically useful treatments: pravastatin and cholestyramine, which can reduce high cholesterol and bile acid levels, respectively.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

ENGINEERING UBX BIOMATERIALS FOR BIOSENSOR, TISSUE ENGINEERING, AND DRUG DELIVERY APPLICATIONS

Alexis J. Carr[1], Jennifer A. Jamison[1], Kenith Meissner[2], Kush N. Shah[3], Carolyn L. Cannon[3], Sarah E. Bondos[1]

[1]Department of Molecular and Cellular Medicine, Texas A&M Health Science Center College of Medicine, College Station, TX

[2]College of Engineering, Swansea University, Swansea, Wales, UK

[3]Department of Microbial Pathogenesis and Immunology, Texas A&M Health Science Center College of Medicine, College Station, TX

Ultrabithorax (Ubx) is a Hox transcription factor found in *Drosophila melanogaster*. *In vitro*, this protein self-assembles into cytocompatible, biocompatible, and nonimmunogenic biomaterials. Ubx is easily functionalized via protein fusions and the incorporation of nanoparticles. We are currently developing Ubx materials for uses in biosensing applications, tissue engineering, and drug delivery. The first goal of this work was to test a method to form linear gold nanoparticle (AuNP) arrays using unmodified Ubx fibers. Many aspects of AuNP-Ubx nanocomposite materials make them suitable for biosensor applications including relatively short AuNP binding times and supramolecular interactions between Ubx and AuNPs resulting in the formation of linear arrays. I have also devised a method to form Ubx hydrogels allowing creation of 3D tissue engineering scaffolds. Ubx and Ubx fusion proteins form thermoreversible hydrogels, thus demonstrating that hydrogel formation is not altered by the presence of a protein fused to Ubx. The thermal stability of the hydrogels appears to be tunable by changing the protein concentration. Lastly, we are developing Ubx hydrogels for antibiotic delivery. *Pseudoalteromonas*-derived marine bacteria produce compounds C58 and C59 which exhibit superior antimicrobial activity over standard-of-care antibiotics against drug-resistant bacteria. Absorbance data of these compounds suggest that we can monitor drug loading/diffusion via UV-Vis spectroscopy. Ubx materials are capable of being engineered for a variety of biomedical applications.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

IN VITRO MECHANICAL STRESS STIMULATES CHOLANGIOCYTE PROLIFERATION AND EXPRESSION OF FUNCTIONAL MARKERS

Mary-Catherine C. Clark, April O'Brien, Madeline Rodriguez, John C. Connolly, Thanh N. Dinh, David E. Dostal, Shannon Glaser
Dept. of Internal Medicine
Texas A&M Health Science Center College of Medicine
Temple, TX

Cholangiocytes are epithelial cells that line the biliary tree and are responsible for the regulation of bile composition. They predominantly do this by the addition of bicarbonate, the changing of water composition, and the secretion of some bile acids. Bile aides in the digestion of fats and allow for their absorption in the digestive tract. We have previously shown that in chronic cholestatic liver diseases, a link exists between bile duct injury, cholangiocyte proliferation and sub-epithelial fibrosis. Typically, chronic cholestasis is modeled through bile duct ligation (BDL) in both mice and rats. Mechanical stretch is an in vitro system that models general cholestasis in humans, similar to animal models of BDL. Thus the AIM of this study was to evaluate the effects of mechanical stress on cholangiocyte proliferative and functional responses. To do this, two adherent cholangiocyte cell lines, Mouse SV-40 Cholangiocytes and Normal Human Cholangiocytes, were lifted from T-75 flasks and plated on 6-well stretch membranes at 50,000 cells per well. Cells were stretched biaxially at various time points. Cells pellets and supernatants were then collected for various assays such as Western Blots, Immunofluorescence, PCR, and dead cell counts. The resultant data showed that when cells are exposed to biaxial mechanical stress there is an increase in proliferative gene expression, tight junction gene expression, and morphological changes. Therefore, our data suggest that mechanical stress provides an *in vitro* alternative for modeling general cholestatic liver diseases, thereby, saving researchers both time and money.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

APOPTOSIS IN CARDIAC ARREST AND THE EFFECTIVENESS OF RESUSCITATION DRUGS

John Connolly[1], Thanh Dinh[1], Brittany Bass[1], Mary-Cate Clark[1], April Kasprick[1], Jason Bowman[2], Matthew McClure[3], David Dostal[1,2].
Department of Medical Physiology
[1]Central Texas Veterans Health Care System
[2]Texas A&M Health Science Center
[3]UNT Health Science Center Texas College of Osteopathic Medicine
Temple, TX

Cardiac arrest remains a public health concern due to a low survival. Epinephrine is the current standard of care (ACLS) treatment for resuscitation. Recently, epinephrine has undergone scrutinization due to the increase in myocardial oxygen consumption, increased cardiac afterload and decreased coronary perfusion. Therefore, we tested whether cardiac myocyte apoptosis will be minimized when treated with a combination of vasodilator sodium nitroprusside (SNP), the arrhythmia drug adenosine, and a calcium sensitizer levosimendan (SNPAL), when compared to epinephrine. We postulated these three drugs provide cardiac benefit by simulating “ischemic postconditioning” (IPC), a maneuver that uses controlled pauses at the end of resuscitation. IPC has been previously shown to significantly improve cardiomyocyte survival and prevent apoptosis in isolated perfused rat hearts. Adult Sprague-Dawley rat hearts extracted from anesthetized rats were perfused with Krebs-Henseleit buffer for 15 minutes to allow for recovery to a normal spontaneous heart rate. To simulate cardiac arrest, perfusion was stopped for 15 min, followed by ACLS or SNPAL drug administration. Following administration, cardiomyocytes were isolated by perfusing with collagenase and analyzed for necrosis and apoptosis using flow cytometry. The SNPAL treatment showed a decrease in apoptosis by 71% $\pm$ 0.1% ($p<0.001$). Ischemic post conditioning with ACLS alone showed reduction in cardiac cell apoptosis by 3% $\pm$ 0.1% ($p=0.01$). The combination of drugs and the ischemic post conditioning demonstrated an increase in cardiac myocyte survival compared to ACLS. In conclusion, these results suggest that a combination of pharmacologic agents will provide a more effective management of cardiac arrest.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

QUANTIFICATION OF α7 NICOTINIC ACETYLCHOLINE RECEPTORS IN TRANSGENIC MICE WITH A 1.2 MB DELETION HOMOLOGOUS TO THE 15Q13.3 MICRODELETION IN HUMANS

Daisy Consuegra Garcia, Katherine Rees, Ursula Winzer-Serhan
Dept of Neuroscience and Experimental Therapeutics
Texas A&M Health Science Center College of Medicine
College Station, TX

The 15q13.3 microdeletion syndrome is a disease in which a small piece of the human chromosome 15 is deleted. This deletion affects 6 genes including the CHRNA7, which codes for α7 nicotinic acetylcholine receptors (nAChRs). These ion channels, which have a role in cognitive functions, are abundant in the hippocampus and are known to bind to α-bungarotoxin with high affinity. Patients who have this microdeletion are predisposed to several neuropsychiatric disorders like: epilepsy, autism, schizophrenia, etc. The mouse model Df(h15q13)/+ has an area homologous to the human portion deleted in the 15q13.3 microdeletion on chromosome 7qC, and heterozygous mice were used in this study. The objectives of this study were to look at α7nAChRs binding levels and mRNA expression in the hippocampus and cortex and compare them to wild-type mice. We hypothesized that both the protein and mRNA levels in heterozygotes would be approximately half of that seen in wild types. mRNA expression was detected with in situ hybridization, and expression of α7 nAChR was evaluated by receptor autoradiography using ^{125}I-αbungarotoxin. Results showed that there were no sex differences in the α7 protein levels in either wild-type or heterozygous mice. The heterozygous mice showed a significant decrease in α7nAChR protein levels with a percentage difference of approximately 50% ($p<0.05$). Hybridization with the α7 anti-sense probe showed reduced mRNA expression in the cerebral cortex, dorsal dentate gyrus and the CA3 part of the ventral hippocampus.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

GENETIC ANALYSIS OF CYCLIN-DEPENDENT KINASE 8 (CDK8) IN *DROSOPHILA*

Kerolayne De Almeida Costa, Xiao Li, Xingsheng Gao, Xiaojun Xie, Jun-yuan Ji
Dept. of Molecular and Cellular Medicine
Texas A&M Health Science Center College of Medicine
College Station, TX

Cyclin-dependent kinase 8 (CDK8) and its regulatory partner Cyclin C (CycC) are mutated, deleted, or amplified in a variety of human cancers, such as melanoma and colorectal cancer. Overexpression of CDK8 alone can promote the growth of these cancers. The growth of melanoma or colorectal cancer cells with gained CDK8 activity is potently blocked when CDK8 is inhibited or depleted, suggesting that CDK8 is an oncoprotein in melanoma and colorectal cancer, making it a promising drug target. Understanding the regulatory network of CDK8-CycC in both normal development and tumorigenesis is essential to successfully develop a pharmaceutical drug that targets CDK8. The goal of this project is to identify both upstream regulators and downstream effectors of CDK8-CycC by performing a dominant modifier genetic screen in *Drosophila*. Overexpressing or depleting CDK8-CycC causes disrupted vein pattern phenotypes. We hypothesized if genes whose products function either upstream or downstream of CDK8-CycC, then reducing their products by 50% would enhance or suppress the CDK8-specific phenotypes in wings. We utilized the Bloomington Deficiency Kit, which uncovers ~98% of *Drosophila* euchromatic genome with molecularly mapped breakpoints. Further mapping of enhancers and suppressors led to identifying the genetic interactions between CDK8-CycC and signaling pathways: EGFR, and Dpp (TGFβ in *Drosophila*). These pathways are essential for normal development in metazoans and are frequently mutated in human cancers. Genetic interactions between CDK8-CycC and these pathways call for further investigation of molecular mechanisms underpinning these interactions *in vivo* and whether these mechanisms are conserved in mammal and human cancer cells.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

PROLINE CODING VARIANT AT THE 72nd CODON OF p53 MAY DELAY MMTV-*Erbb2/neu* DRIVEN MAMMARY TUMORIGENESIS THROUGH A PROSENESCENT MECHANISM

Shruti Dharmaraj, Ramesh T Gunaratna, Andres Santos, Robin S L Fuchs-Young
Department of Molecular and Cellular Medicine & Institute for Biosciences and Technolog
Texas A&M Health Science Center College of Medicine
College Station, TX

Female breast cancer (BrCa) affects nearly one in three women diagnosed with cancer and ranks second in mortality among cancers in the United States. The tumor suppressor p53 is frequently mutated in BrCa, altering key biological functions regulated by p53. A missense single nucleotide polymorphism on the 72nd codon codes for either proline (*p53*^{P72}) or arginine (*p53*^{R72}) and *in vitro* studies show that these variants can also change key biological functions regulated by p53 such as apoptosis, DNA repair, cell cycle arrest and senescence. Studies previously conducted in our laboratory using a humanized p53 model, in which the exon 4 of the human *p53* containing the *p53*^{P72} and *p53*^{R72} variants has been "knocked-in", have provided insight into the impact of these variants on p53-regulated functions in the mammary gland. These studies showed that *p53*^{P72} is better at inducing prosenescent markers such as, PML and *p16*^{INK4a} following IR-induced DNA damage. Additionally, *p53*^{P72} significantly delays MMTV-*Erbb2/neu*-driven mammary tumorigenesis in *Erbb2*$^{WT/++}$;*p53*$^{P/P}$ and *Erbb2*$^{WT/++}$;*p53*$^{R/R}$ mice (log rank test: $p<0.01$). To understand the genetic mechanism behind the difference in tumor latency, we performed qRT-PCR on a panel of selected genes using cDNA from mammary glands of age matched *Erbb2*$^{WT/++}$;*p53*$^{P/P}$ and *Erbb2*$^{WT/++}$;*p53*$^{R/R}$ mice. Interestingly, the genes involved in cell cycle arrest and senescence *p16*^{INK4a} ($p<0.001$), *p21* ($p<0.05$) and *Pai-1* ($p<0.05$) were significantly upregulated in *Erbb2/neu*$^{WT/++}$;*p53*$^{P/P}$ compared to *Erbb2/neu*$^{WT/++}$;*p53*$^{R/R}$ mice. Results from both the current study and the previous work in our laboratory indicate that *p53*^{P72} contributes to delay MMTV-*Erbb2/neu*-driven mammary tumorigenesis through a prosenescence mechanism.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

CELL SURVIVAL PATHWAYS IN CARDIAC ARREST AND THE EFFECTS OF RESUSCITATION DRUGS

Thanh Dinh, John Connolly, Brittany Bass, Jason Bowman, Matthew McClure, David E. Dostal
Department of Medical Physiology
Texas A&M Health Science Center College of Medicine
Baylor Scott & White
Central Texas Veterans Health Care System
Temple, TX

In 2015, more than 300,000 people suffered from cardiac arrest. Of those treated by medical professionals, only 10.6% survived and 8.3% remained neurologically intact. The current standard of care (ACLS) is to administer epinephrine (Epi) and amiodarone in patients undergoing cardiac arrest. However, the efficacy of Epi is disappointing based on several studies. Alternatively, recent studies show that using vasodilators such as sodium nitroprusside (SNP) and adenosine improves the coronary perfusion in the heart, leading to more cell survival. The success of vasodilator treatment is due to the activation of several cardiac survival pathways. Levosimendan has been shown to increase cardiac contractility. In this study, we tested the hypothesis that a combination of SNP, adenosine and levosimendan (SNPAL) will enhance the cardiac survival pathways and therefore, improve the outcome of cardiac resuscitation. Hearts were excised from adult male rats and perfused with Krebs-Henseleit Buffer. The heart tissue was homogenized with lysis buffer and the solubilized proteins were analyzed with Western blot and Immunoprecipitation. The ventricular myocytes were also isolated from the perfused heart, and flow cytometry was used to determine the activation of signaling factors in cardiac survival pathways. One of the major survival factors examined using Western blot analysis was Akt, which plays an important role in cardioprotection. Compared to ACLS, treatment with SNPAL increased the phosphorylation of Akt by five-fold at threonine-308 ($p<0.001$) and two-fold at serine-473 ($p=0.003$), leading to more cell survival. Therefore, mechanisms activated by SNPAL provide a potential replacement to treat cardiac arrest.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

BORRELIA BURGDORFERI ADENYLATE CYCLASE, *CYAB*, ALTERS REGULATION OF VIRULENCE DETERMINANTS AND IS REQUIRED MURINE INFECTION

Yaritza Escamilla, Vanessa M. Ante, Elizabeth Saputra,
Katherine Trevarrow, Jenny A. Hyde
Department of Microbial Pathogenesis & Immunology
Texas A&M Health Science Center College of Medicine
College Station, TX

Borrelia burgdorferi, etiologic agent of Lyme disease, undergoes dynamic gene regulation in response to environmental signals as it navigates the complex enzootic cycle between the *Ixodes* vector and mammalian host. Mechanisms utilized by *B. burgdorferi* for direct detection of environmental cues and the associated regulatory pathways are not well understood. Some bacterial pathogens utilize adenylate cyclase to detect changes in environmental conditions and produce effector molecule cAMP that contributes to virulence and infectivity. We hypothesize *B. burgdorferi* adenylate cyclase (*cyaB*), which has been associated with cAMP production, is involved in the regulation of virulence determinants and has a role in mammalian infection. A *cyaB* deletion mutant (*cyaB*$^{-}$) and *cis* complement (*cyaB*$^{-/+}$) *B. burgdorferi* strains were obtained. Protein production of transcriptional regulator BosR along with lipoproteins DbpA and OspC that are important mammalian virulence factors were assessed under conventional cultivation conditions and in response to changes in temperature, pH, and CO_2. BosR, OspC, and DbpA production were reduced in the absence of *cyaB*. *B. burgdorferi cyaB*$^{-/+}$ strain restored BosR levels similar to wild-type, but OspC and DbpA production were partially complemented. Furthermore, bioluminescent wild-type, *cyaB*$^{-}$, and *cyaB*$^{-/+}$ *B. burgdorferi* strains were evaluated in the murine model using *in vivo* imaging. *B. burgdorferi cyaB*$^{-}$ infectivity phenotype was significantly attenuated relative to wild-type, but the *cyaB*$^{-/+}$ strain was not able to restore infectivity. Overall, this data indicates *cyaB* has a role in the regulation of virulence determinants, murine infection, and suggest additional adenylate cyclase(s) may be present in the borrelial genome.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

EFFICACY OF MICROGLIA-BASED NEUROINFLAMMATION INHIBITION ON EPILEPTIC SEIZURES: COMPARISON OF MATLAB ALGORITHM AND VISUAL ANALYSIS OF EEG DATABASE

Neha Gurram, Ramkumar Kuruba, D. Samba Reddy
Department of Neuroscience and Experimental Therapeutics
Texas A&M Health Science Center College of Medicine
Bryan, TX

Epilepsy is a chronic brain disease characterized by recurrent seizures, which are abnormal electrical discharges from various brain regions. Neuroinflammation plays a key mediatory role in epilepsy and related conditions in the brain. Microglia, which constitute 5-15% of all cells in the brain, are among the major contributors of inflammation in the brain. Seizure analysis is essential for accurate diagnosis and evaluation of antiepileptic therapies. Presently, there are few validated methods for the automatic analysis of epileptic seizures from a 24X7 EEG database. We hypothesized that inhibition of microglia and resulting neuroinflammation modifies epileptogenesis and prevents progression of epilepsy development, and that our new two-step protocol will be accurate and efficient for the detection of epileptic seizures. The proposed hypothesis was tested in the DFP post-status epilepticus model of epilepsy in male rats. The effect of microglia inhibitor and anti-inflammatory agent ibudilast was assessed by recording of 24X7 EEG. The database was analyzed by a valid protocol, which consists of a two-step algorithm, implemented in the Matlab and R programs to find potential epileptic seizures by extracting local energies in different frequency bands of the EEG recordings. We used high-level rule sets to reduce the number of artifacts misclassified as epileptic seizures. Key parameters such as seizure number and seizure duration were precisely recognized using the Matlab program. Our results show this algorithm was able to detect seizures accurately comparable to manual visual screenshot analysis. In conclusion, we found that the seizure analysis of EEG database by software is highly efficient and valid akin to the visual method.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

ANALYSIS OF DEPRESSION FOLLOWING SPINAL CORD INJURY: PHYSIOLOGY, BEHAVIOR, AND MICROGLIAL ACTIVATION

Ashton Hierholzer, Kiralyn Brakel, Miriam Aceves, Alex Aceves, Michelle Hook
Department of Neuroscience and Experimental Therapeutics
Texas A&M Health Science Center College of Medicine
Bryan, TX

Depression in spinal cord injury (SCI) patients is a paramount problem with 16-34% of patients experiencing depressive symptoms. Increased inflammation is also a result of SCI and may contribute to the onset of depression. We hypothesize that the excitation of the immune system due to SCI can prompt depression in a subset of the injured population because of the distinct pro-inflammatory phenotype of these patients. In this experiment, control and test subject male Sprague Dawley rats were implanted with telemetry devices to monitor physiological conditions. Test subjects further received a T12 spinal laminectomy and contusion while controls did not. Locomotor recovery and behavioral tests for depression were conducted over 30 days. Subjects were perfused 30 days post-contusion; brain and spinal cord regions were collected, sectioned and stained. Hierarchical clustering analysis sorted the subjects into “depressed” and “not-depressed” groups based on behavioral data. Telemetry data revealed heart rate increased and heart rate variability decreased in depressed subjects indicating a physiological change in conjunction with behavioral changes. Analysis of the lesion size in the spinal cord showed no difference between the depressed and non-depressed cohorts. The lack of distinction insinuates onset of depression does not hinge on the severity of the injury. This data indicates that individual biological changes unique to each rat procured the presentation of depression. Future analysis of brain regions will assess microglia activation and neurogenesis specifically in the hippocampus and frontal cortex. Future directions would target biological indicators of depression allowing for unique therapeutic treatment for patients.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

HYPERTENSION CAUSES RENAL LYMPHATIC VESSEL DILATION

Kayla Hudson, Katharine Beckman, Catalina Lopez Gelston, Lauren Phillips, Brett Mitchell
Dept. of Medical Physiology
Texas A&M Health Science Center College of Medicine
College Station, TX

Hypertension affects ~70 million adults in America, and there are ~3 million new cases each year. Hypertension is the 2nd leading cause of kidney failure and this occurs due to prolonged inflammation and injury. The lymphatic vascular system plays a central role in removing excess fluid and transporting immune cells from inflamed tissue; however, how hypertension affects renal lymphatic vessels is unknown. We hypothesized that hypertension will cause renal lymphangiogenesis to try to reduce inflammation. Three groups of mice, each containing 3 females and 3 males, were created: 1) control mice receiving tap water, 2) mice treated with N^{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME) for 2 weeks in their drinking water, which induces hypertension by inhibiting the vasodilator nitric oxide, and 3) 2 weeks L-NAME drinking water followed by 2 weeks tap water (L-NAME-washout). Immunofluorescence for the lymphatic endothelial cell markers podoplanin and LYVE-1 did not demonstrate increased numbers of lymphatic vessels in L-NAME mice compared to control mice; however, there was marked renal lymphatic vessel dilation in hypertensive L-NAME mice. VEGF-C and VEGF-R3 are necessary for lymphangiogenesis; however renal expression of these proteins decreased significantly in L-NAME mice. There were no differences between males and females within each group. Studies currently underway include measures of serum cytokines, urinary protein levels, renal gene expression, and whether or not changes observed in L-NAME mice are restored in the normotensive L-NAME-washout mice. The functional effects of renal lymphatic vessel dilation during hypertension are unknown and their modulation may reduce renal inflammation and hypertension.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

DEVELOPMENT OF A NOVEL AFFINITY-BASED APPROACH FOR LARGE SCALE PURIFICATION OF LENTIVIRUS

Casey Hughes, Nagarjun Kasaraneni, Zhilei Chen
Department of Microbial Pathogenesis and Immunology
Texas A&M Health Science Center College of Medicine
College Station, TX

Lentiviruses offer the unique ability to transduce proliferating and resting cells of hematopoietic systems, and are invaluable in the field of gene therapy. However, there is no convenient method for lentivirus purification. Conventional affinity tag-based chromatography, which is used for the purification of non-enveloped viruses, is incompatible with lentiviruses. The overall goal of this project is to develop a novel affinity-based strategy for lentiviral vector purification. This strategy exploits the reversible phase transition property of elastin-like polypeptide(ELP) and the high affinity between two halves of an engineered naturally split DnaE intein from *Nostoc punctiforme*(Npu). One half of the intein(**C**) will be displayed on the surface of lentivirus pseudotyped with vesicular stomatitis virus envelope glycoprotein(**C**-VSV-Gpp) while the other half of the intein(**N**) will be genetically fused to ELP to form(**N**-ELP). Incubation of **C**-VSV-Gpp with **N**-ELP enables strong non-covalent interaction between **N** and **C**, linking **C**-VSV-Gpp with **N**-ELP. High salt concentration induces phase separation of ELP, resulting in the aggregation of ELP and the associated **C**-VSV-Gpp. The aggregate will be filtered via diafiltration, separating **C**-VSV-Gpp from other components. Next, the aggregates will be recovered in a low salt concentration buffer that reverses the phase transition of ELP, resulting in dissolution of the aggregated virus complex. The buffer condition will be further adjusted (dithiothreitol addition or pH reduction) to induce self-cleavage of the intein, releasing VSV-Gpp from the **C**/**N**-ELP complex. Another round of inverse transition cycling will then be performed to separate VSV-Gpp from the **C**/**N**-ELP complex, resulting in pure VSV-Gpp in solution.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

INTEGRAL ROLE OF STEM CELL FACTOR NUCLEOSTEMIN IN HEPATOCELLULAR CARCINOMA DEVELOPMENT AND PROGRESSION

Yi-Hsuan Ku, Kay Pham, Tao Lin, Robert Tsai
Center for Cancer and Stem Cell Biology
Texas A&M Health Science Center College of Medicine
Houston, TX

Hepatocellular Carcinoma (HCC) is among the top three leading causes of cancer-related deaths worldwide. Late-stage HCC is notorious for exhibiting a high resistance to chemotherapies. Our objective is to determine the underlying mechanisms of HCC's ability to resist chemotherapy and the replication-induced genomic damage that normally restricts the proliferative lifespan of most dividing cells. Nucleostemin (NS) is a nucleolar protein with elevated expression in cancer cells that functions by promoting the repair of damaged chromosomes. Loss of NS predisposes progenitor cells to replication-dependent DNA damage. We hypothesize that a key aspect responsible for the malignancy and drug resistance property of HCC involves the upregulation of NS-mediated genome repair mechanisms. NS knockdown was performed in the human HCC cell model, Huh-7, by oligofectamine-mediated transfection of siNS. In the control group, another group of Huh-7 cells was treated with siRNA duplex that targeted a scrambled sequence (siScr). Both groups were treated with Doxorubicin, 5-Fluorouracil, Oxaliplatin, and Olaparib. Results show that Huh-7 cells with NSKD are dramatically more sensitive to 5-FU. TCGA data of HCC were analyzed for NS expression and correlated with clinical parameters and hallmark pathways by statistical tests and gene set enrichment analysis (GSEA), respectively. Our data confirm the clinical importance of NS expression in the survival of HCC patients and reveal the protein's association with DNA repair pathways. NS is a potential target to increasing the chemosensitivity of advanced HCC and further analysis of its underlying mechanisms may pave the way to a rational drug design for HCC treatment.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

LABORATORY ANALYSIS OF PATHOGEN EMERGENCE

Patrick Lynch, Paul de Figueiredo
Department of Microbial Pathogenesis and Immunology
Texas A&M Health Science Center College of Medicine
College Station, TX

Emerging bacterial pathogens pose significant risks to human and animal populations across the globe. However, the molecular mechanisms that drive the emergence of bacterial pathogens remain poorly understood, principally because experimental approaches for interrogating the emergence of new pathogens are limited. Here, we describe one such approach. Specifically, we test the hypothesis that a laboratory strain of *E. coli* (DH5α), which is incapable of intracellular parasitism, can be evolved in the laboratory to survive phagocytosis by macrophages, immune cells that mediate bacterial clearance in mammals. To test this hypothesis, we evolved *E. coli* (DH5α) through successive rounds of exposure to an *in vitro* macrophage cell line. After ten iterations of co-incubation with host cells, host cell colonization, recovery of the intracellular population, *in vitro* cultivation of the recovered bacteria and re-infection of host cells, the intracellular survivability of the evolved *E. coli* was shown to dramatically increase (~100-fold). These findings lead us to conclude that innocuous bacteria are capable of evolving phenotypes (i.e., intracellular survival) characteristic of intracellular bacterial pathogens. Future work will entail sequencing the DNA of evolved and parental strains in order to determine the genetic loci that confer intracellular survival to evolved strains. Comparing findings from this analysis to the genome sequences of intracellular bacterial pathogens (e.g., *Brucella, Coxiella, Legionella*) may provide insights into mechanisms of intracellular parasitism and the evolution of infectious diseases.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

MECHANICAL EFFECTS OF ENGINEERED MATRICES ON ADIPOCYTE BIOLOGY

Naveen Menon, Ben Boyett, Evon Looper, James K. Carrow, Akilesh Gaharwar, Joseph Rutkowski
Dept. of Medical Physiology
Texas A&M Health Science Center College of Medicine
College Station, TX

Obesity, one of the leading causes of preventable morbidity worldwide, is partially defined as the expansion of adipose tissue due to increased cellular lipid uptake. In some instances this enlargement is pathological and can lead to increased inflammation and the formation of excess tissue, fibrosis, in the interstitial gaps of adipose tissue. A model, which replicates the environmental conditions that adipocytes face in fibrotic tissue, is not well defined. The primary aim of this study was to establish a three-dimensional static system to imitate these conditions. The experimental design of our study was tailored to examine the effects of different matrices and the influence of matrix rigidity on adipocyte biology. Our hypothesis is that adipocyte biology is dependent on matrix type and stiffness. Four different matrices were used in this investigation: rat-tail collagen type 1, HyStem-HP (a hyaluronic acid, cross-linked commercial matrix), methacrylated gelatin hydrogel (GelMa) and polyethylene glycol polymer hydrogel (PEG). These different mediums, in which fibroblasts were implanted and differentiated, allowed for testing the adipocyte gene expression of inflammatory, adipogenic and vascularization markers in different matrix compositions and matrix rigors. The analysis of the accrued experimental data suggests that adipocytes tolerate the different matrices similarly. However, the data also suggests that an increase in matrix rigidity inclines the adipocyte functionality to that of a brown fat form, while expressing comparable values of adipocyte inflammation and vascularization. The results of this study will permit for further examination into replicating the conditions that adipocytes face in obesity-induced fibrosis models.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

DELETION OF ZONULA OCCLUDENS-1 IN VASCULAR ENDOTHELIAL CELLS CAUSES EMBRYONIC LETHALITY WITH LYMPHATIC VESSEL DILATION

Pavia Ann Muringathuparambil, Jian Wang, Liangjing Wu, Chenshen Huang, Yang Liu, Binu Tharakan, Xu Peng
Dept. of Medical Physiology
Texas A&M Health Science Center College of Medicine
Temple, TX

Zonula occludens (ZO-1) is a tight junction protein which plays an important function in maintaining cell-cell tension and adherens junctions. Consistent with its essential cellular functions, deletion of ZO-1 resulted in embryonic lethality at around E9.5 day. To determine the role of ZO-1 in blood and lymphatic vessel formation, a new mouse line of vascular endothelial cell specific ZO-1 knockout mice was created by crossing ZO-1/flox mice with Tie2-Cre mice. The results show that ZO-1 is not required for blood vessel formation in the hindbrain at the early developmental stage (E12.5). However, inactivation of ZO-1 in vascular endothelial cells resulted in hemorrhage in the later developmental stage (E18.5). In addition, the inactivation of ZO-1 results in embryonic edema. Immunofluorescent staining of the skin presents extreme lymphatic dilation in the ZO-1 knockout mice. Taken together, ZO-1 plays a key role in lymphatic development during embryogenesis.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

ELUCIDATION OF CDG-3 AGAINST BLAC THROUGH SITE-DIRECTED MUTAGENESIS STUDY ON TEM-1

Jeffrey Ogbudu, R. Tilvawala, J. D. Cirillo
Department of Microbial Pathogenesis and Immunology
Texas A&M Health Science Center College of Medicine
College Station, TX

Mycobacterium tuberculosis (*Mtb*) is the bacterium accounting for 9.6 million of the world's population with tuberculosis. *Mtb* expresses an enzyme *β*-lactamase (BlaC) which hydrolyses β-lactam antibiotics. Previous studies showed that the active site of BlaC consists of unique amino acids ($Thre^{237}$, Gly^{132}, and Asn^{164}) that are absent in any other common class A β-lactamase. In our laboratory, we have developed fluorescent β-lactam probe (CDG-3) which is **specifically** hydrolyzed by BlaC. This substrate enables us to detect *Mtb* faster. We hypothesized that these variations in the active site of BlaC may have led to an expansion of its active site, and thus, enable hydrolysis of CDG-3. Further studies were carried out in TEM-1, a common class A β-lactamase. Single active site mutations A237T, N132G, and R164A of TEM-1, indeed showed increased activity towards CDG-3. To investigate this hypothesis further, in this study, we have successfully incorporated double (N132G/A237T) and triple (N132G/A237T/R164A) mutations into the active site of TEM-1 using site-directed mutagenesis. Further, the plasmids were transformed into *E coli* BL21, protein expression cell line. The mutants were purified by FPLC, after which we confirmed their concentration using Bradford assay. CDG assay was performed on the mutants and results show that triple mutant of TEM-1 show 100-fold increase in activity towards CDG-3 when compared with single and double mutants of TEM-1. We are further purifying the triple mutant TEM-1. In the future, we believe that this project can help improve the design of β-lactam probes that are efficient in detection of *Mtb*.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

NOVEL, CONSERVED RNA SECONDARY STRUCTURES IN MHV-A59, BOVINE CORONAVIRUS (BCoV) AND MERS-CoV

Vinathi Polamraju, Drew Nunn, Julian Leibowitz
Department of Microbial Pathogenesis and Immunology
Texas A&M Health Science Center College of Medicine
Bryan, TX

Betacoronaviruses are a subgroup of viruses in the family *Coronaviradae* known to cause an array of diseases in humans and animals. SARS coronavirus (-CoV) and MERS-CoV are two zoonotic betacoronaviruses that emerged in 2002 and 2012, respectively, which cause severe respiratory diseases in humans. In this study, we aim to determine the RNA secondary structures of Mouse Hepatitis Virus, strain A59 (MHV-A59), the best studied betacoronavirus, and the closely related betacoronaviruses, Bovine Coronavirus (BCoV) and MERS-CoV to identify novel, conserved secondary structures within their genomes. To accomplish this, we passaged DBT cell cultures in vitro and infected them with MHV-A59. Upon clarifying the virus, we tittered the cells and obtained concentrations ranging from 2.6 to $8.0x10^7$ pfu/mL. After purifying the virus by differential and sucrose density gradient centrifugation, we extracted the viral RNA and reacted it with SHAPE-MaP reagent 1M7. 1-methyl-7-nitroisatoic anhydride (IM7) probes for and forms adducts with conformationally flexible ribose 2'-hydroxyl groups in the RNA. The derivatized RNA is reverse transcribed in the presence of Mn^{++} causing misincorporation at adduct sites. This induces mutations in the cDNA transcripts which are incorporated into a cDNA library. Thus deep sequencing of this cDNA library provides us with an avenue to obtain biochemical data to create RNA secondary structure models using Shannon entropy and pairing probability models. RNA secondary structures conserved amongst the three betacoronaviruses are likely to be important for viral replication, shining light on preventing future betacoronavirus infections. Further studies are being conducted to complete SHAPE-MaP analysis of MHV-A59.

IL-3: THE MASTER REGULATOR OF HUMAN BASOPHIL ANAPHYLAXIS

Lovesimrjit Sandhu, Ghamartaj Tavana, James Moore, David P. Huston
Clinical Science and Translational Research Institute
Texas A&M Health Science Center College of Medicine
Houston, TX

Human basophils are effector cells that contribute to atopy and anaphylaxis. To establish the essential requirements for basophil function, basophils were purified by negative selection from leukopaks, and analyzed for mechanisms regulating activation, degranulation, and cytokine production. Using flow cytomerty, basophil purity was >98% by co-expression of FcεR1 and IL-3Rα. Basophil activation was determined by cell surface expression of CD69, degranulation was determined by cell surface expression of CD63, and Th2 cytokine production was determined by intracellular staining for IL-4, IL-5, and IL-13. Results demonstrated that responsiveness to diverse allergic (FcεR1 crosslinking) and non-allergic (C5a and bacterial-derived N-methylformalated peptides) degranulation-inducing agonists only occurred if the basophils were first activated by IL-3, indicating there is a sequential two-signal requirement for basophil anaphylaxis. Furthermore, a quantitative correlation between histamine release, as determined by ELISA, and CD63 expression established that histamine release is restricted to basophils with an MFI >105. This correlation refines the precision of interpreting basophil activation tests used clinically to assess basophil anaphylaxis. In addition, intracellular staining for Th2 cytokines demonstrated that basophils constitutively produced IL-4 that was not modulated by activation or degranulation-inducing second signals, and do not produce IL-5 or IL-13 with or without stimulation by IL-3 and/or second signals. Thus, basophils have the innate capacity to promote Th2 differentiation and B cell IgE isotype switch. In aggregate these studies provide the foundation for the development of therapeutics that target IL-3 or IL-3Rα as strategies to prevent basophil anaphylaxis and basophil promotion of Th2 immunity.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

DETERMINATION OF THE COMPETENCY OF THE TEXAS A&M COLLEGE OF VETERINARY MEDICINE ELECTRONIC HEALTH RECORD DATA TO SUPPORT OUTCOMES FEEDBACK FOR MEDICAL INTERVENTIONS

Amber Schulze, Akintayo A Akinleye, Preston Baker, Seth Polsley, Muppala N Prasanth Raju, Mohammad Atif Tahir, Duane Steward
Dept. of Biomedical Informatics
Texas A&M Health Science Center College of Medicine
College Station, TX

Across the country, doctors commonly use electronic medical records, yet little work has been done to analyze the data that has been collected. The ability to do this, however, requires both an ontology to define the elements and relationships within the system, as well as the ability to extract and congregate useful data from medical records using this ontology. As part of a larger pilot study, we hypothesized that we will be able to find the members and relationships of our current ontology within the medical records of the A&M College of Veterinary Medicine and extract that data to create a meaningful data set to allow for future experimentation. We began by querying the data for the basic elements of our ontology and found 298,444 patients, 116 clinicians, and 302,618 owners; we also determined that encounters could be determined using the admissions table. We then investigated the relevance of the “Call If:” section of discharge summaries to our ontology, and found that while only 2.86% of discharge summaries had this exact heading, the section contained minimal Protected Health Information. As we began to extract data from the Vet School database, we have determined by individually reading documents that we can safely de-identify full-text documents from the vet school with 97.81% sensitivity and 99.99% specificity. As this project continues, our ontology and ability to safely extract data will continue to be validated in other domains, and we will move towards improving the feedback doctors receive on the outcomes of prescribed interventions.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

INVESTIGATION OF STORE-OPERATED CALCIUM ENTRY IN RAT LYMPHATIC MUSCLE CELLS

Michael Schwalm, Xueyang Zhang, Maria Atanasiu, Jian Wang, Hongjiang Si, Sanjukta Chakraborty, David C. Zawieja, Mariappan Muthuchamy, Shenyuan L. Zhang
Department of Medical Physiology
Texas A&M Health Science Center College of Medicine
Temple, TX

An essential function of the lymphatic system is to transport lymph containing immune cells, macromolecules, and lipids etc. throughout the body, in which the spontaneous contraction of the lymphatic muscle cells (LMC) plays an indispensable role. The unique characteristics of LMC resemble a combination of cardiac and smooth muscle cells but the mechanisms underlying the recruitment of LMC during lymphangiogenesis and the regulation of LMC contraction remain unclarified. Ca^{2+} plays a major role in cell migration, proliferation as well as muscle cell contraction. An increase of cytosolic Ca^{2+} level is mainly achieved by the activation of Ca^{2+}-permeable ion channels located on the plasma membrane (PM) or the endoplasmic reticulum (ER)/sarcoplasmic reticulum (SR) Ca^{2+} Release-Activated Ca^{2+} (CRAC) channels are one type of the major Ca^{2+} channels on the PM, which are activated upon the depletion of Ca^{2+} in the ER/SR to mediate Store-Operated Ca^{2+} Entry (SOCE). In recent years, it has been reported that CRAC channels, formed by STIM1 and Orai1, play a vital role in skeletal muscle contraction and smooth muscle cell (SMC) migration and proliferation (e.g., triggered by PDGF). The question we would like to address here is whether CRAC channels also play a functional role in LMC contraction, migration, and/or proliferation. Our Western-blot data indicated the expression of STIM1 and Orai1 proteins in rat lymphatic muscles (rLMC). Besides, our Ca^{2+} imaging data collected from untreated, Orai1-E106A (a dominant-negative Orai1 mutant) transfected, and STIM1/Orai1 RNAi-silenced rLMC demonstrated a necessary role of STIM1/Orai1 in the rising of cytosolic Ca^{2+} levels in LMC treated by thapsigargin (TG) to release SR-stored Ca^{2+}. Furthermore, as previously reported, Substance P (SP) can induce dramatic contractions of lymphatic vessels; therefore, we are testing whether CRAC channels are required for SP-evoked LMC contraction. In addition, we will examine a possible role of CRAC channels in PDGF-mediated migration and proliferation of LMC.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

EXPRESSION AND PURIFICATION OF RECOMBINANT SdrG PROTEINS

Jerry Stewart, Shristee Arora, Magnus Höök
Center for Infectious & Inflammatory Diseases
Texas A&M Health Science Center
Institute of Biosciences and Technology
Houston, TX

Staphylococcus epidermidis is an opportunistic pathogen that is endogenous to the human skin. This Gram-positive bacterium is a common cause for medical device-related infections, which leads to an increase in healthcare cost. *S. epidermidis* consist of virulence factors that can initiate infection by adhering to components of the extracellular matrix via microbial surface component recognizing adhesive matrix molecules (MSCRAMMs). These proteins bind to human proteins such as fibrinogen (Fg), fibronectin and collagen. It has been reported in previous work that SdrG binds specifically to Fg. These studies were performed using a recombinant protein fragment of SdrG known as SdrGn2n3. SdrG consist of an N-terminal A region containing separately folded domains known as N2 and N3. This A region forms IgG-like folds that bind ligands by the "dock, lock and latch" (DLL) mechanism. Additional B repeats are located between the A domain and the serine-aspartate repeat R region. It is our objective to purify a longer recombinant fragment, SdrGAB, in order to show a different binding affinity for Fg, and possibly identify additional unknown ligands. We used different affinity chromatography techniques based on strep tag, his tag, and ion-exchange, to develop a protocol for the purification of SdrGAB. Western ligand blot analysis showed the N-terminal strep tag having an inability to completely bind to strep tactin. We concluded that the degradation rate and folding properties of SdrGAB are the main concerns for purification. Overall, optimization and purification of SdrGAB will help better define the pathogenic mechanism of *S. epidermidis.*

TAMHSC 2016 SUMMER RESEARCH PROGRAM

ANTIMICROBIAL ACTIVITY OF HALOGENATED BIARYL COMPOUNDS AGAINST *STREPTOCOCCUS PNEUMONIAE*

Jordan Sweatt, Carolyn Cannon
Department of Microbial Pathogenesis and Immunology
Texas A&M Health Science Center College of Medicine
College Station, TX

Life threatening infections caused by multi-drug resistant pathogens, such as *Streptococcus pnuemoniae* are increasing at a startling rate, while the discovery of new antimicrobials has decreased significantly. *S. pneumoniae* poses a great threat due to its numerous strains that tend to develop resistance to current standard-of-care antimicrobials. Our objective in the Cannon Lab was to test the effectiveness of novel biaryl compounds against multiple strains of *S. pneumoniae* in order to develop new antimicrobials useful for treatment. We used MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) techniques to determine how well a compound worked against different strains of S. pneumoniae. Starting with the strain TCH8431, four compounds out of the 26 tested, C58, C59, 4-76 and 5-32, consistently inhibited and eradicated the bacteria. Both C58 and C59 exhibited MICs ranging from 4-6 µg/ml with MBCs no higher than 6 µg/ml. 5-32 and 4-76 proved to be the most active as their MIC was repeatedly 1 µg/ml with MBC at 2 µg/ml. Once 5-32 was deemed one of the most active, it was tested against 6 different strains of S. pnuemoniae. Again, it proved to be active against each strain showing similar MIC and MBC results. Standard-of-care antimicrobials, vancomycin and ampicillin were also tested against the 6 strains and showed superior results. These data show that 5-32 and 4-76 along with C58 and C59, following further testing, are potential biaryls that may one day be used among current antimicrobials, such as vancomycin and ampicillin to treat pathogenic-associated chronic infections.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

HOXA11 HYPERMETHYLATION: A NOVEL BREAST CANCER BIOMARKER

Tung Vu, Bingshu Xia, Bing Du, Dekai Zhang
Center for Inflammatory and Infectious Diseases
Texas A&M Health Science Center College of Medicine
Institute of Biosciences and Technology
Houston, TX

Hypermethylation of the CpG islands in the promoter regions of tumor suppressor genes is one of the most common epigenetic alterations in human carcinogenesis. Homeobox A11 (HOXA11), located in the A cluster with other contiguous HOXA genes along the short arm of chromosome 7, which involved in urogenital tract development and limb patterning, has been identified to be hypermethylated at the CpG islands in several types of cancer. However, the clinical significance of HOXA11 methylation in breast cancer still remains elusive. In this study, we investigated the methylation status and expression level of HOXA11 as well as its function in cell proliferation and migration in breast cancer. We identified that HOXA11 is one of most frequently hypermethylated genes in breast cancer. We therefore hypothesize that HOXA11 plays an important role in breast cancer. In breast cancer clinical specimens, we found that HOXA11 is hypermethylated and associated with poor prognosis. This result was confirmed in breast cancer cell lines by utilizing methylation specific PCR (MSP). Moreover, the low expression of HOXA11 could be restored with the cells treated with 5-azadC. To examine the association of HOXA11 and cell migration and proliferation, wound healing assay and MTT assay were performed with breast cancer cell lines. The results indicated that overexpression of HOXA11 in breast cancer cell lines inhibits cell migration and proliferation. HOXA11 is a gene with frequent hypermethylation in breast cancer. Hypermethylation of HOXA11 can be a biomarker for diagnosis and prognosis of breast cancer. Moreover, HOXA11 is a potential tumor suppressor gene and promising therapeutic target for breast cancer.

TAMHSC 2016 SUMMER RESEARCH PROGRAM

POPULATION BASED ASSESSMENT OF 2, 3, 7, 8-TETRACHLRODIBENZO-P-DIOXIN DURING PREGNANCY

Cid'Ni Wilkerson, Melanie Warren, David Threadgill
Dept. of Molecular and Cellular Medicine
Texas A&M Health Science Center College of Medicine
College Station, TX

Dioxin, also know known as 2, 3, 7, 8-tetrachloridibenzo-p-dioxin (TCDD), can be defined as a toxicant with carcinogenic and deleterious effects on the body's tissues and organs. Previous mammalian and aquatic studies pertaining to dioxin exposure during pregnancy do not account for the various genotypes found across the population. We aim to incorporate genetic variability found within the population in assessing exposure risks to dioxin during pregnancy. In order to mimic inter-individual variability we use 36 different inbred mouse strains, with each strain representing specific individual genetic types found in the population. Over a 10 day period, we exposed plug-positive females to various levels of dioxin (0, 0.1, 1, 10, 50, 100 ng/kg/day). On day 10.5 post impregnation, these mice were euthanized and dissected in order to collect their embryos and other tissues/organs. Our current data has determined several strains of the mice are resistant to all doses of dioxin. These mice showed no significance differences in fertilization rates and fetal development compared to controls. In non-resistant strains, high dose exposure resulted in delayed development and a significant decrease of fertilization rate and viable embryos. Furthermore, there are noticeable trends of increased susceptibility as doses increased. Effects of susceptibility were observed to have significant differences across strains, which proves our original hypothesis on the impact of an individual's genetic background on dioxin susceptibility and assessing exposure risks. We intend to identify specific polymorphisms that contribute to the inter-strain susceptibly to determine how they correlate to response across the human population.

2016 Texas A&M Health Science Center
Summer Research Program Participants

Bryan/College Station		
Hayden Anz	Texas A&M University	Dr. Samba Reddy
Andrew Asante	Alabama State University	Dr. Stephen Safe
Preston Baker	Texas A&M University	Dr. Duane Steward
Lauren Beck	Case Western University	Dr. Kayla Bayless
Katharine Beckman	Ave Maria University	Dr. Brett Mitchell
Benjamin Boyett	Texas A&M University	Dr. Joe Rutkowski
Julia Bruggemann	Texas A&M University	Dr. Samba Reddy
Delfina Bur	University of Virginia	Dr. Farida Sohrabji
Alexis Carr	University of Houston	Dr. Sarah Bondos
Daisy Consuegra	University del Este Recinto de Carolina	Dr. Ursula Winzer-Serhan
Kerolayne de Almeida Costa	Texas A&M University	Dr. Jun-yuan Ji
Shruti Dharmaraj	New Jersey Institute of Technology	Dr. Robin Fuchs-Young
Yaritza Escamilla	University of Texas Rio Grande Valley	Dr. Jenny Hyde
Neha Gurram	Baylor University	Dr. Samba Reddy
Ashton Hierholzer	Oklahoma State University	Dr. Michelle Hook
Kayla Hudson	Texas A&M University	Dr. Brett Mitchell
Casey Hughes	University of Texas Rio Grande Valley	Dr. Zhilei Chen
Patrick Lynch	Cornell University	Dr. Paul de Figueiredo
Naveen Menon	Texas A&M University	Dr. Joe Rutkowski
Jeffrey Ogbudu	Alabama State University	Dr. Jeff Cirillo
Vinathi Polamraju	Texas A&M University	Dr. Julian Leibowitz
Amber Schulze	Baylor University	Dr. Duane Steward
Jordan Sweatt	Prairie View A&M University	Dr. Carolyn Cannon
CidNi Wilkerson	Prairie View A&M University	Dr. David Threadgill

Houston		
Yi-Hsuan Ku	University of Texas at Austin	Dr. Robert Tsai
Lovesimrjit Sandhu	University of Texas at Austin	Dr. David Huston
Jerry Stewart	Texas Southern University	Dr. Magnus Hook
Tung Vu	University of Houston	Dr. Dekai Zhang

Temple		
Lauren Canady	Texas A&M University	Dr. Sharon DeMorrow
John Connolly	University of Arkansas	Dr. David Dostal
Thanh Dinh	New Hampshire University	Dr. David Dostal
Pavia Ann Muringathuparambil	University of Texas at Austin	Dr. Xu Peng
Michael Schwalm	Baylor University	Dr. Shenyuan Zhang

2016 Texas A&M Health Science Center
Summer Research Program Seminar Series

Date	Time	Topic	Presenter
5/27	10:00 AM	Roundtable - Record Keeping	
5/31	12:00 PM	TAMHSC Graduate School Overview	Dr. Warren Zimmer
6/3	10:00 AM	Roundtable – Fitting in a Laboratory	
6/7	12:00 PM	CST*R Grand Rounds	Dr. Mansoor Khan
6/10	10:00 AM	Roundtable – Literature Review	
6/14	12:00 PM	Scientific Method	Dr. David McMurray
6/17	10:00 AM	Scientific Misconduct	Dr. Vernon Tesh
6/21	12:00 PM	Biotechnology & Ethics	Dr. Jim Samuel
6/24	10:00 AM	TAMHSC Clinical & Translational Science Track	Dr. David Huston
6/28	12:00 PM	TAMHSC Cardiovascular Track	Dr. Cindy Meininger
7/1	10:00 AM	Roundtable – Writing an Abstract	
7/8	10:00 AM	TAMHSC Neuroscience Track	Dr. Ursula Winzer-Serhan
7/12	12:00 PM	TAMHSC Biochemistry Track TAMHSC Microbial Pathogenesis-Immunology Track	Dr. Sarah Bondos Dr. Jon Skare
7/15	10:00 AM	Roundtable – Organizing Your Poster	
7/19	12:00 PM	TAMHSC Cellular & Molecular Biology Track TAMHSC MD-PhD Track	Dr. Kayla Bayless Dr. Carolyn Cannon
7/22	10:00 AM	Roundtable – How To Give a 10 Minute Talk	
7/25	10:00 AM	Student Presentations	
7/26	10:00 AM	Student Presentations	
7/27	10:00 AM	Student Presentations	
7/28	10:00 AM	Student Presentations	
7/29	9:00 AM- 3:00 PM	Poster Presentations, Reception, and Awards Ceremony	

Program Director

Dr. Brett Mitchell
Department of Medical Physiology
Texas A&M Health Science Center
College of Medicine
Rm. 361A Reynolds Medical Building
College Station, TX, 77843-1114
Email: bmitchell@tamhsc.edu
Phone: 979.436.0751

PLEASE KEEP US UPDATED WITH YOUR CONTACT INFORMATION AND CAREER/SCHOOL DECISIONS AFTER GRADUATION

THANK YOU AND THANKS FOR YOUR HARD WORK THIS SUMMER!