MEDICINE
TEXAS A&M UNIVERSITY

SUMMER RESEARCH PROGRAM

27 JULY 2018 • 9:00 AM

RESEARCH DAY

Health Professional Education Building

Bryan, Texas

SCHEDULE OF EVENTS

July 27, 2018

8:30 AM	**Registration Table Opens** HPEB LL Lobby
9:00AM-12:00PM	**Poster Viewing & Judging** HPEB LL43 A&B
12:00PM-12:30PM	**Lunch** HPEB LL46
12:30PM-1:30PM	**SRP Keynote Speaker** **Dr. Mansoor Khan** Professor and Vice Dean College of Pharmacy Interim Head, Department of Pharmaceutical Sciences **Title: Medication in Healthcare: Challenges and Opportunities with Opioid Products** HPEB LL46
1:30PM-2:00PM	**Presentation of Certificates** Dr. Brett Mitchell, Summer Research Program Director HPEB LL46 **Presentation of Dean's Recognition Awards** HPEB LL46
2:00PM	**Group Picture & Adjourn**

CONGRATULATIONS!

Keynote Speaker

Dr. Mansoor Khan

Professor and Vice Dean
College of Pharmacy
Interim Department Head of Pharmaceutical Sciences

Title: Medications in Healthcare: Challenges and Opportunities with Opioid Products

Dr. Mansoor A. Khan has served US FDA for over 11 years as the Director of Product Quality Research and a Senior Biomedical Research Scientist (SBRS) at CDER , He led research teams on biotech products and small molecules, chemistry and stability, drug delivery systems and bioavailability/bioequivalence, and chemistry reviews of new and generic drugs for several complicated products. In Sept of 2015, he joined Texas A&M University as Professor and Vice Dean at Texas A&M Health Science Center College of Pharmacy in their College Station Campus. He aslo serves as the Interim Head of the Department of Pharmaceutical Sciences. Prior to joining FDA in 2004, Dr. Khan was a Professor of Pharmaceutics and Director of Graduate Program in the School of Pharmacy at Texas Tech University Health Science. He is a registered pharmacist, and earned his Ph.D. degree in Industrial Pharmacy from the St. John's University School of Pharmacy in 1992. He has published over 295 peer-reviewed manuscripts, five texts including "Pharmaceutical and Clinical Calculations" and “Quality by Design for Biopharmaceutical Drug Product Development, 25 book chapters, 250 poster presentations, and more than 250 invited presentations world-wide. Dr. Khan's research focus is primarily in the area of formulations design and development, and biopharmaceutics. He led the FDA new drug review team that approved the first 3D product on Aug 3, 2015.

Dr. Khan has held several leadership positions at the AAPS including elected chair of pharmaceutics and drug delivery (PDD) and the founding chair of formulations design and development (FDD). He serves on the editorial board of Pharmaceutical Technology, International Journal of Pharmaceutics, AAPSPharmsciTech, and the Drug Delivery and Translational Research. He has received FDA/CDER 2015 outstanding ANDA reviews award, over ten FDA/CDER Team Excellence Awards, FDA/CDER Scientific Achievement Award, and FDA/CDER Exemplary Performance Awards, outstanding alumni award from St. Johns University, College of Phamacy, Excellence Award from Texas A&M University Health Science Center,. He received the 2012 AAPS Research Achievement Award in Formulations Design and Development. He is also an AAPS and AAiPS Fellow.

Acknowledgements

The Texas A&M Health Science Center College of Medicine's Summer Research Program continues to attract the top students from the best colleges and universities all across the country. This year we had 49 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 the applications.

- **Pooneh Bagher, Ph.D.**
- **Kayla Bayless, Ph.D.**
- **Sanjukta Chakraborty, Ph.D.**
- **Sharon DeMorrow, Ph.D.**
- **Cedric Geoffroy, Ph.D.**
- **Shannon Glaser, Ph.D.**
- **David Huston, M.D.**
- **Jun-Yuan Ji, Ph.D.**
- **Jason Karpac, Ph.D.**
- **Kristin Patrick, Ph.D.**
- **Xu Peng, Ph.D.**
- **Samba Reddy, Ph.D.**
- **Mendell Rimer, Ph.D.**
- **Joseph Rutkowski, Ph.D.**
- **Andreea Trache, Ph.D.**
- **Emily Wilson, Ph.D.**
- **Shenyuan Zhang, 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 was made possible by the following people who provided funding and sponsorships:

- **Carrie Byington, M.D. – Senior Vice President, Texas A&M Health Science Center**
- **Mike O'Brien, Ph.D. – Provost, Texas A&M University - San Antonio**
- **Tami Annable – Executive Director, Temple Health and Bioscience District**
- **Dennis Daniels, MPH, Dr.PH – Prairie View A&M University**
- **Van Wilson, Ph.D. – Associate Dean, Texas A&M College of Medicine**
- **Filomeno Maldonado, M.A. – Texas A&M Health Science Center**
- **American Heart Association Summer Undergraduate Research Fellowship**

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, Heather Francis, Shannon Glaser, Sharon DeMorrow,** 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** and **Cierra Singleton** in Bryan/College Station, **Tammy Kocurek** in Houston, and **Amelia Rodriguez** 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 for a memorable summer – Gig 'em!

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

Abstracts

TAMHSC 2018 SUMMER RESEARCH PROGRAM

INTRANASAL ADMINISTRATION OF MSC-DERIVED EXOSOMES MODULATE STATUS EPILEPTICUS INDUCED ABNORMAL PLASTICITY OF NEWLY BORN NEURONS AND MICROGLIAL ACTIVATION IN THE HIPPOCAMPUS

Mariam Atobiloye, Sahithi Attaluri, Daniel Gitai, Maheedhar Kodali, Dong-Ki Kim, Darwin J. Prockop Ashok K. Shetty

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Status epilepticus (SE) is a self-sustaining seizure episode that causes adverse alterations in the hippocampus, which lead to a state of chronic epilepsy. We investigated the effects of mesenchymal stem cell (MSC)-derived exosomes (small extracellular vesicles, EVs) to ameliorate the most prominent early changes induced by SE such as the abnormal plasticity of newly born neurons and activation of microglia. F344 rats were subjected to Kainic-acid-induced SE for 2 hours and then intranasally treated with a dose of exosomes (100 billion) or vehicle (VEH). In animals receiving VEH after SE (n=5), 20% of doublecortin (DCX)-positive newly born neurons displayed basal dendrites at 7 days post-SE. Furthermore, the percentage of microglia with ramified morphology (resting microglia) was severely depleted in the CA1 subfield (6%) of these animals because of their activation. In contrast, in animals receiving EVs after SE (n=8), only 5% of DCX+ newly born neurons exhibited basal dendrites ($p<0.0001$). Also, these animals displayed a higher percentage (20%) of ramified microglia than animals receiving VEH, implying a reduced level of activation ($p< 0.05$). Thus, intranasal administration of MSC-derived exosomes after SE is efficient for dampening the abnormal plasticity of newly born neurons and modulating microglial activation in the hippocampus. Basal dendrites promote abnormal connectivity between dentate granule cells and hilar/CA3 pyramidal neurons, as well as enhance granule cell to granule cell connectivity. Since both abnormal plasticity and neuroinflammation contribute to the occurrence of spontaneous seizures in the chronic phase, the results have significance. *Supported by a DOD grant to A.K.S.*

TAMHSC 2018 SUMMER RESEARCH PROGRAM

THE THERAPEUTIC EFFECTS ON LIVER FIBROSIS, BILIARY PROLIFERATION, AND CELLULAR SENESCENCE AFTER KNOCKOUT OF Melatonin 1 AND MELATONIN 2 RECEPTOR GENES

Paul Baker Jr., Nan Wu, Konstantina Kyritsi, Julie Venter, Fanyin Meng, Pietro Invernizzi, Francesca Bernuzzi, Keisaku Sato, Eugenio Gaudio, Tianhao Zhou, Heather L. Francis, Paolo Onori, Antonio Franchitto, Shannon S. Glaser, Gianfranco Alpini**.**

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Cholangiocytes are liver epithelial cells of the bile duct. These cells proliferate during cholestasis and help regulate the amount of bile in the liver. Knocking out of MT1/MT2 receptor genes will differentially regulate liver fibrosis, biliary proliferation, and cellular senescence in bile duct ligated mice. Sirius Red staining was used to observe liver fibrosis, immunohistochemistry was used to observe proliferation, qPCR was used to observe senescence through gene expression (p16/p21), and immunofluorescence was used to observe biliary melatonin receptor expression. Bile duct ligation was performed on mice to develop into BDL to simulate liver injury. The mice received had globally knocked out genes. Small hairpin RNA was used to silence the genes *in vitro*. Antibodies were then used to select for cells with the plasmid. Liver fibrosis increased in MT2 KO (Knock out) and MT1/MT2 DKO (Double knock out) BDL mice but decreased in MT1 KO BDL mice compared to WT normal and BDL mice. Biliary proliferation decreased in normal and BDL MT1 KO mice but increased in normal and BDL MT2 and MT1/MT2 DKO mice. Biliary senescence remained constant and low in all types of normal mice. BDL WT mice showed elevated levels of cells with senescence compared to the normal mice. MT1 KO BDL mice showed less senescence than WT BDL while MT2 KO showed more compared to the WT BDL. In conclusion, the removal of the MT1 melatonin receptor gene is a potential therapeutic technique used to reduce liver fibrosis and cellular senescence during liver injury.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

ROLE OF IL-6 LEVELS IN THE DEVEPLOMENT OF DEPRESSION PRIOR TO SPINAL CORD INJURY

Amelia Blydenburgh, Kiralyn Brakel, Ravali Dundumulla, Michelle Hook

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Approximately 30% of spinal cord injury (SCI) patients develop depression. Although likely caused in part by changes in psychosocial factors, new evidence suggests that inflammation, which is characteristic of SCI, may also contribute to the development of depression. Like humans, rodents that display depression-like symptoms after SCI have increased serum pro-inflammatory cytokine levels. Intriguingly, they not only have increased cytokines post-injury, but interleukin-6 (IL6) expression is also significantly upregulated in depressed, relative to not-depressed, rats *prior to injury.* We hypothesized that elevated IL-6 levels prior to injury might increase rodents' susceptibility to developing depression after SCI. To test this, male Sprague-Dawley rats received IL-6 (1.6ug/day) for 7 days prior to a spinal contusion injury (T12 vertebra). Rats were assessed with a battery of depression tests (sucrose preference, open-field, social activity, burrowing, forced swim) before injury and on days 2, 9, and 20 post-injury. Using data from the post-injury tests, rats were characterized as depressed or not-depressed using hierarchical clustering. The rats clustered into two groups: 43% displayed depression-like behaviors. Supporting our hypothesis, the incidence of depression was significantly higher in the IL-6 treated rats, compared to controls: 60% of the IL-6 treated rats were depressed, compared with 27% of the controls. ELISAs of cytokine levels in the frontal cortex and hippocampus at 30 days' post-injury revealed no significant differences between the groups. Supraspinal inflammation may not be necessary for the long-term maintenance of depression. However, these data suggest that inflammatory cytokines, such as IL-6, do increase susceptibility to depression post-injury.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

FnBPA SEQUENCE VARIANCES OF *S. AUREUS* STRAINS AND THEIR RELATION TO FIBRINOGEN BINDING AFFINITY

Laura Chang, Kelly Churion, Magnus A. Höök

Center of Infectious and Inflammatory Diseases
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Houston, Texas

Staphylococcus aureus is a bacteria found naturally in 20% of the human population and is the cause of infections like pimples and abscesses, but can lead to more dangerous infections like endocarditis when the bacteria enters the bloodstream. The bacteria's ability to create biofilm by exploiting the host's fibrinogen with its microbial surface component recognizing adhesive matrix molecules (MSCRAMMs), specifically fibronectin binding protein (FnBPA and FnBPB), has been a barrier for antibiotics to combat such infections, especially from methicillin resistant *S. aureus* (MRSA). The aim of this study is to determine the relation between sequence variations and mechanisms of effective fibrinogen binding of different *S. aureus* strains in order to provide insight on producing an effective antibiotic. In correlation, differing fibrinogen affinity due to sequence variations may indicate which strains have more tendency to form biofilm. By growing and purifying FnBPA/B in the lab, we can conclude optimal binding values of fibrinogen to FnBPA/B through isothermal titration calorimetry (ITC). Moreover, by using docking and modeling programs, probable interactions between fibrinogen and FnBPA/B can be analyzed and compared to ITC data. Thus, the future direction of this study is to determine whether or not sequence variation in different *S. aureus* FnBPA/B strains play a significant role in changing the mechanism and affinity for fibrinogen binding and thus, biofilm formation. It is also worth investigating whether dimerization of FnBPA/B affects binding of fibrinogen and if it correlates to biofilm formation ability.

This work is supported by NIH grant, R01 AI020624. LC was supported by the A&M Summer Research Program.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

DEVELOPMENT OF PLGA/PLGA-PEG NANOPARTICLES FOR THE TARGETED DELIVERY OF SILVER IBUPROFENATE, A NOVEL ANTIMICROBIAL COMPOUND

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The overuse of antibiotics has led to a decline in efficacy of standard-of-care antimicrobials due to the rapid emergence of multi-drug resistant (MDR)-bacteria. Infections with MDR-bacteria pose major problems for patients, and the shrinking antimicrobial pipeline hasn't kept pace with the need for new antibiotics. An alternative to traditional antibiotics, silver cations exhibit broad-spectrum antimicrobial activity against MDR-bacteria. To enhance the efficacy of silver, we've developed a novel silver-salt of ibuprofen (AgIBU) with potent antimicrobial activity. Because the antimicrobial properties of silver depend on its +1 ionic state, and AgIBU is aqueously insoluble, bioavailability and biodistribution of effective concentrations of AgIBU to treat MDR-bacterial infections remain major challenges. We aim to develop targeted poly(lactic-co-glycolic acid)/poly(lactic-co-glycolic acid)-polyethylene glycol (PLGA/PLGA-PEG), hydrophobic-core nanoparticles for the controlled release of AgIBU. Nanoparticles were produced using nanoprecipitation, characterized for drug loading using UV/VIS-spectroscopy, and tested for size distribution and zeta-potential using dynamic light scattering and phase analysis light scattering, respectively. Conjugation of maltotriose, which targets the maltodextrin transporter exclusively found on outer surfaces of bacteria, involves a five-step synthesis process. Optimization resulted in nanoparticles with up to 4.9% drug loading (%m/m), average diameters from 2799 to 3900 nm, and zeta-potentials of -40.52 mV to -34.51 mV. Purification and verification using ^{1}HNMR for two steps of the maltotriose conjugation were completed. Further optimization should improve both loading and size distribution for a target loading of ~7-10% and size of 200-400 nm. Future studies will test maltotriose functionalized, drug-loaded nanoparticles for selective binding to, and killing of MDR-bacteria.

Funding sources: Men of Distinction Foundation, Texas A&M Health Science Center Summer Research Program Fellowship

TAMHSC 2018 SUMMER RESEARCH PROGRAM

SPECIALIZED PROTEINS IN *COXIELLA BURNETII* MAY IMPROVE REPLICATION IN MACROPHAGES

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Coxiella burnetii, a gram-negative intracellular bacterium, is the causative agent of the zoonotic disease, Q fever. Interestingly, *Coxiella* replicates within the acidic phagolysosomes of cells, especially those of macrophages in mammals. Currently, six Type 4 Secretion System effector-producing genes—*CBU1639, CBU1636*, *CBU2007*, *CBU2016*, *CBU2028*, and *CBU1217*—have been identified that may be key to *Coxiella* survival in macrophages. However, the functions of these effectors are unknown. As we have already produced transposon insertion mutants for each of these genes, we hope that the introduction of complementing plasmids will restore the normal replication of the *Coxiella* in macrophages, confirming the current hypothesis. Genetic complements will be produced by isolating and cloning a wild type copy of the disrupted genes into a tetracycline-inducible *Tn7* transposon to re-introduce a chromosomal copy of each into the appropriate mutant strain of *Coxiella*. Here we describe the cloning process for each of the complementing plasmids. The *E. coli* clones that harbor the plasmids will be screened through colony PCR, and the plasmids confirmed by DNA sequencing. Future directions include observing the effects of the proteins in yeast through an epistatic miniarray profile (E-MAP) in an effort to predict their functions. When this study is completed, we believe we will have confirmed the contribution of each of the macrophage-specific effectors to *Coxiella* pathogenesis.

This work was supported by Defense Threat Reduction Agency grant #HDTRA1-13-1-0003 and NIH/NIAID grant #5R01AI090142-01A1.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

THE EFFECT OF TRAUMATIC BRAIN INJURY ON NEUROGENESIS IN ALCOHOL-DEPENDENT MICE

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Traumatic brain injury (TBI) is a major cause of death and disability worldwide, contributing to nearly 30% of all injury deaths. More than half of adolescents and adults treated in rehabilitation for TBI have prior histories of substance use disorders, and TBI is also known to lead to an increased addiction susceptibility. TBI can result in pathogenic neuroplasticity, including neurogenesis and neuronal turnover, which may contribute to addiction. While alcohol has been shown to reduce hippocampal neurogenesis and neuronal turnover in animal models, TBI has demonstrated an increase in neurogenesis in short periods post-injury, as well as the aberrant growth of basal dendrites from the immature neurons. However, studies have not yet explored the effects of combing ETOH and TBI on neurogenesis and aberrant plasticity in the hippocampal, dentate gyrus (DG) region of the brain. This study explores this effect by quantifying the number immature granule cells, and their basal dendrites that grow into the hilus of the DG. Mice were provided free access to ETOH every other day for 6 weeks, then half were subjected to a lateral fluid percussion injury (FPI). Mice were then allowed free access to alcohol again for 4 more weeks. The data demonstrated an increase in the number of immature neurons following TBI in alcohol-dependent mice, compared to sham TBI mice. The data also showed that the newborn neurons from the TBI + ETOH mice had more basal dendrites extending into the hilus compared to sham + TBI mice.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

GUT MICROBIOTA MEDIATED METABOLITES ALTER LYMPHATIC INFLAMMATORY MECHANISMS

Sabrina DeLeonibus, Catalina Lopez Gelston, Cassidy Weeks, Sahar Eshghjoo, Katherine Kelly, Arul Jayaraman, Robert Alaniz, Sanjukta Chakraborty.

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Diet induced changes in gut microbial composition and dysregulated immune responses are key players in the progression of metabolic syndrome (MetSyn) and obesity. The lymphatics that are directly exposed to the gut microbiota in the mesenteric bed are central factors in inflammatory and immune responses and are impaired during MetSyn. However, it remains undefined how alterations in microbial metabolites impact lymphatic inflammation. Endogenous tryptophan (Trp) derived gut microbiota metabolites have been shown to alter inflammation and immune response. We hypothesize that microbiota influence the lymphatic mesenteric microenvironment via such discrete metabolites to impact inflammatory mechanisms. We evaluated the effects of two key Trp metabolites—indole and tryptamine—in vitro and in vivo using lymphatic endothelial cells (HDLECs) and a rat model of LPS that induced acute inflammation. HDLECs were treated with indole and tryptamine in the presence or absence of LPS and levels of inflammatory cytokines were determined. Further, rats were injected with LPS for 24hrs with or without indole and assessed for inflammation. Our results show that indole decreases levels of several inflammatory mediators induced by LPS in the HDLECs, the mesenteric arcades, and lymph nodes. Similar effects are also observed with tryptamine. Flow cytometric analysis also shows that indole significantly suppresses the population of inflammatory MHCII+/cd45+cells that are induced in LPS treated animals. Hence, our data demonstrates that tryptophan metabolites suppress inflammatory pathways induced in inflamed lymphatics and, thus, maybe viable therapeutic targets for inflammation associated lymphatic dysfunction during MetSyn.

This work was supported by AHA grant #17SDG33670306.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

COMPARING MAS-RELATED G-PROTEIN COUPLED RECEPTOR MEMBER X2 (MRGPRX2) IN HUMAN BASOPHILS AND MAST CELLS

Thao Doan, Ghamartaj Tavana, James Moore, David P. Huston

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Human basophils are leukocytes that mediate inflammation and anaphylaxis. Basophil anaphylaxis occurs in a two-step process that requires interleukin-3 (IL-3) activation and then degranulation via second signals, such as bacterial-derived N-methylformalated peptides (FMLP), cross-linkers of FcεR1 (CRA1), or complement degradation anaphylatoxin (C5a). Mas-related G-protein coupled receptor member X2 (MRGPRX2) is reported to be expressed by human mast cells and mediates IgE-independent activation and degranulation using cationic ligands, such as neuropeptides, antimicrobial peptides, and drugs. Recent controversy suggest MRGPRX2 is not exclusive to mast cells and may also be present on basophils, but it has not been clearly demonstrated. We aimed to establish whether MRGPRX2 has constitutive or inducible expression by basophils in the presence and absence of IL-3, second signals, or IL-3 + second signals. Additionally, basophils were tested for response to known MRGPRX2 agonists, substance P and compound 48/80. Human basophils were purified from leukopaks through negative selection, and flow cytometry was used to analyze MRGPRX2 with IL-3 and second signals, CRA1, FMLP, and C5a. Basophil activation and degranulation were measured by expression of CD69 and CD63, respectively. Our results indicated basophil expression of MRGPRX2 is not constitutive or inducible by IL-3, second signals, or IL-3 + second signals. Basophils did not respond to MRGPRX2 stimulants, substance P or compound 48/80. PCR data indicated MRGPRX2 mRNA is expressed by LAD2 mast cells but not basophils. Our results definitively show basophils do not express MRGPRX2. Cationic ligands that induce anaphylaxis through MRGPRX2-mediated pathways function by targeting mast cells only.

Supported by NIH grant RO1AI097372.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

OPTOGENETIC CONTROL OF EARLY ENDOSOME LABELING USING ENGINEERED FYVE DOMAINS (OPTOENDO)

Brendan D'Souza, Lian He, Yubin Zhou

Center for Translational Cancer Research, Institute of Biosciences and Technology, College of Medicine, Texas A&M University, Houston, TX

Endosomes play important roles in endocytic and exocytic pathways, and control of these organelles implies the ability to influence how cells digest, process, and recycle materials. The goal of this study was to develop innovative tools (designated OptoEndo) to label and interrogate endosomal compartments in living cells. We hypothesized that this could be achieved by fusing a photo-sensory domain with a minimized FYVE (Fab 1, YOTB, Vac1, and EEA1) domain. Upon light simulation, OptoEndo would undergo dimerization to enable its interaction with the phosphatidylinositol-3-phosphate PI(3)P lipids that are abundantly distributed on early endosomal membranes. To investigate an optimal configuration for OptoEndo, FYVE domains from various proteins were aligned with a light-sensitive domain from cryptochrome 2 (CRY2) and the fluorescent marker mCherry (mCh). OptoEndo was then transfected into HeLa cells, and colocalization between OptoEndo and an endosome marker was measured before and after illumination with blue light. Through optimization, we found that the FYVE domains from FYVE, RhoGEF, and PH domain-containing proteins 2 and 4 (FGD2 and FGD4) successfully showed a light-dependent association with early endosomal membranes. We further revealed that these protein domains are very sensitive to changes in length, with various sequence lengths preceding the FYVE domains providing unique photo-sensitivities. In the future, further modifications can be made to the FGD2 and FGD4 FYVE domains to reduce pre-aggregation, increase light sensitivity, and promote the reversibility of OptoEndo. Afterward, other enzymatic domains may be attached to provide novel controls on endosomal function, movement, and behavior.

This work was supported by the National Institutes of Health (R01GM112003 and R21GM126532) and the Welch Foundation (BE-1913).

CHARACTERIZATION OF CARDIAC FUNCTION IN PRESENILIN-1 TISSUE-SPECIFIC KNOCKOUT MICE

Charles Edgar, Michelle Kaspick, Stephen Jokerst, Graham Rector, David Dostal

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Presenilin-1 (PSEN-1) is an integral protein located in the endoplasmic reticulum which forms the γ-secretase complex, mutations in which can corrupt the function of γ-secretase leading to formation of amyloid plaques associated with Alzheimer's Disease. PSEN-1 also acts as an ER Ca^{2+} leak channel, and while it has been speculated that interferences in this function causes development of Alzheimer's pathologies, its effects have not been studied in relation to heart disease. Because of the necessity of Ca^{2+} leak channels in activating ryanodine receptors and initiating myocardial contraction, we hypothesized that mutations in the gene, simulated by CRE-induced PSEN cardiac knock-out mice, would cause loss of sarcoplasmic calcium release culminating in diastolic dysfunction and dilated cardiomyopathy, and that PSEN-1 function would be modified by cardiac stretch mechanisms previously characterized by Dostal lab. Tamoxifen or a sham corn oil treatment was orally administered to cardiac PSEN knockout mice at 12 weeks of age. Echocardiography was used to track various measures of cardiac function over several weeks after treatment. Systolic and diastolic blood pressures were measured using tail cuff plethysmography. Cardiac myocytes were isolated from adult mouse hearts and used for PSEN staining and Western blot analysis. We found that loss of PSEN-1 function led to dilated cardiomyopathy, diastolic dysfunction, and arrhythmia; and that PSEN function was modulated by JNK, Akt, and p38, supporting our hypotheses. Langendorf perfusion, EKG analysis, proteomic analysis, and measurements of calcium trafficking will now be used to conclusively identify the mechanism of PSEN-1 knockout induced heart failure.

This work was supported by the Veterans Affairs Administration of Central Texas through a cooperative grant with Baylor University. CE & SJ were supported by Temple Health & Bioscience District Research Fellowships.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

DOWNREGULATION OF HEPATIC STEM CELL FACTOR BY VIVO-MORPHOLINO TREATMENT INHIBITS MAST CELL MIGRATION AND DECREASES LIVER DAMAGE IN MDR2$^{-/-}$ MICE

Jenée Farrell, Laura Hargrove, Lindsey Kennedy, Vik Meadows, Julie Ann Tolefree, Abigail Joy Garcia, Julia Mirabel, Nicole Francis, Heather Francis.

Department of Medical Physiology
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Temple Health and Bioscience District Research Fellowship
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Primary sclerosing cholangitis (PSC) is an idiopathic, autoinflammatory disorder characterized by increased mast cell (MC) infiltration, biliary damage and hepatic fibrosis. In human PSC and Mdr2$^{-/-}$ mice (PSC model), damaged cholangiocytes secrete increased levels of stem cell factor (SCF), a chemoattractant for c-kit, that induces hepatic MC migration. MCs contribute to biliary damage and hepatic fibrosis. Our present study evaluates if blocking SCF inhibits MC migration, biliary damage, and hepatic fibrosis in Mdr2$^{-/-}$ mice. Wild-type (WT) and Mdr2$^{-/-}$ mice (12 weeks) were treated with mismatch or SCF Vivo-Morpholino by tail vein injection. Hepatic damage was assessed by H&E staining and ductular reaction was evaluated by immunohistochemistry for CK-19. Biliary SCF expression was measured in cholangiocytes by immunofluorescence. MC migration was measured by mouse MC protease-1(mMCP-1) staining and MC activation was determined using serum chemistry to measure histamine (HA) levels. Hepatic fibrosis was determined by Sirius Red staining and qPCR for the fibrotic marker α-SMA. Inhibition of SCF/c-kit interaction reduced hepatic damage, MC migration/presence, ductular reaction and hepatic fibrosis in Mdr2$^{-/-}$ mice treated with SCF vivo-morpholino compared to WT and Mdr2$^{-/-}$ mismatch. Since MCs exacerbate PSC-induced damage, inhibition of MC infiltration or targeting MC mediators, may be a therapeutic option to alleviate PSC progression.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

LPS-INDUCED ALTERATIONS IN HIPPOCAMPAL GENE EXPRESSION IN A MOUSE MODEL OF HETEROZYGOUS 15Q13.3 MICRODELETION SYNDROME

Tucker J.F. Fischer, Katherine A. Rees, Desiree A. Garza, Alexandra L. Tan, Ursula H. Winzer-Serhan

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College Station, TX

The 15q13.3 microdeletion syndrome has a prevalence of 1 in 40,000 people and increases the risk of developing epilepsy and psychiatric disorders. This deletion encompasses 6 genes including *CHRNA7*. *CHRNA7* codes for the α7 subunit of the nicotinic acetylcholine receptor, which modulates GABA's developmental switch from depolarizing to hyperpolarizing by regulating expression of the Cation-Chloride Cotransporters KCC2 and NKCC1. Furthermore, α7 nAChRs modulate neuro-inflammatory responses. We hypothesize that in a mouse model of the microdeletion, heterozygous (Het) mice will have altered expression of KCC2 and NKCC1 compared to wild-type (WT), and that Het mice will have an enhanced immune response to LPS with differential activation of microglia and astrocytes. In our study, we injected WT and Het mice with LPS or saline. After three hours, hippocampi were dissected, total mRNA extracted, and expression of target proteins measured using qPCR. We found that 1) KCC2 expression was increased in Het mice, and NKCC1 expression was decreased by LPS treatment in both genotypes. 2) Iba-1 expression was upregulated 14-fold in WT mice in response to LPS but no change was seen in Het mice. 3) Levels of GFAP, C1qA and C3 were not affected by genotype or treatment. These results indicate that GABAergic signaling is altered by the microdeletion and perhaps affected by neuroinflammation, and microglial responsivity to an inflammatory challenge is diminished in Het mice with no significant difference in the innate immune response. Further experiments will assess the difference in microglial activation and elucidate KCC2's potential role in this syndrome.

This work was supported by Texas A&M Health Science Center Summer Research Program Fellowship.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

CYTOKINE EXPRESSION IN THE CENTRAL NERVOUS SYSTEM IN A MOUSE MODEL FOR THE 15Q13.3 MICRODELETION

Desiree Garza, Tucker Fischer, Alexandra Tan, Katherine Rees, Ursula Winzer-Serhan

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15q13.3 microdeletion syndrome is associated with symptoms such as neuropsychiatric impairments, behavioral problems, intellectual deficits, seizures and autism. The 15q13.3 deletion occurs on chromosome 15 and affects six genes, including the *CHRNA7* gene that encodes α7 subunit of the nicotinic acetylcholine receptors (nAChRs). The α7 nAChRs regulate the immune system via the cholinergic anti-inflammatory pathway and reduce an inflammatory response to immunogens. Reduced expression of α7 nAChRs should therefore result in increased immune activation in response to immunogens. A preliminary study indicated that an LPS dose of 100 µg/kg significantly elevated serum pro-inflammatory cytokine levels. The α7 nAChR also regulates the cholinergic anti-inflammatory pathway in the central nervous system (CNS). Thus, reduced expression of α7 nAChRs should result in enhanced neuroimmune activation to an LPS challenge in the 15q13.3 mouse model *(Df(h15q13)/+).* Adult heterozygous (Het) and wild-type (WT) female mice were injected with either 100 µg/kg LPS or saline. Quantitative PCR was used to measure the relative expression of the pro-inflammatory cytokines TNF-α, IL-1β, IL-6, RANTES and HMGB1 in the hippocampus 3 hours after the injection. LPS significantly increased mRNA expression of TNF-α, IL-6 and RANTES, but there was no significant treatment effect for IL-1β and HMGB1. Furthermore, there was no significant genotype effect for any of the cytokine measured in this study. In conclusion, LPS treatment caused significant activation of a neuro-inflammatory response but there were no significant differences between WT and Het mice indicating similar neuroinflammatory responses.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

MICE LACKING VIMENTIN EXHIBIT DECREASED ANGIOGENIC RESPONSES IN EARLY PREGNANCY

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Angiogenesis is the formation of new blood vessels from preexisting structures. Angiogenesis occurs during wound healing, tumor growth, and most severely, pregnancy. Sufficient angiogenesis is vital to a healthy pregnancy and results in better delivery of nutrients required for fetal growth, whereas insufficient angiogenesis can increase chances for preecclampsia and fetal growth restriction. Previous studies suggest the intermediate filament protein, vimentin, is essential for angiogenesis *in vitro*. Vimentin-null mice deliver an average of four viable pups per litter, while wildtype mice have six pups per litter. In this study we utilize mouse uterine tissue samples collected during timed pregnancy studies in vimentin-null and wildtype mice and compare angiogenic rates among these mice at day 5.5 of pregnancy. We believe that vimentin helps stabilize transmembrane receptors necessary for the initiation of new blood vessel growth. To measure angiogenic responses, we cut tissue samples using a cryostat, stained using hematoxylin and eosin to identify sections containing implantation chambers, performed immunofluorescent staining of tissue sections, created binary masks and quantified signal intensity using NIS Elements AR and ImageJ and imaging using an epifluorescent microscope. Using ImageJ, an acute area is identified to measure the amount of endothelial cells surrounding implantation chambers, where endothelial cell staining using PECAM-1-specific antibodies indicated angiogenic vessels. Another calculation is performed to normalize the endothelial growth to outer erosion area of implantation chamber. Results show that vimentin-null mice undergo significantly less angiogenesis during decidualization than wildtype mice. Future studies will investigate additional timepoints for a more thorough analysis.

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TAMHSC 2018 SUMMER RESEARCH PROGRAM

MIR363-3P TREATMENT HAS NO EFFECT ON COGNITIVE FUNCTION IN MIDDLE-AGED FEMALE RATS AT DAY 100, BUT CA1 HIPPOCAMPAL CELL ATROPHY MAY CONTRIBUTE TO POST STROKE VASCULAR DEMENTIA AND COGNITIVE IMPAIRMENT

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Stroke is the leading cause of serious disability in the United States with over ¼ of survivors developing either vascular cognitive impairment (VCI) or vascular dementia (VD). Thus, we hypothesize that administration of mir363-3p treatment, which we have shown as a neuroprotectant for stroke in middle-aged female rats, will improve the long-term cognitive outcomes post stroke in these rats. Sprague-Dawley female rats were subjected to stroke using stereotaxic injection of a vasoconstrictor, Endothelin-1, and randomly assigned to two treatment groups: scrambled oligos or mir363-3p mimic. At 100d, all animals were subject to the Novel Object Recognition Test (NORT), a test of cognition. Rats were then anesthestized and injected with a retrograde tracer, Fluorogold, into the left and right striatum. After 4 days, the rats were overdosed with anesthetic, perfused with saline and formaldehyde. The brain was cryosectioned and fluorogold-labeled cell in the CA1 hippocampus of the left and right hemisphere were analyzed for size and label density. Our data shows that there was no differences in the number of retrogradely labeled cells. Thereafter, cells were binned into large medium and small and then analyzed by 2 way ANOVA for cell size and treatment. Large bin cells were mostly found in the sham animals whereas medium and small sized cells in stroked (scrambled and Mir363) animals. This suggests stroke may cause progressive cell shrinkage in the CA1 hippocampus, which could attribute to VD and VCI and may in a longer time period of time could show behavioral changes in the rats.

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TAMHSC 2018 SUMMER RESEARCH PROGRAM

THE RELIABILITY OF CRE REPRESENTATION OF DOPAMINE D1 AND D2 NEURONS IN THE CORTEX OF D1-CRE AND D2-CRE MICE.

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Dopamine signals mainly through D1 receptors (D1Rs) and D2 receptors (D2Rs). Transgenic mice express Cre recombinase under the control of the D1R or D2R promoter, i.e., D1-Cre or D2-Cre mice, have enabled cell type-specific access to these D1R-expressing or D2R-expressing neurons. However, when these mouse lines crossed with a reporter line, e.g. Ai14, the fluorescent protein tdTomato may not reliably represent the D1 and D2 neurons outside the striatum. For instance, if the promoter is active when animals are young, Cre will be active and turn on the Cre-dependent reporter, tdTomato, expression. In the adult, the promoter may be turned off in cortex, and there may be no D1Rs or D2Rs expression in the neurons, but the reporter gene of tdTomato will express throughout the mouse's life. To address this question, we examined the reliability of tdTomato representation of dopamine D1 or D2 neurons are identical in D1-Cre;Ai14 and D2-Cre;Ai14 mice. To do so, AAV-Flex-GFP was injected bilaterally into the mPFC region of D1-Cre;Ai14 and D2-Cre;Ai14 transgenic mice. Then, fluorescent images of D1-Cre;Ai14 and D2-Cre;Ai14 were acquired using a confocal microscope and were analyzed using the IMARIS program to count the number of tdTomato-positive neurons (red neurons), GFP-positive neurons (green neuron), and the overlap (yellow neurons). We found that the percentages of yellow neurons over red neurons are indistinguishable in D1-Cre;Ai14 and D2-Cre;Ai14 mice. These data suggest that the tdTomato representation of dopamine D1R- and D2R-expressing neurons is identical in the cortex of the D1-Cre;Ai14 and D2-Cre;Ai14 mice.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

THE EFFECTS OF INCREASING RENAL LYMPHATIC VESSEL DENSITY ON ANGIOTENSIN II-DEPENDENT HYPERTENSION

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Hypertension affects 1 in 2 adults in the U.S. Hypertension is associated with renal immune cell infiltration and accumulation resulting in inflammation. The function of the lymphatic system is to help transport immune cells throughout the body, thus we hypothesized that increasing renal lymphatic vessel (RLV) density in angiotensin II-induced hypertension will reduce accumulated immune cells in the kidney and blood pressure. To test this hypothesis, we used mice that undergo kidney-specific over expression of VEGF-D (KidVD), which when given doxycycline overexpress VEGF-D resulting in renal lymphangiogenesis. An angiotensin II pump was subcutaneously implanted for 3 weeks. A week after the implantation, doxycycline was administered in the drinking water for 2 weeks and the mice were then euthanized. Systolic blood pressure (SBP) was measured every week following pump implantation using the tail-cuff method. In both male and female mice, the KidVD (+) mice showed a significantly lower SBP (SBP: 115 ± 2; 119 ± 4) relative to the KidVD (-) mice (SBP: 157 ± 20; 141 ± 9) respectively. RT-qPCR on kidneys was then performed and there was an increase in gene expression of RLV markers such as Lyve1, Prox1, and VEGF-D indicating an increase in RLV density. Further, gene expression of immune cell markers, chemokines, and kidney injury markers in KidVD (+) mice were not significantly different from those of controls. We therefore concluded that augmenting renal lymphatics may be a possible treatment for hypertension as increasing RLV density in mice with angiotensin-II dependent hypertension reduced SBP, renal immune cells, and inflammation.

Geina was supported by an American Heart Association Summer Undergraduate Research Fellowship.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

EFFECT OF HDAC INHIBITOR VORINOSTAT ON CHRONIC NEUROINFLAMMATION AFTER TRAUMATIC BRAIN INJURY IN MICE

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Chronic neuroinflammation is a maladaptive biomarker of many neurological diseases such as epilepsy and traumatic brain injury (TBI). TBI-induced post-traumatic epilepsy can develop from a host of underlying mechanisms, through a heterogeneous process called epileptogenesis. Current treatments are aimed at reducing symptomatic seizures rather than the underlying neuropathological cascades. Recent evidence suggests the use of epigenetic histone deacetylase (HDAC) inhibitors to prevent epileptogenesis (Reddy et al., 2017). In this study, we investigated the neuroprotective effect of vorinostat (SAHA) administration, a HDAC inhibitor, following TBI in mice. TBI was induced by a controlled cortical impact paradigm; SAHA (25 mg/kg) was administered twice daily for 21 days starting 1 hour post-injury. At 4 months post-injury, mice were perfused and coronal slices of the brain were stained for GFAP(+) and IBA1(+) immunohistochemistry. Area fractionation densitometry was utilized to analyze the extent of neuroinflammation in the dentate gyrus (DG), CA1, CA3, and amygdala. A subjective scoring method was used to quantify neuroinflammation in extra-hippocampal regions of the brain. Induction of TBI resulted in a chronic increase of both GFAP(+) astrogliosis and IBA1(+) microgliosis expression in regions of interest. In SAHA-treated TBI mice, there was significant attenuation of astrocytosis and microgliosis in a region-specific manner, specifically in GFAP(+) expression in the hippocampus CA3 and DG subfields, and IBA1(+) expression in the amygdala. These results indicate the protective potential of HDAC inhibition to reduce chronic neuroinflammation after TBI, demonstrating a therapeutic promise of neuroprotective therapy for brain injury, PTE and other related neurological diseases.

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TAMHSC 2018 SUMMER RESEARCH PROGRAM

BRAIN-WIDE MAPPING OF INPUTS TO DIRECT- AND INDIRECT-PATHWAY NEURONS IN THE POSTERIOR DORSOMEDIAL STRIATUM

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The dorsomedial striatum (DMS) is heavily involved in drug and alcohol use disorders due to its strong role in goal-directed learning. The posterior DMS (pDMS), which is primarily involved in action-outcome associations and causative expressions in goal-directed learning, receives numerous extra-striatal inputs and is comprised of dopamine D1 receptor-expressing (D1Rs) medium spiny neurons (D1-MSNs) and D2 receptor-expressing MSNs (D2-MSNs). Following excessive alcohol consumption, these neurons demonstrate aberrant synaptic plasticity. However, the afferent inputs onto pDMS D1- vs. D2-MSNs remain unknown. Using a pseudotyped rabies virus-mediated monosynaptic retrograde tracing method, we analyzed presynaptic neurons projecting onto either D1- or D2-MSNs in the pDMS by labeling presynaptic neurons throughout the entire mouse brain. We measured the extent to which presynaptic neurons projecting onto pDMS D1-MSNs (or D2-MSNs) also expressed D1Rs (or D2Rs). Our mapping results identified several distinct projections from the cortical regions, thalamus, amygdala, and midbrain. Surprisingly, we observed that most extrastriatal D1-MSN-projecting neurons did not contain D1Rs and D2-MSN-projecting neurons did not contain D2Rs. Additionally, we observed limited expression of D1-MSN-projecting neurons that contain D1Rs in cortical and thalamic regions, as well as limited D2-MSN-projecting neurons that express D2Rs in the cortical, thalamic, and midbrain regions. These results suggest that linked corticostriatal and thalamostriatal neurons do not express the same type of dopamine receptors. We believe this study, along with future characterization of these pathways, will advance knowledge of the pDMS in drug and alcohol addiction.

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TAMHSC 2018 SUMMER RESEARCH PROGRAM

DORSOMEDIAL STRIATUM-PROJECTING NEURONS PREFERENTIALLY EXPRESS DOPAMINE D2, BUT NOT D1, RECEPTORS

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Dopamine signals regulate multiple aspects of brain function, mainly through D1 receptors (D1Rs) and D2Rs. D1Rs and D2Rs are highly expressed in the dorsomedial striatum (DMS), as well as the other DMS-projecting brain areas. Both D1Rs and D2Rs were found on presynaptic terminals in the DMS, but with a less extent of D1Rs. We hypothesized that D2R-expressing inputs would be stronger than D1R-expressing inputs. To address this, we generated two lines of double transgenic mice (D1-Cre;Snap25 and D2-Cre;Snap25) where D1R-expressing neurons and D2R-expressing neurons were labeled by the green fluorescent protein, respectively. AAV-Retro-tdTomato was infused into the DMS, which labeled DMS-projecting neurons by red fluorescent protein, tdTomato. Fluorescent images were acquired using a confocal microscope. We found that D2R-expressing neurons projecting to the DMS outnumbered the D1R-expressing neurons projecting to the DMS. This result suggests that more D2R-expressing neurons project to the DMS than D1R-expressing neurons. We also discovered that the relative percentage of D2R-expressing inputs to the DMS is higher in the anterior sessions than posterior sessions, while the relative percentage of D1R-expressing inputs to the DMS is higher in the posterior sessions than the anterior sessions. These data suggest that D2R-expressing inputs are stronger than D1R-expressing inputs in the DMS, and that presynaptic D2Rs, but not D1Rs, may play a strong regulatory role in the DMS.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

THE EFFECTS OF AN NR4A1 ANTAGONIST ON CANINE CANCER CELLS

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Nuclear receptor 4A1 (NR4A1) is a protein that has been implicated in the growth, invasion, and metastasis of many types of cancer. As NR4A1 presents a target for chemotherapy, diindolylmethane analogs (C-DIMs) have been developed as antagonistic ligands for NR4A1. The effects and mechanisms of C-DIMs in human cancers have been previously studied by this lab, so for this study our focus was to determine whether canine cells responded similarly. We investigated the effects of C-DIM 8 on NR4A1 and related proteins expression in osteosarcoma, mammary carcinoma and melanoma canine cells. After performing a growth inhibition assay to determine the IC50 for each cell line (10, 11, and 12μM for melanoma, osteosarcoma, and mammary carcinoma respectively), cells were treated for 24 hours with either DMSO or 15μM C-DIM 8 and then Western blotting was used to determine the level of protein expression. We found that NR4A1 was expressed in all three cell lines, although we did not observe any NR4A1 degradation after 24 hours. We also observed that for the oncogenic proteins EGFR and survivin, which are regulated in part by NR4A1, protein expression was downregulated in C-DIM 8 treated cells. Also, an Annexin V apoptosis assay for the osteosarcoma and mammary carcinoma cells confirmed that treatment with C-DIM 8 resulted in a higher rate of apoptosis compared to control samples. These results are similar to those found after testing C-DIMs on human cancers and show that canines are a feasible model for testing C-DIM analogs in a clinical trial.

Texas A&M Health Science Center Summer Research Program Fellowship

TAMHSC 2018 SUMMER RESEARCH PROGRAM

EFFECT OF HDAC INHIBITOR VORINOSTAT ON CHRONIC NEURODEGENERATION AFTER TRAUMATIC BRAIN INJURY IN MICE

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Post-traumatic epilepsy concerns the development of spontaneous recurrent seizures following traumatic brain injury (TBI). This occurs via epileptogenesis, the process by which a normal brain becomes epileptic due to a number of processes including inflammation, hyperexcitability, and epigenetic modifications. Epigenetics refers to changes in phenotype controlled by alterations in gene expression. Several therapeutic interventions have been proposed to interfere with the maladaptive epigenetic modifications associated with TBI. Opposing HAT and HDAC enzymes control the addition and removal of acetyl groups from histone proteins. This study examines the neuroprotective effects of vorinostat (SAHA) administration, a potent HDAC inhibitor, following TBI in mice. TBI was induced using a controlled cortical impact model and SAHA (25 mg/kg) was administered twice daily for 21 days, starting 1 hour after injury. At 4 months post-TBI, mice were perfused and brains were processed for parvalbumin (PV+) immunohistochemistry and Nissl histology. An unbiased stereological quantification method was used to determine the extent of GABAergic PV(+) interneuron loss in the CA1, CA2, CA3, DG, and DH subregions of the hippocampus. A neuropathology scoring method was used to quantify neurodegeneration in extra-hippocampal regions. TBI was associated with robust PV+ interneuron loss (49%) in the contralateral hippocampal subfields accompanied by a massive lesion in the ipsilateral hemisphere. SAHA treatment significantly reduced hippocampal interneuron loss (10%) and lesion volume compared to the control group. These results indicate the neuroprotective potential of HDAC inhibition after TBI, especially to reduce the extent of chronic degeneration of GABAergic interneurons in the hippocampus.

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TAMHSC 2018 SUMMER RESEARCH PROGRAM

SIGNIFICANCE OF TYPE VI AND XII COLLAGEN TO OSTEOCHONDRAL PROCESSES AND BONE REPAIR

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Medical care associated with the treatment of bone disorders is a multi-billion dollar industry. Nevertheless, current technologies employed for bone repair are frequently inadequate. Human mesenchymal stem cells (hMSCs) present an attractive alternative to current technologies, but operate via mechanisms which are not fully understood. It is known that Type VI and XII collagen are essential mediators of cell-cell communication pathways between osteoblasts during osteogenesis. To further examine the role of collagen VI and XII in osteochondral processes, we transcriptionally blocked their expression in hMSCs. Previously, our lab found that this inhibited direct-osteogenesis. However, osteogenic repair often involves a chondrogenic intermediate. We therefore examined chondrogenesis by hMSCs using *in vitro* assays of hMSC-derived chondrocytes that generate cartilage. Histological analysis of cartilage micromasses revealed little morphological variation in collagen VI and XII knockdown populations (KD VI, KD XII) as compared to controls. This suggests that collagen VI and XII are vital to direct-osteogenesis rather than formation of chondrogenic intermediates during bone repair. In parallel to this, KD VI, KD XII and control hMSC-derived osteoblasts were immunostained for type VI and XII collagen to allow visualization of cell-cell contacts. Microscopy revealed matrix-bridge attachments composed of both proteins – suggesting their contribution to osteoblast communication. However, quantification and comparison of matrix bridges between osteoblast populations could not be performed as planned because hMSC populations did not grow at equivalent densities. Further work will quantitatively assess the roles of collagen VI and XII in hMSCs to allow for optimization of hMSC-based treatment for bone injuries.

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TAMHSC 2018 SUMMER RESEARCH PROGRAM

THE EFFECTS OF ANGIOTENSIN II ON LYMPHATIC ENDOTHELIAL CELL BIOLOGY

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Approximately 103 million Americans and nearly half of all African American adults are affected by chronic high blood pressure, or hypertension, having a blood pressure greater than 130 over 80 mmHg. Not only are those who have hypertension at a higher risk for heart disease and stroke, but for kidney failure and atrophy as well. There is a well-known relationship between high levels of angiotensin II (AngII) and hypertension, as renal interstitial levels of AngII are doubled in hypertension, suggesting a potential direct interaction with lymphatic endothelial cells (LECs). We hypothesized that AngII will have a direct effect on LEC activation, adhesion, and proliferation. After growing a sufficient stock of LECs, we treated them with 0nm, 5nm, 10nm, and 100nm of AngII for 24 hours. We lysed the cells, isolated the RNA and created cDNA for qPCR in order to compare gene expression. Our findings suggest that Ang II increased genes that are involved in the activation, adhesion, and proliferation of LECs, such as MCP1, ICAM, and VEGFR-3, while VCAM and iNOS tended to be increased. These results suggest that interstitial levels of AngII directly affect LEC biology.

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TAMHSC 2018 SUMMER RESEARCH PROGRAM

A PHOTOSWITCHABLE CRISPR/DCAS9 SYSTEM FOR TRANSCRIPTIONAL REPROGRAMMING

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CRISPR/dCas9 is a novel genome editing system that provides a therapeutic platform for countless health issues. After entering the cell, dCas9 must translocate from the cytosol to the nucleus in order to alter the expression and subsequent function of various genes. However, without tight regulation over the system's movement, dCas9 can have adverse off-target effects. This project highlights the development of CaRROT, a tool that controls the translocation of dCas9 to the nucleus with blue light illumination. In our study, we tested five different CaRROT constructs, designated as V1-V5. V1-V2 were strictly light dependent, and the constructs were transfected into HeLa cells. V3-V5 used calcium dependent versions of CaRROT and regulated calcium levels by pairing CaRROT with a light dependent tool known as Opto-CRAC. Therefore, for V3-V5, CaRROT and Opto-CRAC constructs were co-transfected into HeLa cells. Confocal imaging was then used to record dCas9 translocation over a thirty minute time period after blue light activation. The V5 construct, with calcium-responsive NFAT (nuclear factor of activated T-cells) fragments and without light-responsive NLS (nuclear localization signals), allowed dCas9 to translocate to the nucleus upon blue light illumination and is CaRROT's most promising foundational design. With further research, CaRROT will be able to activate gene expression for applications such as regenerative medicine and disease treatment. Reversibly, this tool can halt the expression of harmful genes such as oncogenes. CaRROT paired with Opto-CRAC can also be used with a reporter gene like GFP (green fluorescent protein) in order to record gene regulation activities.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

EFFECT OF HDAC INHIBITOR VORINOSTAT ON CHRONIC EPILEPTIC SEIZURES AFTER TRAUMATIC BRAIN INJURY IN MICE

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Epigenetics refers to a set of functional changes to the genome that can impact gene expression without altering the underlying DNA sequence. These plastic changes, such as DNA methylation and histone modification, have been implicated as critical drivers in many disease states. Traumatic brain injury (TBI) is a major cause of mortality and neurological diseases, such as the development of post-traumatic epilepsy (PTE). As PTE patients often do not respond to currently available antiepileptic drugs, there is a need for the development of treatments that reduce epileptogenesis after TBI. This study expands on our recent report that suggests epigenetic HDAC inhibition reduces epilepsy development (Reddy et al., 2017). Here we tested the effect of vorinostat (SAHA), a broad-spectrum HDAC inhibitor, to reduce epilepsy development after TBI in mice. TBI was induced in adult mice by a controlled cortical impact paradigm. Animals were treated with SAHA (25 mg/kg) twice daily for 21 days post-TBI. Cohorts were recorded by video-EEG system for 120 days. The EEG data was analyzed with a MATLAB algorithm, then each detected seizure was manually verified. Epileptic seizures developed progressively in the untreated TBI group, with a peak increase in seizure frequency 2–3 months post-TBI. Progression and overall incidence of spontaneous seizures were significantly reduced in the SAHA cohort, with only 57% animals developing epilepsy, compared to 87% in the untreated (control) cohort. These results suggest that HDAC inhibition reduces the risk of developing epilepsy following TBI, confirming the antiepileptogenic potential of HDAC inhibitors for attenuating epilepsy.

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TAMHSC 2018 SUMMER RESEARCH PROGRAM

ADMINISTRATION OF SEROTONIN RECEPTOR 2B ANTAGONIST DECREASES DUCTULAR REACTION AND LIVER FIBROSIS IN THE MDR2$^{-/-}$ MOUSE MODEL OF PRIMARY SCLEROSING CHOLANGITIS (PSC)

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Serotonin (5HT) exerts its effects through 16 receptor isoforms. Previous studies have shown that: (i) activation of 5HTR 2B increases ductular reaction and liver fibrosis in normal and bile duct ligated (BDL) rats. To define the role of 5HT2B on changes in biliary mass and fibrosis in BDL rat model and Mdr2$^{-/-}$mice. We evaluated the expression of 5HTR 2B, by (i) immunofluorescence in liver sections (costained with CK-19, biliary marker, or desmin, marker of hepatic stellate cells, HSCs), isolated normal isolated rat cholangiocytes and, murine cholangiocytes (IMCLs) and Human Hepatic Stellate cells (HHSTeCs) and by (ii) immunohistochemistry on liver sections. Liver damage was assessed by: (iii) intrahepatic ductal mass (IBDM) by immunohistochemistry for CK-19 and liver fibrosis by Sirius red staining in liver sections; and (iv) the expression of fibrosis (α-SMA, Col1a1, Fn1, TIMP1 and TIMP2) markers in isolated cholangiocytes by *q*PCR. **Results**: Antagonizing serotonin receptor 2B there is a decrease in liver damage as assessed by reduced biliary proferation, liver fibrosis, and hsc activation. : (i) cholangiocytes, HSCs, NRC, ICMLs and HHSTeCs express 5HTR 2B; *In vivo*, administration of 5HTR 2B antagonist to BDL rats and Mdr2$^{-/-}$ mice decreases IBDM, and fibrosis compared to vehicle-treated BDL rat and Mdr2$^{-/-}$ mice. Antagonist of 5HTR 2B decreases ductular reaction and liver fibrosis in BDL rats and Mdr2$^{-/-}$ mice. Inhibition of 5HTR 2B may be an important target for ameliorating cholangiopathies including PSC.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

GALANIN`S ROLE IN THE STIMULATION OF CHOLANGIOCYTES AND HEPATIC STELLATE CELLS IN MDR2KO MICE LEADING TO HEPATIC CHOLESTASIS

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Hepatic fibrosis is the accumulation of extracellular matrix proteins which occurs in most chronic liver diseases. Fibrosis often leads to cirrhosis, which can subsequently develop into liver cancer. When stimulated, cholangiocytes in the liver can activate hepatic stellate cells (HSC) which causes them to undergo a phenotypic change and lay down collagen and other fibrotic proteins. Few studies have investigated the effects that galanin, a 29-amino acid neuropeptide recently detected in the liver, could have on the stimulation of cholangiocytes and HSC's. In the present study we used Mdr2-knockout (Mdr2KO) mice, a transgenic mouse model to mimic human cholestasis. A controls, we used FVBN mice. Our aim was to investigate the expression of galanin and its receptors in the liver and the effects of galanin treatment on liver fibrosis, cholangiocytes, and HSC's. The experimental procedures included animal treatment with galanin, collection of liver tissue, assessment of liver histology by immunohistochemistry, immunofluorescence, confocal microscopy, Sirius red staining, laser capture microdissection and qPCR. The results showed that galanin can be found in cholangiocytes and hepatic stellate cells. Galanin receptors GalR1 and GalR2 were expressed differentially, with both GalR1 and GalR2 in cholangiocytes and only GalR2 in HSC's. Galanin treatment of FVBN and Mdr2KO mice caused a significant increase in cholangiocyte proliferation, HSC proliferation and activation, and fibrotic markers. Galanin's effects were greater in FVBN mice than in Mdr2KO, suggesting that galanin may have a more prominent role in the initiation phase of liver fibrosis than in its progression.

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TAMHSC 2018 SUMMER RESEARCH PROGRAM

EXPLORING INTERACTIONS BETWEEN BACTERIAL EFFECTOR PROTEINS AND THE HOST RETROMER COMPLEX IN *SALMONELLA* PATHOGENESIS

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Salmonella enterica is a major human gastrointestinal pathogen. *S. enterica* utilizes two T3SS secretion systems to invade the host cell and enable intracellular survival in a vacuole. Effector proteins are translocated through T3SSs to manipulate host cell processes. Previous genetic profiling of *S. enterica* effectors and host proteins uncovered a previously-unknown interaction between sseC, a component of the SPI-2 T3SS translocon, and the retromer, a human protein complex involved in endosomal protein sorting, retrograde trafficking and autolysis. This research documents preliminary exploration of effector-retromer interactions, particularly involving sseC and the retromer subunit VPS35. Scramble-knockdown (SCR) and VPS35-knockdown (VPS35KD) HeLa cells were infected with wild-type *Salmonella* Typhimurium and were then fixed at 30-minute, 1-hour, 2-hour and 4-hour timepoints. Cells were then stained to observe colocalization of *Salmonella* and the autolysis marker LC3. Further, bacterial RNA expression in infected SCR and VPS35KD cells at these timepoints was measured with qPCR. Additionally, a reporter plasmid containing the sseC promoter and GFP was designed through Gibson cloning and transformed into wild-type *Salmonella*. SCR and VPS35KD cells were then infected and GFP expression was measured at 15-minute intervals. Colocalization data indicates increased targeting of *Salmonella* to the autolysis pathway in VPS35KD cells. Interactions between many different *Salmonella* effectors and the retromer are suggested by qPCR data. GFP-reporter data demonstrates consistent sseC underexpression in VPS35KD cells. This work increases understanding of a newly-described interaction between the *Salmonella* effector sseC and the human retromer complex, providing further detail on mechanisms of *Salmonella* survival in the host cell.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

QUANTIFYING LYMPHANGIOGENESIS IN ADIPO-VD MOUSE ADIPOSE TISSUE

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Inflammatory adipose tissue expansion is the root of the metabolic syndrome in obesity. Insulin-resistant adipocytes and dysregulated lipolysis results in adipose tissue inflammation, increased circulating lipids, ectopic lipid deposition, and systemic insulin resistance. Lymphangiogenesis - the expansion of the lymphatic vessel network - is a necessary process in clearing fluid and immune cells during inflammation. We hypothesized that enhanced adipose lymphangiogenesis may reduce local inflammation and improve metabolism in obesity. We generated transgenic mice with inducible, expression of murine VEGF-D under a tightly-controlled Tet-On promoter to stimulate tissue-specific lymphangiogenesis. Crossed with adipocyte-specific adiponectin-rtTA mice (Adipo-VD), VEGF-D overexpression by adipocytes induced *de novo* lymphangiogenesis in murine white adipose tissues and massive expansion of brown adipose tissue lymphatics. Our previous studies suggest that an increase in lymphatic density promotes “healthy” adipose tissue in obesity. We intend to understand the mechanism of lymphatic function in adipose tissue inflammation by utilizing a four month timecourse study in Adipo-VD mice and characterize the adipose tissue physiology.

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TAMHSC 2018 SUMMER RESEARCH PROGRAM

EVOLUTION OF *E. COLI* CAUSES AN INCREASE IN SURVIVABILITY IN MURINE MACROPHAGE CELLS

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Endosymbiosis is a symbiotic relationship in which one organism lives inside of the other organism. A key feature of endosymbiosis involves the invasion of host cell immune surveillance. We hypothesized that evolved *E. coli* bacteria, in both generational and continuous evolution methods, would demonstrate higher microbial survival rates in murine macrophage cells in comparison to wild-type strains. Generational evolution occurs over multiple generations whereas continuous evolution occurs over several days. To test intracellular survivability, macrophage cells were infected with strains of evolved *E. coli* at a MOI of 1:100 and were incubated for one hour before the introduction of gentamicin media to kill extracellular bacteria. The macrophages were then lysed and plated on LB agar plates. The plates were incubated overnight to allow for bacterial growth and bacterial colonies were counted at multiple dilutions. Results for generationally evolved *E. coli* showed a significant increase in survivability rate between G25/G20 and G0 at 48 and 72 hours post-infection, while continuously evolved samples only showed a significant increase at 24 hours. We concluded that generational evolution of *E. coli* increases the survivability rate of *E. coli* in murine macrophage cells, while continuous evolution of *E. coli* only increases survivability rate at 24 hours. Future work will focus on further generational evolution, as well as analyzing genome sequencing data for evolved *E. coli* samples to identify reoccurring pathogenic mutations. Reoccurring mutations suggest these mutations aid in aversion of host cell immune surveillance and could lead to a better understanding of pathogenesis.

Sadmaan was supported by the Texas A&M Health Science Center Summer Research Program Fellowship.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

A BIOLUMINECENT REPORTER TO STUDY BACTERIAL GROWTH

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Bioluminescence allows a rapid, real-time evaluation of infection in a living animal. While the traditional method of enumerating bacteria using colony forming units (CFU) is the standard, there are pathogens such as *Mycobacterium tuberculosis* that take weeks to grow. To validate the use of bioluminescence as a tool to follow infections, a lab strain of *Escherichia coli* was used to construct a bioluminescent reporter strain. *E. coli* XL1blue electro-competent cells were transformed with the plasmid pJDC181 containing the click-beetle red luciferase (CBRlux) gene by electroporation. The impact of plasmid insertion into the parent strain was determined by comparing the growth curves of the parent strain and bioluminescent clone. No significant difference was observed in the growth rate of the bioluminescent clone compared to the parent strain. A bioluminescence assay was performed at different growth phases of the bioluminescent clone, and a consistent increase in the signal from lag to log phase was observed. This confirms that luminescence is a direct indicator of metabolic activity of bacterial cells expressing it. The strategy described here is with a non-infectious lab strain, but this technique can be applied to any infectious bacteria which can then be used for vaccine and drug efficacy studies.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

CREATION AND ANALYSIS OF A NON-HODGKIN LYMPHOMA DATABASE

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Non-Hodgkin lymphoma (NHL) ranks among the top ten estimated new cases and deaths for cancers in the United States in 2018. 50% of these diagnosed lymphomas are categorized in the distant stage; there is a need to diagnose lymphoma earlier. The objective is to determine genetic mutations associated with NHL that could serve as biomarkers for detection of the cancer and to determine how these genetic mutations impact metastasis and survival. The framework for a lymphoma specific database was created through the Informatics for Integrating Biology and the Bedside (i2b2) Research Data Warehouse. i2b2 is tool used in translational research where de-identified patient data can be found and uploaded. Patient data including sex, age, survival status, and mutation information were compiled from projects such as the Cancer Genome Atlas. The five most common mutated genes included PIM1, CD79B, MPEG1, IRF4, and MYD88 and are related to serine/threonine-protein kinase, B-cell antigen, macrophage expression, interferon regulatory factor, and immune system cell signaling, respectively. This concludes that mutations in genes involving the immune system can lead to lymphoma. The next step in the project is to upload the rest of the already transformed data into i2b2 and determine their affects on survival. In the long term, the results found could be utilized to assess an individual's risk of developing NHL over their course of their lifetime and aid in early detection, ideally before the cancer metastasizes.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

IDENTIFICATION OF NOVEL SURFACE AND SECRETED PROTEINS IN STAPHYLOCOCCUS EPIDERMIDIS

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Staphylococcus epidermidis is a gram-positive opportunistic pathogen that colonizes human skin. It is one of the leading causes of nosocomial infections in the U.S. However, its pathogenic mechanisms are not fully understood. Pan-genome analysis revealed that *S. epidermidis* has 20% variable genes. These variable genes are known to transcribe novel surface and secreted proteins, which can contribute to virulence through biofilm formation, evasion of host immune responses, invasion of host cells and adhesion to host cells and tissues. Finding novel virulence factors would enable us to better understand *S. epidermidis* pathogenic mechanisms. The goal of my project is to identify novel surface and secreted virulence factors in *S. epidermidis*. To this end, I developed a bioinformatics workflow to identify and characterize novel proteins using *S. epidermidis* MB2193 (ST-2) clinical isolate obtained from blood of a cancer patient at MD Anderson, Houston, TX. Proteins were characterized as extracellular proteins, lipoproteins, cell wall anchored/associated proteins, or peptides using web programs PsortB, TMHMM, PRED-Lipo and MatureP. The presence of these proteins in other species was determined using BLAST. The proteins were further distributed into 4 groups: Group A which includes proteins present in multiple genus, Group B which contains proteins present primarily in Staphylococci, Group C which contains proteins present primarily in CoNS, and Group D represents proteins present in primarily *S. epidermidis*. Through this workflow, I identified two novel putative cell wall associated proteins. Further studies are warranted to confirm the presence of these cell wall associated proteins on the surface.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

OPIOID-IMMUNE INTERACTION AFTER SCI

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Opioids are widely used for the management of acute pain after spinal cord injury (SCI). Unfortunately, we have found that morphine administration after SCI has significant adverse effects on recovery of function. In a rat model, even a single administration of morphine on the day after SCI increases long-term pain and undermines locomotor recovery. We have shown, using agonists and antagonists for specific opioid receptors, that kappa-opioid receptors (KORs) mediate the adverse effects of morphine. Additionally, these adverse effects can be blocked by minocycline (glial inhibitor). Using flow cytometry, we found that KOR expression on microglia is increased by SCI and morphine. The current study aimed to determine the downstream pathways in microglia that may be engaged by morphine and KOR activation. There are multiple ways that KOR activation may decrease recovery and increase cell death. First, KOR-mediated activation in microglia may lead to β-arrestin recruitment and the production of excitotoxic levels of pro-inflammatory cytokines. Alternatively, morphine may activate the $G\alpha_i$ signaling pathway and increase dynorphin (KOR agonist) expression to neurotoxic levels. To test this, after 3 days of i.v. morphine administration, we collected injured spinal tissue and isolated CD11b+ (microglia/ macrophages) cells using anti-CD11b magnetic beads. Preliminary western blot analyses on the isolated immune cells support the observations made using flow cytometry. We see a trend toward increased expression of OPRK1 and dynorphin in the microglia of morphine-treated SCI subjects. Further investigation is required to confirm morphine's role in biasing signaling toward Gαi activation or β-arrestin recruitment.

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TAMHSC 2018 SUMMER RESEARCH PROGRAM

THE MICROBIOTA-DERIVED METABOLITE INDOLE IS AN ACTIVATOR OF AMPK AND REGULATES IMMUNE CELL METABOLISM

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The human body is colonized by trillions of highly-diverse microorganisms, collectively known as the human microbiota. Perturbations in the intestinal microbiota are associated to pathology in several diseases, such as auto-inflammatory disorders and inflammatory bowel disease. Recent studies suggest that microbiota-derived metabolites are involved in the proper development of immune cells, and homeostasis of the immune system. However, how metabolites impact the metabolism of immune cells remains poorly understood. Novel research has provided insight into the role of adenosine monophosphate-activated protein kinase (AMPK) in inflammation and immunity. Here we determine the impact of indole, a microbiota-derived tryptophan metabolite, in the metabolism of dendritic cells. We hypothesize that indole activates AMP-activated protein kinase in DC2.4 cells. DC2.4 cells were exposed to indole concentrations of 0, 0.1, 0.5, and 1.0 mM for 8, 16, and 24 hrs. Whole protein lysates were generated, and western blotting was performed using antibodies directed against phosphorylated-AMPK and AMPK. Protein levels were analyzed by densitometric analysis using Image Lab 6.0.1. Indole-conditioned dendritic cells exhibited higher levels of p-AMPK in a dose-and time-dependent manner. Indole also increased AMPK protein expression in dendritic cells. These results suggest that indole activates AMP-activated protein kinase in DC2.4 cells. If activated, AMPK inhibits anabolic processes that consume ATP, and promotes catabolic processes that regenerate ATP. By altering immune cell metabolism, indole has the potential to be utilized as an anti-inflammatory pharmacological agent.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

A DOMINANT MODIFIER GENETIC SCREEN FOR FACTORS THAT INTERACT WITH CDK8-CYCLIN C

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Dysregulations of Cyclin-dependent kinase 8 (CDK8) and its regulatory partner Cyclin C (CycC) have been linked to a variety of human cancers. Because gain of CDK8 activity can drive tumorigenesis in melanoma and colorectal cancer patients, there are considerable interests in developing CDK8 inhibitors that will translate into targeted therapies for oncology. The objective of this experiment was to identify the presence of genes that could impact the regulatory network of the CDK8-CycC complex in both normal development and tumorigenesis using the *Drosophila melanogaster* model. A genetic screen was performed to identify dominant modifiers of the wing veined phenotypes caused by over-expression and depletion of CDK8. Twenty-six genomic loci were found to interact with the CDK8-CycC complex by enhancing or suppressing wing vein patterns. The aforementioned genomic regions were analyzed through crosses with *nub-Gal4* recombined with *UAS-cdk8*$^{+}$, *UAS-cdk8-RNAi*, *CycC-RNAi*, and *cdk8-CycC RNAi* lines. Overlapping deficiency lines were used to identify functional interactions between the CDK8-CycC complex and other genes. After the process of deficiency mapping, the wings of the flies containing suppressors and enhancers were dissected and imaged through a 5x objective lens. Deficiency mapping revealed that overlapping stocks 9544 and 9696 uncovered the narrowest genomic region that contained nine possible genes which could affect CDK8-CycC interactions. Further mapping could reveal a specific gene that could account for modification of the CDK8-specific phenotype by these two deficiency lines. Studying the signaling pathway of the gene could determine how the CDK8-CycC complex directly affects transcriptional regulation.

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TAMHSC 2018 SUMMER RESEARCH PROGRAM

BEHAVIORAL CHARACTERIZATION OF A MOUSE MODEL FOR THE 15Q13.3 MICRODELETION SYNDROME

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The 15q13.3 microdeletion is characterized by a heterozygous deletion of six genes on human chromosome 15, and is associated with mental and cognitive deficits as well as increased susceptibility to schizophrenia, epilepsy, and autism. The mouse model (*Df(h15q13)/*+) has a homologous microdeletion and similar behavioral phenotype, including learning deficits. We predicted that the heterozygous (Het) mice would have spatial learning deficits compared to wild-type (WT) mice. Spatial learning was assessed in the Barnes Maze test in adult male WT and Het mice. During initial learning, Het mice showed significantly increased distance travelled and latency to the target hole compared to WT, indicative of impaired spatial learning. There was no difference in average speed, nor significant differences during reversal learning. One of the genes affected by the MD is *CHRNA7*, which codes for α7 subunit of nicotinic receptors. *CHRNA7* is implicated in the anti-inflammatory cholinergic pathway. Lipopolysaccharide (LPS) causes neuro-inflammation and sickness behavior, and might augment behavioral differences between WT and Het mice. Additional behavioral experiments measured burrowing and nest-building behaviors following an LPS (100 µg/kg) injection. LPS treatment significantly affected the average amount burrowed in WT and Het mice, and LPS-injected Het mice burrowed significantly less than LPS-WT mice. However, LPS did not affect nesting scores in either WT or Het mice. Results indicate that the heterozygous 15q13.3 MD impairs spatial learning and that the Het mice exhibit increased susceptibility to inflammation-induced behavioral deficits, which could significantly affect the manifestation of neurological disorders that are often associated with neuroinflammation.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

THE EFFECTS OF TGR5 AGONISTS: BETULINIC ACID, TCG-1005, AND GPBAR-A ON NEUROINFLAMMATION

Alison Thomas, Gabriel Framptom, Matthew McMillin, Stephanie Grant, Elaina Williams, Anca Petrescu, Sharon DeMorrow

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Hepatic encephalopathy (HE) describes the array of neurological complications, that arise due to severe liver impairment. A key feature of HE is the activation of microglia and resulting neuroinflammatory cascade which is regulated by the release of the proinflammatory chemokine ligand 2 (CCL2) from neurons. Research from our laboratory suggests that aberrant bile acid signaling plays a role in the pathogenesis of HE. The activation of the bile acid receptor Takeda G protein-coupled receptor (TGR5), is neuroprotective during HE unlike other bile acid receptors, but its activation on microglia activation via neuron signaling has not been demonstrated. This study investigated the effects TGR5 agonists: betulinic acid, TC-G 1005, and GPBAR-A have on neuroinflammation and whether TGR5 can inhibit microglia activation directly or indirectly through the release of CCL2 via neurons. Betulinic acid was the most effective agonist in reducing CCL2 expression in HT-22 cells and primary neurons. When considering microglia signaling, both TC-G 1005 and GPBAR-A were effective in decreasing phagocytotic behavior in EOC-20 cells and primary microglia. EOC-20 cells and primary microglia treated with TGR5 agonists reduced the expression of proinflammatory cytokines, and both IL-1ß and TNFα expression decreased in treated EOC-20 cells and primary microglia. With these findings, the activation of TGR5 with agonists: betulinic acid, TC-G 1005, and GPBAR-A may alter microglia activation either directly, or indirectly by influencing neuron signaling so that microglia are quiescent in HE.

This work was supported by a NIH R01 award and a VA Merit award.

TAMHSC 2018 SUMMER RESEARCH PROGRAM

INHIBITION OF MAST CELL-DERIVED TGF-β1 DECRESES BILIARY DAMAGE AND HEPATIC FIBROSIS IN A MURINE MODEL OF PRIMARY SCLEROSING CHOLANGITIS

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Primary Sclerosing Cholangitis (PSC) is characterized by biliary damage, inflammation and hepatic fibrosis. Mast Cells (MCs) infiltrate the liver during PSC and promote damage. Multidrug resistance 2 knockout ($Mdr2^{-/-}$) mice mimic human PSC. L-histidine decarboxylase (converts histidine to histamine) knockout mice ($HDC^{-/-}$) contain fewer/altered MCs. $Mdr2^{-/-}HDC^{-/-}$ double knockout (DKO) mice have decreased MC number/activation, biliary damage, inflammation and liver fibrosis; reintroduction of MCs restores these parameters. Transforming growth factor (TGF)-β1 promotes hepatic fibrosis; MCs secrete TGF-β1. We evaluated the role of MC-derived TGF-β1 on liver and biliary damage in DKO mice. DKO mice received tail vein injections of cultured MCs treated with PBS or LY2157299 (TGF-β receptor inhibitor (MC-TGFβRi)) 3 days prior to sacrifice. We evaluated: (i) liver damage, (ii) biliary proliferation, (iii) inflammation, (iv) liver fibrosis/hepatic stellate cell (HSC, key contributor of fibrosis) activation, and (v) TGF-β1 secretion. *In vitro*, MCs were treated with 0.1% BSA or LY2157299 (10 μM); TGF-β1 secretion was measured. Supernatants from treated MCs were placed on cultured cholangiocytes or HSCs prior to measuring proliferation and fibrogenesis. DKO mice have reduced damage and TGF-β1 secretion compared to $Mdr2^{-/-}$ mice. Damage and TGF-β1 secretion are restored following MC reintroduction; however, these changes were mitigated in DKO mice injected with MC-TGF-βRi. *In vitro,* MC-TGF-βRi had decreased TGF-β1 secretion. Cholangiocytes treated with MC-TGF-βRi supernatants had decreased proliferation. HSCs treated with MC-TGF-βRi supernatants had reduced fibrogenesis. We identified a novel pathway wherein MC-specific TGF-β1 influences PSC-induced damage. Targeting MC-derived TGF-β1 may be a therapeutic target for PSC patients.

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TAMHSC 2018 SUMMER RESEARCH PROGRAM

DESIGN AND EXPRESSION OF GFP-FUSION CONSTRUCTS OF *MYCOBACTERIUM TUBERCULOSIS* PROTEIN KINASE D

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Extrapulmonary infection occurs in 1 in 5 tuberculosis cases, but the precise mechanisms of dissemination from the lungs have yet to be fully characterized. Protein Kinase D (PknD), which contains intracellular (N-terminal) kinase, transmembrane, and extracellular (C-terminal) sensory domains, has been linked to dissemination. The hypothesis of this project is that tagging each domain with GFP will allow for their visualization by microscopy. Three separate vectors containing genes for the PknD-GFP fusion constructs and hygromycin resistance were made by overlap-extension PCR, restriction digest, and ligation. The three fusion constructs are currently at different stages of development. Overlap-extension PCR successfully amplified the transmembrane construct. The N- and C-terminal vectors were also amplified, then transformed into *Escherichia coli* for replication and screened by plating on hygromycin plates and PCR. Transformation of both constructs into *E. coli* was confirmed. The N-terminal plasmid was extracted from positive colonies and transformed into *Mycobacterium smegmatis*, a non-pathogenic species. After screening for the N-terminal plasmid by PCR and fluorescence, *M. smegmatis* clones were visualized by confocal microscopy. Microscopy showed GFP expression in all *M. smegmatis* cells, with strong expression in a small percentage of bacteria. Future plans include expression of the C-terminal and transmembrane constructs in *M. smegmatis*, visualization by microscopy and comparison to the N-terminal construct, and confirmation by DNA sequencing. The constructs can then be transformed into PknD-deficient M. tuberculosis, which will allow for examination of PknD localization and expression in its native species and the protein's role in extrapulmonary dissemination.

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TAMHSC 2018 SUMMER RESEARCH PROGRAM

GENETICALLY ENGINEERING LYME DISEASE RESISTANCE VIA CHIMERA PRODUCTION

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The North American Deer Mouse, or *Peromyscus maniculatus*, serves as a reservoir for various diseases including Lyme disease and Hantavirus. Among noted ailments, Lyme disease is currently the fastest growing vector-borne infectious disease in the U.S. Because current findings on assisted reproduction of this species are limited, a chimera will be used to generate this disease resistant *P. maniculatus*. Before a chimera can be produced, antibiotic selection of an inserted fluorescent marker will be used to differentiate *P. maniculatus* iPSCs from the *Mus musculus* blastocyst. RT-PCR, bright field images, and fluorescent images are used to evaluate stem cell pluripotency and expression of fluorescent markers. Future studies will include verification of successful chimeric blastocyst implantation, and isolation of genes responsible for this disease vector. While this project is ongoing, we hope to eradicate *P. maniculatus* enabled diseases using this highly innovative approach to gene editing.

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Please keep us updated with your contact information and career or school decisions after graduation.

Thank you for your hard work this summer!

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