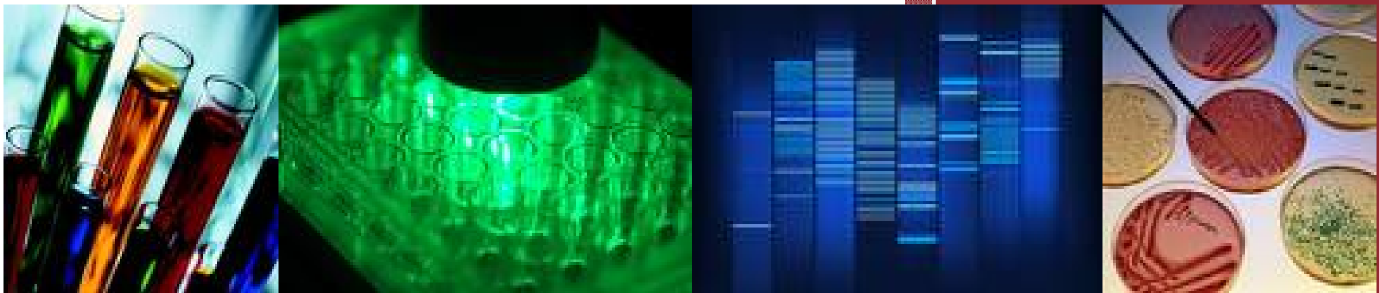
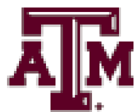
Poster Session and Reception

2013

Summer Research Program



August 07, 2013
9:00am - 4:00pm
Health Professions Education Building
Bryan, TX



HEALTH SCIENCE CENTER
TEXAS A&M UNIVERSITY

Schedule of Events

August 07, 2013

8:30am	**Registration Table Opens** HPEB L1 Lobby
9:00am-4:00pm	**Poster Viewing** HPEB LL43 A&B
11:30am	**Lunch** HPEB LL11 A&B
12:15-1:00pm	**CST*R Grand Rounds – SRP Featured Speaker** **John W. Belmont, M.D., Ph. D.** *Integrative Genomics Analysis of Human Influenza Vaccine and Infection: Clinical Research Trials at Texas A&M* HPEB LL46
1:00-2:30pm	**Poster Presentations – Students Presenting** HPEB LL43 A&B
2:30-4:00pm	**Closing Remarks and Presentation of Certificates** Dr. Warren Zimmer, Summer Research Program Director HPEB LL46
	Presentation of Dean's Recognition Awards HPEB LL46
4:00pm	**Adjourn**

Speaker's Biography

John W. Belmont, M.D., Ph.D.
Professor
Department of Molecular and Human Genetics
Baylor College of Medicine
Houston, Texas

Dr. Belmont has been helping patients understand the genetic basis of birth defects over his 25+ year career. Specifically his major interest is in genetics of the cardiovascular system with a particular focus in cardiovascular malformations and connective tissue disorders. This is also a focus of his research - from his lab website "Significant congenital cardiovascular malformations (CVM) occur in about 8 per 1000 live births. We are interested in identifying genetic factors that cause CVM or contribute to the risk for their occurrence. One project focuses on the underlying basis for CHARGE Syndrome. CHARGE is a complex phenotype that involves the development of the eye, ear, cranial nerves, brain, genitourinary system, and heart. Recently a gene, *CHD7*, has been found to cause CHARGE in about 65 percent of patients. Using a large set of CHARGE cases we have characterized the mutational spectrum in *CHD7*. We are using microarray and nextgen sequencing technology to screen for new genetic loci involved in the CHARGE-like phenotype." His research has expanded into other genetic malformations of the heart and is leading to explorations of how to treat patients with cardiovascular disorders.

A second area of interest is the analysis of how host genetics affect the responses to vaccines. He and his colleagues have worked with the Influenza Research Center to gain an understanding of the complex genetics of response to seasonal influenza vaccine. These studies aim to use genetics to predict the magnitude of host antibody response and help define a new gene – environment interaction that will be important in the development of effective vaccines. These studies help form the basis of his talk today entitled ***Integrative Genomics Analysis of Human Influenza Vaccine and Infection: Clinical Research Trials at Texas A&M***.

In addition to his home department, Dr. Belmont is also a professor in the Departments of Pathology and Immunology and Pediatrics as well as a member of the Program in Developmental Biology, Stem Cells and Regenerative Medicine, and the USDA/ARS Children's Nutrition Research Center. He is a member of several societies including the American Society of Human Genetics and the Society for Pediatric Research. He is an elected member of American Society of Clinical Investigation and the Association of American Physicians. He holds degrees from the University of Texas at Austin (BA, 1974), and Baylor College of Medicine (MD, PhD 1981). He was a Pediatrics Resident at the Children's National Medical Center (1983) and at Baylor College of Medicine (1984) and a Fellow in Medical Genetics at Baylor College of Medicine (1986). Dr. Belmont's lecture is presented in cooperation with the Texas A&M Health Science Center Clinical Science and Translational Research Grand Rounds Program.

Acknowledgements

The Summer Research Program continues to grow and the quality of the students that participate is second to none. Our application numbers were again strong (~6 applications/spot) and the quality of applications equally strong making the competition for a spot in the program especially keen (please see a list of the participants on page 46). The SRP committee evaluated each application and with an NIH study section-like meeting came to a consensus on which students to offer a spot. This task is becoming more difficult each year and I applaud the committee for their diligent work. For the second year we have increased the number of participating students from the generous input from Dean Shomaker who created the Prairie View Scholars positions and Mr. Jack Hart from the Temple Health and Bioscience District with the THBD Scholar Program. As we continue to cultivate new resources we will be able to continue a growth and strengthening of the program. Of course, it is the faculty that give their time both as mentors and presenting stimulating/informative lectures (please see page 47 for a listing of the speakers and topics) that are the real drivers of the program. They are the real VIPs and each deserves a hardy "job well done". The program would not be able to sustain its quality and existence without them.

Even though we serve two distinct student populations, medical students between their first and second years and undergraduates from around the country, all participate equally in the program. This enriches the experience as each group can learn from the other and share their ups and downs of laboratory experience. Each has worked extremely hard this summer and the posters displayed at today's reception are the products of this hard work. Please take the time to visit the posters and ask the students what they did during their summer vacation; be prepared to be amazed by their work and their abilities!

We obtained funding from a number of sources and would like to thank Dr. Shomaker, College Dean; Dr. Wesson, Vice Dean of the Temple Campus (Scott and White Research); Dr. David Carlson, Vice President for Research and Graduate Studies, and Mr. Jack Heart representing the THBD, for major contributions to our budget. It is a down year for research funding but the support of department chairs is also acknowledged. It is difficult, but not impossible, to provide content simultaneously to three locations. The work of Drs. Murray (College Station), Mitchell (Temple) and Huston (Houston) as site coordinators keeping things running efficiently is greatly appreciated. Finally, I would like to thank Dr. Van Wilson and his staff in College Station, **Amanda Watkins-Borths**, **Hannah Boyer**, **Marsh Miller**, **Peggy Hazelwood**, and **Mary Ann Wolff**.

Dr. Huston's staff in Houston, **Anna Wirt;** and the Dean's staff in Temple**, Loria Lynce** and **Cari Cummings** for making certain that the entire program got off the ground and running effortlessly.

Warren Zimmer, PhD
Director, SRP

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TAMHSC SUMMER RESEARCH PROGRAM

August 7, 2013: 9:00 AM - 4:00 PM
Health Professions Education Building
Bryan, Texas

Cardiac myosin binding protein-C phosphorylation: The answer to heart failure with preserved ejection fraction?

Mohamed Abdalla[1], Yang Liu[1] MD, PhD, David Kidwell[2] MD, MBA, Kevin Duong[1], David Weber[1], Adam Willms[1], Richard L. Moss[3] PhD, Carl W. Tong[1] MD,PhD.

[1]Texas A&M HSC College of Medicine; [2] Scott & White Hospital, Department of Internal Medicine; [3]University of Wisconsin School of Medicine and Public Health

Introduction & Background: Heart failure is a growing major public health problem especially with the nation's aging population. Upon reaching age 40, 1 in 5 Americans will develop heart failure in the remaining years of their lives. Heart failure with preserved ejection fraction (HFpEF) accounts for 50% of all heart failure cases and there is no effective treatment to combat HFpEF.

Hypothesis & Objective: We hypothesize that phosphorylation of cardiac myosin binding protein-c (MyBPC3) enhances the heart muscle's ability to relax (lusitropy); therefore, MyBPC3 phosphorylation can be a useful treatment for HFpEF. MyBPC3 is part of the thick filament within the heart muscle and is thought to slow cross-bridge cycling by inhibiting acto-myosin interaction. When phosphorylated, MyBPC3 releases its inhibition on this interaction and allows for accelerated cross-bridge cycling. In doing so, MyBPC3 phosphorylation enhances muscle relaxation and therefore has the potential to be used as a treatment for HFpEF.

Materials and Methods: Three mice lines were transgenically derived from a MyBPC3(KO) background: MyBPC3(tWT) with normally phosphorylatable MyBPC3, MyBPC3(t3SA) with a non-phosphorylatable MyBPC3 mutant, and MyBPC3(t3SD) with a constitutively phosphorylated MyBPC3 mutant. Comparisons were then made using echocardiography, running wheels, and body measurements.

Results: We found that MyBPC3(t3SD) hearts show normal wall thickness while MyBPC3(t3SA) hearts show increased wall thickness thus exhibiting hypertrophy. MyBPC3(t3SD) hearts exhibit markedly enhanced lusitropy by echocardiographic tissue Doppler measurement of peak myocardial relaxation velocity at mitral valve annulus (Ea). Furthermore, both MyBPC3(t3SD) and MyBPC3(t3SA) hearts show normal systolic function (ejection fraction). Conversely, MyBPC3(t3SA) hearts show decreased diastolic function represented by depressed myocardial relaxation velocity (Ea), elevated peak blood flow velocity Doppler/myocardial relaxation velocity ratio (E/Ea), and elevated isovolumetric relaxation time (IVRT).

Conclusions: These data suggest that MyBPC3(t3SA) hearts develop hypertrophy to compensate for lack of phosphorylated MyBPC3 but in doing so worsens diastolic dysfunction. Moreover, lower average voluntary running distance, increased heart/body weight (HW/BW), and increased lung/body weight of MyBPC3(t3SA) mice demonstrate signs of heart failure that MyBPC3(t3SD) mice do not exhibit. Thus, combination of MyBPC3(t3SA) mice resembling HFpEF and MyBPC3(t3SD) mice exhibiting enhanced lusitropy demonstrates that phosphorylation of MyBPC3 is crucial for normal diastolic function.

TAMHSC SUMMER RESEARCH PROGRAM

August 7, 2013: 9:00 AM - 4:00 PM
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Bryan, Texas

Evaluation of Novel Allosteric Modulators of the Endocannabinoid System using Rat Neural Stem Cells

Claire Adkins[3], Bharathi Hattiangady[1,3], Dai Lu[2,3], Ashok Shetty[1,3]

[1]Institute for Regenerative Medicine, Department of Molecular and Cellular Medicine, [2]Department of Pharmaceutical Sciences , [3]College of Medicine, Texas A & M University Health Science Center

Introduction: Neural stem cells (NSCs) exist in two important regions in the adult brain and contribute to ongoing neurogenesis. The hippocampal NSCs play an important role in learning, memory and mood, while sub-ventricular NSCs are important in olfactory function and probably in brain repair in pathological conditions such as stroke. The extent of NSC proliferation and adult neurogenesis are regulated by many physiological and pathological conditions. The endocannabinoid (eCB) system has been found to play a role in neurogenesis as neural stem cells have cannabinoid receptors and the modulation of their expression can induce neural stem cell proliferation, regulate neuroblast migration and determine neural cell fate. In this study we tested four different micro compounds that are allosteric modulators of cannabinoid receptors for their ability to stimulate NSC activity in vitro.

Specific aim: To screen four micro compounds (ORG27569, LDK1240, LDK1241, and ICAM-b) that are allosteric modulators of cannabinoid receptors for their ability to stimulate neural stem cell activity in vitro.

Methodology:

Neurosphere assay: We screened four different CBN receptor modulator micro compounds namely LDK1240, LDK1241, ICAM-b and ORG27569 (provided by our collaborator Dr. Dai Lu) for their ability to influence neural stem cell activity. Neural stem cells (NSC) were obtained from PND-2, GFP+ transgenic rat, anterior sub-ventricular zone (aSVZ). We plated 10,000 cells in 6 well plates using proliferation medium containing all four micro compounds in three different concentrations (1000nm, 100nm and 10nm). Normal control and DMSO controls were run parallel. On day 7, the yield of neurospheres was quantified under the epiflurescence microscope for all 14 groups (4 compounds x3 dilutions, normal control and DMSO control). Based on the results of above experiment, we selected two promising compounds (LDK 1240 and LDK 1241) at 100nm concentration and further confirmed their ability to stimulate NSC activity with a low-density neurosphere assay (100 cell per well in 24 well plates; n=24).

Differentiation culture: To further confirm whether, both LDK 1240 and LDK 1241 also influence neuronal differentiation, we expanded aSVZ NSCs in proliferation medium containing these two drugs at 100nm concentration for 7 days and then differentiated them on a Poly-L- Lysine coated 24 well plate. At day 6 cultures were fixed in 2% paraformaldehyde and stained for TUJ-1(marker for neurons), GFAP (marker for astrocytes) or O1 (marker for oligodendrocytes). We quantified and compared the % of neurons, astrocytes and oligodendrocytes in treated, DMSO or naïve group.

Culture in the absence of trophic factors: In order to test whether, these two micro compounds maintain NSC activity in the absence of EGF and FGF-2, we ran another experiment where NSCs were plated in proliferation medium containing no EGF and FGF2.

Results and summary: Our initial screening experiment with high density neurosphere assay revealed that, out of four micro-compounds tested, two of them (LDK1240 and LDK 1241) are promising with a significantly higher yield of neurospheres. The yield of neurospheres at day 7 was 50% more in the presence of LDK1240 and was 54% higher in the presence of LDK 1241. Further confirmation using low density neurosphere assay also showed similar trend. The results of differentiation assay however, did not show any difference in terms of yield of neurons. We found that ~ 25-29% TUJ-1+ neurons in both treated and control groups differentiated into neurons.

Further, the cultures grown in the absence of EGF and FGF2 did not show neurospheres in any groups until day 10. However, a parallel group that was expanded in the presence of EGF and FGF-2 had neurospheres. Thus, our additional experiment in the absence of trophic factors confirmed that none of these compounds are effective in NSC proliferation in the absence of trophic factors.

Cytotoxic Effects of Eosinophils against Tumor Cells

Zhaleh J. Amini-Vaughan, and David P. Huston
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Introduction: Eosinophils participate in host defense against helminths and are also considered important in the pathogenesis of asthma and allergic diseases. Eosinophil differentiation and survival is predominantly regulated by IL-5, and eosinophil migration responds largely to the chemokine eotaxin-1. Recent studies have also indicated an association between eosinophilia and improved tumor prognosis in colon, breast, colorectal, nasopharyngeal, oral, gastric, and head & neck cancers. More recently, murine studies have indicated that eotaxin is necessary to clear melanomas and that eotaxin deficient mice have lower tumor clearance. Furthermore, IL-5 transgenic mice have decreased tumor metastasis. A tumoricidal role for eosinophils against a human colorectal cancer cell line has been suggested, *in vitro,* and was cell contact-dependent, requiring the adhesion molecule CD11a/CD18.

Hypothesis: We hypothesize that eosinophils have the potential for tumoricidal activity against a broad spectrum of tumors, and thus have the potential to be harnessed as a novel cancer therapeutic.

Methods: ATCC human cancer cells (PC3-prostate, RKO-colon, TFI-erythroleukemia, and A549-lung) were maintained in culture. Human eosinophils were isolated from leukopacks via Robosep negative antibody selection. Eosinophil purity was determined by dual staining for CD16 and CD66b using flow cytometry. Eosinophils were co-cultured with tumor cells in varying effector-to-target ratios for 4 hours. Tumor cell viability was assessed by Annexin V and propidium iodide staining using flow cytometry.

Results: A549 cells had the highest sensitivity to eosinophil co-culture with the percent viability dropping by 79% at a 5:1 eosinophil to cancer cell ratio. RKO and TF1 cell lines were less sensitive to co-culture. RKO viability decreased by 39% in experiment 1 and by 25% in experiment 2. TF1 viability decreased by 22% in experiment 1 and by 33% in experiment 2. PC3 cells had the lowest sensitivity decreasing in viability by 8% in experiment 1 and by 13% in experiment 2.

Conclusion: These studies demonstrate the cytotoxic potential of eosinophils across a spectrum of tumors. Eosinophils had highest tumoricidal activity against A549 cells followed with intermediate sensitivity in RKO and TF1 cells. PC3 cells had the lowest sensitivity to tumoricidal effects. Cell death was largely due to apoptosis rather than necrosis. Overall, the optimum effector-to-target cell ratios were approximately 5:1. These studies suggest that eosinophils have the potential to be harnessed as a cancer therapeutic.

TAMHSC SUMMER RESEARCH PROGRAM

August 7, 2013: 9:00 AM - 4:00 PM
Health Professions Education Building
Bryan, Texas

Anthrax Lethal Toxin-Induced Metabolic Dysfunction

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Division of Molecular Cardiology, Cardiovascular Research Institute
Texas A&M University Health Science Center, College of Medicine, Temple;
Central Texas Veterans Health Care System, Temple, Texas

Introduction: We previously discovered a cardiogenic mechanism for anthrax lethal toxin (LT), by which LT directly impairs cardiac myocyte function through disruption of the MEK7-JNK1-PP2A-B56α-Akt-phospholamban (PLB) signaling axis and dysregulation of diastolic intracellular calcium levels, resulting in diastolic dysfunction *in vivo*. The effects of LT on myocardial metabolism, however, are currently unknown.

Hypothesis and Specific Aims: We hypothesized that acute anthrax LT toxicity dysregulates cardiac metabolism by impairing metabolic substrate sensing and energy utilization through inhibition of the MEK7-JNK1-Akt-PLB signaling pathway. Our aims were to (1) determine the time- and dose-dependent effects of LT on metabolic parameters of mitochondrial respiration (oxidative phosphorylation, ox-phos) and glycolysis in neonatal rat ventricular myocytes (NRVM), and (2) determine whether ox-phos, glycolytic metabolism, and mitochondrial reactive oxygen species (ROS) production are modulated by MEK7-JNK1-Akt-PLB signaling in NRVM.

Methods: We established time-dependent responses (0, 30 min, 1 h, 2 h and 4 h) of anthrax LT at doses of 0.10 ng PA + 0.05 ng LF, 0.05 ng PA + 0.025 ng LF and 0.01 ng PA + 0.005 ng LF on the metabolic profile of isolated NRVM (Seahorse Bioscience XF Analyzer) to determine whether ox-phos or glycolysis is predominantly affected by LT toxicity. We then established the gain-of-function/loss-of-function effects of sarcoplasmic reticulum (SR)-targeted Akt (Akt→SR) and B56α adenoviral expression in the presence of LT. Furthermore, we determined whether adenoviral expression of constitutively-active (CA)-MEK7 in the presence and absence of dominant-negative (DN)-JNK1 or DN-JNK2 provided a cardioprotective effect during LT toxicity. Mitochondrial ROS production was assessed by flow cytometry (BD Bioscience FACSCalibur) as an indicator of mitochondrial stress/electron transport chain (ETC) uncoupling. Isoelectric focusing was performed (NanoPro 1000) to determine isoform-specific cell signaling interactions.

Results: We demonstrated that ox-phos is most affected by LT at 30 min – 1 h, whereas glycolysis is most affected by LT at 2 h – 4 h. We observed that CA-MEK7 overexpression protects against the ox-phos effects of LT through MEK7-JNK1 signaling. Glycolytic impairment by LT is reversed by Akt->SR expression, which also prevents mitochondrial ROS accumulation. Isoelectric focusing data further suggests that the protective effects of Akt->SR signaling involve mTOR-mediated energy sensing. Thus, integration of the JNK signaling module with Akt regulation represents an important stress-activated signalosome that may confer protection to sustain cardiac metabolism through nutrient availability/sensing during different aspects of cellular stress.

August 7, 2013: 9:00 AM - 4:00 PM
Health Professions Education Building
Bryan, Texas

Factors Influencing Outcomes of Lumbar Fusion

Nathan Boes, Antoine Scott, Kristen Arndt and Research Mentor: Jonathan Friedman, MD
Dept. of Surgery and Neuroscience and Experimental Therapeutics
Texas A & M University Health Science Center College of Medicine
The Texas Brain and Spine Institute of Bryan/College Station

Introduction: Lower back pain, LBP, is a common disorder with a lifetime prevalence of 85%[1]. LBP is a problem for many working-aged adults in America that contributes to a loss in productivity, increased hospitalization rates/medical expenses, decrease in quality of life, and chronic pain. With many possible physiopathological factors involved in LBP, it is often difficult to give a definite diagnosis[2]. Optimal treatment options therefore range from conservative management to surgical intervention based on the patient's symptoms and physical exam findings.

Surgical spinal fusion is one method used to eliminate LBP in correcting irregular motion of the spine by joining two or more vertebrae. It is the goal of this research to determine clinical, radiographic, and surgical factors that were associated with improved outcomes in patients who underwent lumbar fusion.

Hypothesis: Certain clinical and radiographic features will predict a more favorable outcome from lumbar fusion.

Methods: We are conducting a retrospective chart review of 361 patients over the past 6 years treated with lumbar fusion at a single center, by a single surgeon. Inclusion for this study requires minimum of six months of atraumatic chronic LBP and stratification into subgroups based on the following parameters: age, gender, BMI, occupation, attempted nonsurgical treatments, duration of chronic back pain, and compounding comorbidities. Outcomes were assessed eight weeks post- procedure with inclusion of radiographic findings, complications, duration of recovery, rehabilitation regimen, and subsequent patient complaint.

Results: We will be using data analysis to predict favorable surgical fusion outcomes among patients with LBP. Patient demographic information, past medical history, examination findings, pre/post operative imaging results, surgical factors including unilateral vs. bilateral hardware implantation, use of interbody device, one level vs. multilevel procedures, and follow up care will be used as predictors of outcome.

1. 1: Spoor AB, Oner FC. Minimally invasive spine surgery in chronic low back pain

patients. J Neurosurg Sci. 2013 Sep;57(3):203-18. PubMed PMID: 23877267.

2. White, A. A., Gordon, S. L. 1982. Syn- opsis: workshop on idiopathic low back pain. Spine 7:141-49

Rnd3 as a Novel Heart Failure Regulator

Conan Chen, Tingli Yang, Xiaojing Yue, Xiangsheng Yang, Jiang Chang
Institute of Biosciences and Technology
Texas A & M University Health Science Center College of Medicine, Houston, Texas

Introduction: Heart failure is the condition in which the heart cannot pump adequate amounts of blood into circulation. Heart failure has been associated with cell apoptosis, and inflammatory response, among other contributing causes. Our lab previously demonstrated Rho signaling pathway is critical for heart failure development. Activation of Rho kinase activity leads to cardiac apoptosis and fibrosis, eventually leading to heart failure. Rnd3, a small GTPase, is an endogenous Rho kinase repressor whose biological function in the heart is unknown. This study investigates the role of Rnd3 in heart failure and its associated molecular mechanism. In preliminary studies, it was found that: 1. human failing hearts express significantly lower Rnd3 protein levels than do normal human hearts, and 2. when inducing myocardial infarction in single-knockout $Rnd3^{+/-}$ mice, the infarction site was observed to have a massive inflammatory response. These two observations suggest that Rnd3 has a role in suppressing the heart inflammatory response, one of the factors leading to heart failure. Thus Rnd3 represents a potential novel suppressor of the heart inflammation response.

Hypothesis: Since p38 MAPK and p65 NFkB are two crucial regulators of pro-inflammatory cytokines and the inflammation response, it is hypothesized that Rnd3 recruits protein phosphatase one (PP1) to dephosphorylate p38 and/or p65, which are only active in this pro-inflammation role when phosphorylated.

Methods: HEK 293 T Cells were used as an *in vitro* model of the inflammation response in body cells. The Rnd3 knockdown and overexpression cell lines were generated by transfecting Rnd3-specific siRNA and Rnd3 plasmid vectors, respectively, into the cells. A negative cell line, a line without any Rnd3 alteration, was used as a control. Each of the knockdown, overexpression, and negative cell groups had an additional positive PP1 cell line. Proteins were extracted from the cell groups, run through a Western Blot, and then probed for P-p38, P-p65, p38, and p65 using their respective antibodies. The protein levels of the knockdown group were compared to those of the respective control groups to determine the effect of Rnd3 on p38 and p65.

Results: Current results are inconclusive. Based on comparisons between siRNA and the negative control, Rnd3 knockdown gives an increase in P-p38 and P-p65, consistent with our hypothesis. However, overexpression of Rnd3 also gives an increase in phosphorylation. Whether or not PP1 participates in this dephosphorylation is unclear since some parts of the data show and increase, others a decrease, in phosphorylation.

Value of Repeat Surgical Resections for Pediatric Gliomas of the Brainstem, Thalami, and Basal Ganglia

Elizabeth Coffee[1], Nicholas Wetjen[2]
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Introduction: Brain tumors are the leading cause of childhood cancer-related deaths. Of childhood primary tumors in the central nervous system, approximately 20% are found in the brainstem and 4% originate in the thalamus, with even more in surrounding structures such as the basal ganglia. Extent of surgical resection remains the most significant prognostic factor amongst operable gliomas in these locations. Adjuvant therapies are often used in conjunction with surgical resection, while some institutions advocate for repeat surgical resection to manage residual or recurrent tumor, with or without adjuvant therapy. However, there is no comprehensive data available evaluating the impact of "second-look" surgery on survival in this population.

Hypothesis: We propose that the utility of repeat surgeries in pediatric patients with gliomas of the brainstem, thalami, or basal ganglia can be determined by a systematic review of the literature accompanied by a meta-analysis of survival data.

Methods: In order to compile all relevant data, a broad search was completed in MEDLINE using grouped terms for pediatrics, surgery, glioma, and outcomes. Searches will also be completed in EMBASE and PubMed. All abstracts and potentially relevant papers will be screened by two separate reviewers. Survival data from all relevant papers will be compiled to perform a meta-analysis using Kaplan-Meier survival estimates and Cox regression models to compare repeat surgical groups to controls. Radiation therapy and chemotherapy groups will also be included in both variable and control categories.

Results: As of yet, 1781 abstracts have been identified and are in the screening process. Should the data allow, the utility of second-look surgeries in the pediatric population will be determined for gliomas of each the brainstem, thalami, and basal ganglia. These data can be used to weigh the risks and benefits of different treatment modalities for pediatric patients diagnosed with these tumors, and will influence the recommendations by which these tumors are managed in a clinical setting.

TAMHSC SUMMER RESEARCH PROGRAM

August 7, 2013: 9:00 AM – 4:00 PM
Health Professions Education Building
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Effect of Intracellular Angiotensin II on Proliferation of HL-1 Cardiomyocytes

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Introduction: The classical Renin angiotensin system, or the RAS, is an endocrine system that regulates blood pressure. Angiotensin II (Ang II) is the principal effector molecule of the RAS and binds the known receptors AT_1 and AT_2. Ang II is also present as part of an intracellular RAS and has been shown to increase cardiomyocyte hypertrophy independent of AT_1 and AT_2, suggesting the involvement of other binding partners. Our lab identified Xinα and STAT1 as binding partners of intracellular Ang II (iAng II) using a mouse atrial cardiomyocyte (HL-1) cell line transfected with a plasmid expressing Ang II. In order to study the effects of iAng II and its binding partners, we needed to develop a biological assay. We tested whether cell proliferation can be used to study iAng II interaction with the binding partners.

Hypothesis: We hypothesize that we will observe a higher rate of proliferation in the HL-1 cardiomyocytes transfected with iAng II compared to native cells.

Methods: Design of transfected cell model: HL-1 cells were transfected with a plasmid expressing FLAG-Ang II peptide (FA cells) or an inert FLAG-scrambled-Ang II peptide (FSA cells) to act as control. ELISA was used to measure iAng II expression. Novel binding partner identification: Co-immunoprecipitation using anti-FLAG beads, followed by mass spectrometry, was used to identify binding partners of iAng II. Functional assay development: HL-1 cells were plated in 24-well dishes. AlamarBlue containing serum-free media was added after 24 hours and fluorescence and absorbance readings were taken at next 0, 24, 48 and 72 hours. Click-iT® EdU Cell Proliferation Assay was used to determine the rate of DNA replication. Briefly, cells were plated in 8 replicates on 96 well plates. After 4 hours, the media was exchanged for serum-free media containing EdU. Fluorescence readings were taken at 24, 48 and 72 hours to quantify the amount of EdU incorporated.

Results: The FA cell line expressed about 6 times more iAng II than the HL-1 wild-type and FSA control cells. For cell proliferation assay, the culture conditions were optimized for cell plating density, glucose, and serum concentration. Using AlamarBlue, we observed that the FA cells metabolized significantly more AlamarBlue ($p < .05$), suggesting an increased rate of proliferation. Using Click-iT® EdU, we found that the FA cells incorporated significantly more EdU ($p < .05$), showing an increased rate of DNA replication; thus, implying an increased rate of cell proliferation. These results demonstrated that the cell proliferation in FA cells can be used as a functional assay to study biological role of iAng II interaction with newly identified binding partners.

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Mission BREATHE: Better Recognition of Exacerbating Asthma Triggers in the Home and Environment *(Mejor reconocimiento de los exacerbantes de Asma en el hogar y en el medio ambiente)*

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Introduction and Background: The most common barriers to adequate asthma care among low-income families are economic, social, literacy, and language barriers.[1] In 2008, the per capita income in McAllen, TX, a border community with many surrounding *Colonias*, was $20,395 compared to a national average of $40,947.[2,3] In 2009, the average yearly cost of treatment for an asthmatic child was $1,039.[1] In addition, the McAllen area continues to be a vulnerable population due to higher prevalence of minorities, especially Hispanics.[4]

Research has shown that a majority of the asthma related problems in impoverished communities is caused by environmental exposures.[5] According to the IOM, exposure to cigarette smoke, house dust mites, indoor pets, mold, and cockroaches can exacerbate asthma symptoms.[6] More specific to McAllen, TX, research indicates that individuals who reside in border cities are especially vulnerable to asthma due to the poor air quality caused by pollutants and use of pesticides.[7] Previous educational interventions have taken a substantial amount of time, ranging from 2 hours to once a week sessions for 5 weeks.[5,8] While several of these have been successful,[5,8] the time needed to complete these programs may present a barrier to working parents.

Hypothesis: A shorter asthma education program will be beneficial to the health of a child with asthma, more appealing for parents to participate in, and will ultimately assist with the problem of poorly controlled childhood asthma by increasing parental awareness.

Methods: A community based participatory research approach will be used in equal partnership with a *Promotora*, a trained community healthcare worker, and the Rio Grande Regional Hospital for the purposes of addressing cultural differences and language barriers. Participant enrollment will be obtained with the cooperation of the Rio Grande Regional Hospital Respiratory Therapy Services and local physicians. After obtaining consent and meeting all inclusion/exclusion criteria, participants will be asked to complete the following procedures/assessments for Day 1, which include an asthma control survey, Asthma knowledge pre-test, 30-minute education session, and Asthma knowledge post-test, and Day 14-28, which includes a follow-up phone call post-asthma control survey. This study will assess the level of control 30 participants (parents) from this area have over their child's asthma as well as promote increased asthma knowledge to improve the quality of life for a child with asthma.

Results: The results for this study are pending. The recruitment process began on July 23, 2013 and the intervention will take place between August and December 2013.

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Roles of Cardiac Myosin Binding Protein-C Phosphorylation in Pressure-Overload Heart Failure

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Introduction: Cardiac myosin binding protein-C (MyBPC3) is a muscle thick filament protein that inhibits actin-myosin cross bridge interaction. Phosphorylation can release MyBPC3 inhibition to improve cardiac contractility and lusitropy. MyBPC3 phosphorylation levels are decreased in heart failure.

Hypothesis: Cardiac myosin binding protein-C (MyBPC3) phosphorylation mitigates pressure-overload induced heart failure.

Methods: We tested our idea by using severe trans-aortic constriction (TAC) induced pressure overload to challenge mouse models of phosphorylation deficient MYBPC3 (S273A, S282A, S302A)-MYBPC3(t3SA), phosphorylation mimetic MYBPC3 (S273D, S282D, S302D)-MYBPC3(t3SD), and WT-control MYBPC3(tWT) of MYBPC3.

Results: Prior to TAC or sham surgery, MYBPC3(t3SA) hearts exhibited hypertrophy, predominantly diastolic dysfunction (elevated E/Ea ratio, E=mitral blood flow velocity Doppler, Ea = myocardial relaxation velocity tissue Doppler), preserved ejection fraction (EF>50%), and elevated Akt, Erk, and p38 activation. In contrast, pre-surgery MYBPC3(t3SD) hearts demonstrated enhanced diastolic function (smaller E/Ea). At TAC+3 weeks, MyBPC3 (t3SA) showed depressed EF and increased E/Ea only in comparison to MYBPC3(t3SD). At TAC+5 weeks, MYBPC3(t3SA) deteriorated further to exhibit reduced EF, increased E/Ea, and left ventricular dilation vs. MYBPC3(tWT) and MYBPC3(t3SD). Furthermore, MYBPC3(t3SA) demonstrated increased TAC+5 weeks mortality. Thus, we conclude that loss of MYBPC3 phosphorylation directly contributed to heart failure and combination of (S273-P, S282-P, S302-P) improved preservation of cardiac function.

Cytomegalovirus Assembly and Phosphoinositide Metabolism

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Introduction: Human cytomegalovirus (HCMV) is a member of the herpesvirus family and a major cause of morbidity and mortality in newborns and immunocompromised persons. Like other herpes viruses, HCMV particle assembly occurs in two phases: nuclear encapsidation of the viral DNA and envelopment at a stable juxtanuclear structure referred to as the cytoplasmic viral assembly center (VAC). In spite of being an active area of research for many years, the pathways regulating HCMV assembly are not completely understood.

Phosphoinositides (PIPs) are phosphorylated derivatives of phosphatidylinositol (PtdIns). PIPs are concentrated at the cytosolic face of membranes. Reversible phosphorylation of the PtdIns head group at positions 3, 4, and 5 gives rise to the 7 derivatives observed in eukaryotic cells. The modification of PIPs is regulated by the action of kinases and phosphatases that add or remove phosphate groups and by phospholipases, which cleave lipids. Together, the activities of these enzymes regulate production of phosphoinositides both spatially and temporally. Although they comprise a very minor pool of phospholipids in cellular membranes, PIPs are critically important not only as lipid precursors for second messengers, but also as signaling molecules in vesicular transport. PIPs serve as binding platforms for the recruitment of proteins and thus confer identity to the various organelles of the secretory pathway. Rab GTPases can bind to specific PIPs and together they function as regulators of membrane traffic at exocytic and endocytic sorting hubs.

Hypothesis and Aims: Little is known about the interactions between enveloped DNA viruses, including HCMV, and the PIP system. We hypothesize that HCMV infection subverts the PIP system to control the flow and recruitment of virion proteins into the VAC. We explored this hypothesis with the following aims:

- Test whether infection with the laboratory strain Towne alters localization of phosphoinositides in primary fibroblast by immunofluorescence.
- Use chemical inhibitors to manipulate the function of PIP kinases and test whether changes in activity affect formation of the VAC by immunofluorescence and extracellular virus production by plaque assay.

Methods:

- **Cells, Virus Infection, and Measurement of Extracellular Virus Production**: Primary human fibroblasts were infected with HCMV Towne at a multiplicity of 3-5 (MOI). Twenty-four hours post-infection (p.i.) cultures were treated with PIP inhibitors. Media and drugs were replenished every 24 h thereafter. Culture supernatants were collected every 24 h and frozen.
  Plaque Assay*:* For measurement of virus titer, ten-fold dilutions of culture supernatants were used to infect primary fibroblasts, then cells were overlayed with agarose 24 h p.i. The number of plaques that developed were counted at day 9.

- **Immunofluorescence**: HCMV Towne- and mock-infected primary human fibroblasts were seeded onto coverslips, which were fixed in 2% formaldehyde at 72 h p.i. For PIP staining, cells were permeabilized with digitonin and incubated with GST lipid-binding domain fusion proteins at 5 μg/mL. Coverslips were incubated with anti-GST and EEA1, gm130, or HCMV gM mouse monoclonal antibodies followed by corresponding isotype-specific, fluorescently-labeled secondary antibodies.

- **Western Blot**: Towne-, TRB-, and mock-infected cell lysates were separated by SDS-PAGE and transferred to nitrocellulose. Actin was used as a loading control. Blot was blocked in 5% milk in .05% TBST. Primary antibodies were run for 3 hours using mouse IgG2B at 1:600 for Vps34 and antiactin at 1:4000 for actin. After three washes in .05% TBST, secondary antibody of anti-mouse at 1:2000 for Vps34 and 1:4000 for actin was run for 1 hour. After three washes in .05% TBST, film was developed using standard SuperSignal for Vps34 and half strength SuperSignal for actin.

Results: The following results support our hypothesis.

- HCMV gM, a marker for the assembly center, colocalizes with PI3P- and PI4P-positive membranes.
- Treatment of infected cells with 3-methyladenine, a Vps34 inhibitor, results in increased virus production. This effect is dose dependent but independent of serum concentration.
- Treatment of infected cells with AS604850, a PI3Kγ inhibitor, leads to decreased virus production. This effect is dependent on inhibitor and serum concentration.

Ethanol exposure during pregnancy induces a sex and region specific reduction in blood flow of adult mice offspring

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Introduction: Alcohol consumption during pregnancy causes brain defects, cardiovascular abnormalities, limb deformation and behavioral problems in the developing fetus. These range of deleterious effects caused by prenatal ethanol exposure is known as fetal alcohol spectrum disorder or FASD (Jones et al., 1973; Goodlet et al., 2005). 12% of women admit to consuming alcohol at some time in their pregnancy and up to 5% of children born in the United States are affected by prenatal alcohol exposure (Brady et al., 2012). Previous studies have shown that ethanol exposure during second trimester pregnancy reduces blood flow in the developing mouse fetus (Bake et al., 2012), and decreases differentiation of neural stem cell ($CD24^{+}$) population (Tingling et al., 2013). Impaired blood flow may be considered as a sign of altered development of the heart and vasculature, and may lead to multiple cardiovascular complications or even premature death if left untreated.

Hypothesis: The present study tested the hypothesis that prenatal ethanol exposure in mice will have a significant impact on developing fetal vasculature and will reduce blood flow in different organs of adult offspring.

Methods:

Animals: The adult mice used in this study are the offspring of C57 Bl pregnant mice purchased from Harlan Laboratories (Houston, TX). The multiple-binge exposure model was used, in which the pregnant mice were exposed to ethanol (3g/Kg body wt.) via. intragastric gavage on gestational day 12.5, 13.5, 14.5, and 15.5. This gestational age is equivalent to the second trimester of human pregnancy, a period in which neurogenesis and angiogenesis play a critical role in brain development. The treated and control offspring were weaned at 3-4 weeks of age and assessed for ethanol's effect on blood flow at 6 months of age. A total of 30 male and female adult mice were examined **in** this study.

Ultrasound Imaging: Mice were anesthetized using isoflurane (4-5%), maintained with 1.5% on a temperature-controlled platform on supine position. Neck, chest, abdomen and a small area on thigh were cleaned using depilatory cream. High-resolution ultrasonography was used to analyze blood flow in the arteries of control and treated mice. Pulse wave Doppler measurements for carotid arteries, ascending aorta, descending aorta, renal arteries, lobar arteries within kidneys, and femoral arteries were obtained using a high-frequency VEVO2100 ultrasound imaging machine coupled to a MS550D Microscan transducer.

Data Analysis: Data from the pulse wave Doppler imaging scans were analyzed using the VEVO2100 measurement and analysis software (Visualsonics,Canada). 3 ultrasound scans were measured per artery in each animal examined. Within each scan, 5 individual pulse waves were quantitated. Waves were analyzed for change in acceleration, peak velocity, and the area under the velocity envelope (VTI) of each artery in each animal.

***Statistical analysis*:** Data was analyzed by 2-way-ANOVA (Sex and treatment as independent variables) using SPSS and were considered significant at $p<0.05$.

Results: *In utero* ethanol exposure resulted in significantly decreased blood flow velocity (acceleration, peak velocity) in the carotid and renal lobar arteries of female adult offspring, but not males. Decreased blood flow, especially within organs like the kidney may result in decreased glomerular filtration and deficiencies in erythropoiesis which in turn may result in variety of secondary consequences for the function of other organs like the cardiovascular system and brain. Poor blood flow can be a predisposing factor for many disease conditions during a lifespan and could potentially increase the risk for occurrence of vascular disorders such as stroke, especially in the adult population. Thus, the findings of this study suggest that prenatal ethanol exposure could contribute to developmental origin of adult diseases in a sex-specific manner.

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Infection of C57BL/6 Mice with Theiler's Murine Encephalomyelitis Virus as a Model for Epilepsy: Assessment of different routes of infection

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The International League Against Epilepsy (ILAE) defines epilepsy as "a neurological disorder of the central nervous system that is marked by seizures". Tonic-clonic, or *Grand mal* seizures are the most serious form of seizures. The individual will lose consciousness, fall, and skeletal muscles contract toward the body. Common viral infections have been known to cause seizures in children, which may lead to epilepsy later on in life. Theiler's Murine Encephalomyelitis Virus (TMEV) is a naturally occurring virus in mice and it causes seizures in the C57BL/6 strain, thus it is an appropriate animal model for investigating the pathogenesis of virus-induced epilepsy. In this study, eighty male C57BL/6 mice were divided into eight groups and were injected with TMEV via either intracranial, intraperitoneal, oral, subcutaneous, intradermal, tongue, intramuscular, or the hypoglossal nerve routes, respectively. The mice were observed, weighed, and assigned a clinical score over a 7 to 20 day period, and were then sacrificed. Brains and spinal cords were collected for histological examination. We focused our attention in the hippocampus since it is known to be involved in epilepsy. The intracranial group was the only group that exhibited seizures as expected, but a majority of the remaining groups exhibited hind-limb paresis with one mouse exhibiting paralysis. Histological examination determined that mice showing seizures had significant damage to the CA1 and CA3 areas in the hippocampus, as well as the dentate gyrus. Although the mice infected intracranially were the only experimental group to develop seizures, the fact that other groups developed paresis/paralysis suggests that the virus may still be gaining access to the CNS via these routes.

Keywords: Immunology, Epilepsy, Theiler's Murine Encephalomyelitis Virus, Neuroscience

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August 7, 2013: 9:00 AM - 4:00 PM
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Insights into Sexual Dimorphisms through Epigenetics

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Introduction: **Stroke** is the leading cause of death in the United States and claims more than 129,000 lives each year (Kockaneck et al. 2011). Previous studies have shown that mature **adult female rats have smaller infarcts** as compared to middle-aged females, mature males, and middle-aged males five days after middle cerebral artery occlusion (Selvamani & Sohrabji, 2012). **Epigenetic alterations**, including histone modifications and changes in DNA methylation, occur during normal aging and in disease conditions. The extent of nucleosome modification (i.e. DNA methylation, histone acetylation and/or deacetylation) plays an important role in regulating gene expression.

Hypothesis: The present study is designed to test the hypothesis that sex and age differences in stroke severity are associated with fundamental changes in epigenetic regulation of the genome. Here we used changes in expression and function of key regulatory enzymes that modify DNA topology.

Methods:

Animals: Sprague Dawley rats were purchased from Harlan Laboratories

- Adult males and females (6 months)
- Middle-aged males and females (9-11 months)

Females were smeared daily to determine cycle length or acyclicity.

Animals were deeply anesthetized and through a stereotaxic surgery the **left middle cerebral artery (MCAo) was occluded.** Animals were sacrificed and both left and right hemispheres of the **cerebral cortex were collected**. Both contralateral and ipsilateral cortex samples were prepared for **epigenetic assays** using a nuclear extraction kit (Epigentek). Enzymes that lead to Histone deacetylation (HDAC, HDAC 6, & SIRT), acetylation(HAT's), and DNA methylation (DNMT-1) were measured using kits purchased from Epigentek.

Results:

HDAC and HDAC 6

- The ischemic hemisphere had a greater amount of HDAC 6, as well as higher levels of HDAC activity.
- Studies have shown that HDAC's are increased in the cortex following ischemia, and that cortical neurons from HDAC 6 knock down animals were protected from oxygen and glucose deprivation (Chen et al. 2012).
- Elevated HDAC activity in the ischemic cortex is thus consistent with the increased cell death in this region.

SIRT

- Middle-aged males had lower SIRT activity levels than adult males.
- Previous studies have shown decreased levels of SIRT1 in the hippocampus during aging (Quintas et al. 2012).

DNMT1

- Middle-aged females had greater amounts of DNMT1 than adult females in both hemispheres.
- Previous studies have shown that mice with reduced levels of DNMT1 are protected from cerebral ischemia and that treatment with a DNMT inhibitor in wild-type animals resulted in protection (Endres et al. 2001, 2000).

The early epigenetic changes observed in the current study may provide insights into the age and sex differences in infarct severity previously observed five days after stroke.

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Effect of Erk1/2 on Muscle Fiber Morphology and Differentiation

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Introduction:

Fiber Types: Muscle fibers can be classified into two distinct types. Type 1 fibers, also called slow fibers, are characterized by small size and great resistance to fatigue due to rich myoglobin content and mitochondria. Type 1 fibers contract slowly and do not generate much force. Type 2 fibers can be subdivided into three separate categories. Type 2B fibers are generally the largest in size and can generate a great amount of force, but due to low mitochondrial count, they fatigue easily. Type 2A units are an intermediary between slow and 2B, and 2X fibers fall midway between 2A and 2B.

Extracellular-signal Regulated Kinase: Extracellular-signal regulated kinase 1 and 2 (Erk1/2) are part of a signaling cascade involved in many different cellular processes. Erk1/2 can act as an inhibitor or an activator during skeletal muscle differentiation. It has also been implicated as playing a role in the regulation of fiber type differentiation. However, literature reports conflicting results regarding the need of Erk1/2 signaling for slow or fast fiber differentiation.

Animal Model: C57BL/6 mice were used to generate a variety of genotypes: total *Erk1* knockouts (*Erk1*$^{-/-}$), conditional *Erk2* knockouts in skeletal muscle (*Cre*$^{+}$*Erk2*$^{f/f}$), and a double knockout cross between these two (*Cre*$^{+}$*Erk1*$^{-/-}$*Erk2*$^{f/f}$). *Cre*$^{-}$ siblings were used as controls.

Hypothesis: Elimination of Erk1/2 (separately and concurrently) will affect muscle fiber morphology and development.

Methods:

Fiber-typing: Cross-sections of sternomastoid (STN) and tibialis anterior (TA) muscles from 14-week-old mice (n=3 per genotype) were subject to immunohistochemical staining with myosin 2B antibody (undiluted) or myosin slow antibody (undiluted) plus dystrophin (1:300). Images were taken to encompass the entire area of a single whole muscle section with overlapping myofibers and then compiled into a single picture. Total fibers were counted with dystrophin, and unstained fibers were counted on the color merge. The number and percentage of stained fibers were calculated from these values.

Fiber Area: The dystrophin-stained images for control and *Cre*$^{+}$*Erk1*$^{-/-}$*Erk2*$^{f/f}$ from the above-described compilations were analyzed for myofiber area using the integrated morphometric analysis tool in MetaMorph. The entire section was analyzed and myofibers overlapped by multiple images were only analyzed a single time.

Results:

Fast 2B Fibers: The 2B fiber type comprises 50% or more of the total fibers in both STN and TA for all genotypes and are visually observed to be larger than unstained (non-2B) fibers. No statistical significance was found between the controls and various mutants. Loss of Erk1/2 does not seem to have a major effect on 2B expression.

Slow Fibers: Neither STN nor TA has many slow fibers. In STN there is a slight reduction in slow fibers in all mutant genotypes as compared to control with significance observed for *Erk1*$^{-/-}$ only. On the other hand, there is an increase in slow fibers in all mutant genotypes with significance in *Cre*$^{+}$*Erk1*$^{-/-}$*Erk2*$^{f/f}$ as compared to control in the TA. While loss of Erk1/2 has some impact on slow fibers, these are such a small portion of the total that it likely does not account for any of the observed phenotypic changes in these animals.
Area: There is an observed shift in distribution in myofiber area between the control and *Cre*$^{+}$*Erk1*$^{-/-}$*Erk2*$^{f/f}$, in which *Cre*$^{+}$*Erk1*$^{-/-}$*Erk2*$^{f/f}$ has a decrease in large fibers and an increase in small fibers as compared to the control. Together with total fiber count, this likely contributes to both the smaller muscle size and lower observed weights.

Future Directions: Future work will include continuing fiber type analysis on 2A and 2X in STN and TA of the four genotypes and looking for correlation to observed neuromuscular junction phenotypes (data not shown). The necessity of Erk1/2 signaling for slow fiber differentiation will be studied in the soleus muscle.

Fibroblast-Endothelial Cell Interactions in the Heart

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Introduction: Normal cardiac function depends on interactions between many types of cells, including fibroblasts, endothelial cells, myocytes, and components of the extracellular matrix (ECM). Each of these cells communicates in multiple ways, whether it be via chemical signals, mechanical signals or electrical signals. It is dynamic interactions between different cell types that allow for proper form and function of the heart. These interactions are able to maintain the vasculature in the normal heart and allow for vascular remodeling following cardiac injury. Our goal is to understand these cell-cell interactions and how they are altered in the disease state. When we better understand the signaling that takes place in the heart, we should be able to stimulate angiogenesis in the pathological heart, which will provide therapeutic remedies to those with heart disease. Specifically, we have been looking at the role of microRNAs (miRNAs), and their mode of transfer between cells, as well as their effect on angiogenesis.

Hypothesis: We hypothesize that small signaling proteins or miRNAs can pass freely through gap junctions that link fibroblasts and endothelial cells (ECs). Our initial studies have focused on identifying miRNAs involved in angiogenesis that may pass between cells. After identifying Let-7F as an angiogenic signaling molecule, we will continue to study the effects of its over- and under-expression.

Methods: Many different methods were employed, including cell culture of ECs and fibroblasts, co-cultures between these two cell types, DNA extraction and purification, cloning and transformation of E. Coli, transfection of neonatal rat cells with our engineered Let-7F DNA, cell adhesion assays, cell aggregation assays, and 3-D collagen tube formation assays.

Results: We have demonstrated that fibroblast-EC interactions involve N-cadherin, as well as other cell surface molecules. In tube formation assays, co-culturing ECs with fibroblasts results in enhanced vessel formation, while disruption of these interactions results in decreased tube formation. Moreover, our studies involved IL-6 and vascular endothelial growth factor (VEGF). These changes in IL-6 and VEGF expression require direct cell-cell interactions. We have previously shown that fibroblasts and ECs can exchange intracellular material through tight gap junctions. Indeed, we have demonstrated that the miRNA, Let-7F, is able to pass between fibroblasts and ECs and may play a key role in vascular remodeling in the heart. We will continue to characterize the exchange of intracellular materials between cells and examine their roles in the vascular remodeling process following cardiac injury.

Role of the brain bile acid system in TBI-induced pathology

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Introduction: Traumatic brain injury (TBI) is a condition which may bring about varied pathology. Despite the fact that millions of people suffer from TBI each year, biomarkers and therapeutic options are unavailable. This is likely due to the lack of a mechanistic understanding of the associated pathology. The majority of TBI research focuses on the direct effects on the brain. Less studied are the systemic contributions which may contribute to TBI related pathology. The hepatic-neuroimmune axis is involved in the response to infection and injury. There is scant evidence of hepatic alterations following TBI. Considering this, further examination of potential hepatic mechanisms to TBI is warranted. Our preliminary data show that in the liver, TBI induces the acute phase response, inflammation, and bile acid system changes. Interestingly, the brain also contains bile acid machinery that includes enzymes, receptors and transporters. Previous studies of hepatic dysfunction in non-TBI models have implicated the brain bile acid system in cognitive dysfunction. Thus, alterations to the brain bile acid system could also contribute to TBI-related pathology. In this study, we explore the impact of TBI on the bile acid system in the brain.

Hypothesis: We hypothesize that TBI induces changes to bile acid machinery in the brain, and that modulating this system will improve functional outcomes.

Specific Aims: Aim 1: Assess alterations to bile acid receptors and transporters in various brain regions using western blot. Aim 2: Determine cell-specific expression of bile acid receptors and transporters in the brain using immunocytochemistry

Methods: We utilize a recognized rodent model of TBI known as fluid percussion injury (FPI), which yields quantifiable measures of cognitive and neuropathological dysfunction and has been established in our lab. Tissue samples were harvested at 6h, 24h, 3d, and 7d post-FPI and used for western blot analysis of three bile acid system proteins: FXR, ASBT, and OATP. The cerebral cortex, hippocampus, hypothalamus, piriform cortex, and cerebellum (control) are regions of interest.

For immunocytochemistry, the cortex and hypothalamus were examined for FXR and ASBT, respectively. Fixed sections were incubated in primary antibodies for 24h at RT. Following PBS rinses, tissue was incubated in secondary fluorescent-conjugated antibodies. In serial sections, tissue was double-labeled for neuronal (NeuN) and glial (GFAP) markers to determine which cells also label for the bile acid proteins.

Results: The results show neuronal and glial expression of bile acid proteins, and indicate alterations to the brain bile acid system following TBI.

Mechanosensor Regulation of Contractile Function in Cardiac Myocytes

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Introduction: Heart failure is the leading cause of morbidity and mortality in the Veteran population, which is characterized by reduced left ventricular (LV) calcium (Ca^{2+}) uptake in the sarcoplasmic reticulum (SR), decreased contractility and prolonged relaxation time. A common feature of heart failure is the dysregulation of calcium cycling in cardiac myocytes, in which the peak systolic calcium transient is diminished and the intracellular Ca^{2+} concentration is increased. A key factor in these observations is decreased SR calcium content. Interestingly, hypo-phosphorylation of phospholamban (PLB) has been observed in both human and animal models of heart failure, predicting a more pronounced inhibition of sarcoplasmic reticulum Ca-ATPase (SERCA2a) activity, resulting in the decrease in SR calcium concentration. Increased PLB activity would lead to diastolic and systolic dysfunction, as less Ca_i^{2+} is available for subsequent contraction.

Hypothesis: That p38 and Akt serve as important regulators of intracellular Ca^{2+} and Na^{+} in cardiac myocytes.

Methods: These studies involved combining the use of primary cultures of neonatal rat cardiac myocytes (NRVM) and papillary muscles, which were used to connect the signaling pathways, activated by mechanosensors, to intracellular calcium and sodium mobilization, as well as mechanical function. This work employed the use of pharmacologic inhibitors for p38 (1 μM SB580), Akt (100 nM PF), and JNK (1 μM SP190) to determine the role of p38, Akt and JNK on intracellular calcium and sodium ion concentrations in both NRVM and papillary muscles. Intracellular calcium and sodium ion concentrations were obtained in paced (1 Hz) NRVM using an IonOptix fluorescent microscopic imaging system, whereas contractile responses in paced papillary muscles will be measured using an Aurora small-muscle contraction system.

Results: Intracellular calcium and sodium measurements obtained in NRVM indicate that both p38 and Akt are important for preventing increases in intracellular calcium, whereas JNK was an important for preventing decreases in intracellular calcium. Although the changes in intracellular sodium paralleled that of intracellular calcium, these changes appear to be compensatory.

Conclusions: The stress-activated protein kinases p38 and JNK have differential roles in the regulation of intracellular calcium, in which p38 has actions which mirror that of Akt. Future studies will determine whether p38 may be an upstream regulator of Akt.

TAMHSC SUMMER RESEARCH PROGRAM

August 7, 2013: 9:00 AM - 4:00 PM
Health Professions Education Building
Bryan, Texas

Smooth Muscle γ-Actin Expression in Prostate Epithelia

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Introduction: Prostate cancer is one of the most pronounced, non-cutaneous malignancies diagnosed in men. In 2010, over 200,000 men were diagnosed with prostate cancer in the United States. Studies have shown there are many potential drivers of the malignant cells causing prostate cancer; for example, NKx3.1 acts as a vital regulator towards prostate cancer development. Among its many activities NKx3.1 also contributes to the regulation of smooth muscle gamma actin (SMGA) in prostate cancer cells.

Previous studies have shown that SMGA transcripts are expressed in normal and cancerous prostate cells. Curiously, SMGA protein is not apparent in the prostate epithelia. Since, SMGA mRNAs are expressed in the prostate epithelial, **the aim of this study is to investigate why SMGA protein not expressed in the prostate epithelia**. Preliminary studies have shown that SMGA specific antibody clone B4 cannot identify SMGA protein in prostate epithelia. Therefore, we designed a study and hypothesized that **(A)** SMGA protein is present in prostate epithelium, however, not recognizable because of a fold in the protein structure that makes it unrecognizable by actin specific protein and **(B)** that the presence of a competitive inhibitor binds to SMGA protein in prostate epithelium.

To prove our hypothesis, we tested different antibodies which are actin-specific using PC-3 prostate cancer cells. These cells were obtained from a bone metastasis of a grade IV prostatic adenocarcinoma (ATCC CRL-1435). The PC-3 cells were cultured with F-12K media for about 3 weeks and lysate was prepared from cells. Western blot analysis was carried out using actin-specific antibody to bind actin within the PC-3 lysate. Purified actin was used as the control to prove that the antibody bounded to actin. Actin was then immunoprecipitated from the lysate and cut out from the gel for proteomics analysis.

Hypothesis: SMGA protein is present in prostate epithelium, however, not recognizable because of a change in the protein structure that makes it unrecognizable by actin specific antibody.

Methods: To test our hypothesis, immunoprecipitation, western blot and tandem mass spectrometry were carried out. Western blot was used to test the efficacy of the actin specific antibody HUC1-1 and to prove that actin was present in the prostate cancer cell line.

Immunoprecipitation is a technique used to identify and is a purification method for a specific protein of interest. Smooth muscle gamma actin was immunoprecipitated from lysate using the muscle specific HUC 1-1 antibody bound to magnetic beads.

For Tandem Mass Spectrometry the band near 43 kDa was cut out of the gel and separately prepared in solution with an arg-lys protease. The digested peptides were isolated by a mass spectrometer, measuring their mass to charge ratio.

Results: Based on our result from western blot, actin was expressed in the PC3 lysate, thus HUC1-1 antibody was effective in binding to actin. Also, Actin was immunoprecipitated from lysate and was seen at 43kDA. Our results for mass spectrometry are still under analysis. We hope to gain understanding on why there is SMGA mRNA expressed in the prostate epithelial but SMGA observed in mature smooth muscle cells, is not observed in the prostate epithelia.

Nuclear Receptor RARα-Mediated Regulation of Cardiac Remodeling in Diabetes

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Introduction: Diabetes cardiomyopathy (DCM) is one of the leading causes of increased morbidity and mortality in patients with diabetes. Activation of the retinoic acid receptor (RAR) and retinoid X receptor (RXR) has an anti-diabetic effect; but, the role in cardiac remodeling remains unclear. Recently, we reported that activation of RARα-mediated signaling ameliorates hyperglycemia-induced cardiac remodeling, through regulating oxidative stress/apoptosis, hypertrophy, cardiomyocyte metabolism and fibrosis. We also observed that expression and transcriptional activation of RARα is significantly suppressed in the hearts of Zucker Diabetic Fatty (ZDF) rats. Therefore, elucidating the molecular mechanisms of RARα deletion-induced cardiac dysfunction in diabetes will result in predicting the structural and functional cardiac consequences and in developing therapeutic approaches for the treatment of diabetic cardiomyopathy.

Hypothesis: Impaired RARα signaling induces diastolic heart failure and diabetic cardiomyopathy, through promoting hypertrophy, oxidative stress, ROS generation and disrupting cardiac glucose/lipid homeostasis.

Methods: We utilized the Cre/loxP conditional cardiac gene deletion mice model. The cardiac RARα gene deletion was achieved by injecting tamoxifen (0.5 mg/mice/day) in mice for 4 days. Type I diabetes was induced through the destruction of pancreatic beta cells by STZ (steptozotocin; 250 mg total/mice). The cardiac specific conditional RARα knockout male mice (6 wks old) and age matched wild type (WT) controls were randomized into 5 groups: Cre (+); Cre (-) with tamoxifen; Cre (+) with tamoxifen; Cre (+) with oil + STZ (streptozotocin) and Cre (+) with tamoxifen + STZ group. A significant reduction in protein expression of RARα was observed in RARα knockout mice (RARαKO) heart as compared to WT mice. Cardiac tissue samples were isolated at 16 wks post-STZ injection and stored at -80°C. Echocardiography was performed to monitor cardiac function at 0, 4, 8, 12, 16 weeks. Analysis for apoptotic and oxidative stress marker proteins were performed by Western blotting. The cardiac hypertrophic, glucose and lipid metabolic gene expression was analyzed by real time RT-PCR using GAPDH as internal control. Dihydroethidium (DHE) staining was used to measure intracellular reactive oxygen species (iROS) generation in the myocardium.

Results: Echocardiographic data showed a decreased E/A ratio in RARαKO mice at 16 weeks (wks) with an early decrease (8 wks) of ratio in the diabetic RARαKO mice group, as compared to WT animals. These data suggest that diastolic dysfunction is a characteristic of RARα gene deleted mice and also impairment of RARα-mediated signaling exacerbates the development of diastolic dysfunction in the diabetic animal model. Further, a significant increase in the hypertrophic gene expression of ANP and BNP was observed in RARαKO mice heart which was significantly higher in the diabetic RARαKO myocardium, suggesting that lack of RARα mediated signaling promotes cardiac hypertrophic changes. Increase in oxidative stress and ROS generation was observed in RARαKO mice heart; which increased further under diabetes. Dysregulation of glucose and lipid handling was also observed in the myocardium of the RARαKO mice heart.

Conclusions: 1) Lack of RARα-mediated signaling induces cardiac remodeling through oxidative stress induced apoptosis, hypertrophy and dysregulation of cardiac metabolism.

2) Lack of RARα-mediated signaling accelerates diabetes-induced cardiac remodeling.

Effect of bile acid treatment on hypothalamic-pituitary-adrenal axis in mouse hypothalamic neurons

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The HPA axis describes a complex set of positive and negative influences between the hypothalamus, the pituitary gland and the adrenal gland. It forms a major part of the neuroendocrine system that controls the body's reaction to stress, and has regulatory influences on digestion, the immune system and mood and behavior. The goal of this project was to assess the effects of bile acids on HPA axis activity. During cholestasis there is an increased accumulation of bile acids in the liver and a spillover of bile acids into the systemic circulation. These elevated serum bile acids levels have been associated with hepatotoxicity, hepatic fibrosis, cardiomyopathy, and vasodilation. In this study, we assess the effects of bile acid signaling in the regulation of the HPA axis by determining an appropriate concentration for bile acid treatment of M-hypo A-21 and M-hypo A-29 neurons. We also look at the effect of bile acid administration on corticotropin releasing hormone (CRH) expression and secretion from hypothalamic neurons. We treated confluent M-hypo A-21 and M-hypo A-29 neurons with varying concentrations of bile acids (10μM, 1μM, 100nM, 10nM and 1nM). We find that glucocorticoid receptor is localized to the nucleus in M-hypo A-21 cells after treatment with bile acids. Additionally relative expression levels of CRH receptor 1, CRH receptor 2, and CRH binding protein levels are decreased after taurocholic acid (TCA) treatment. This indicates a suppression of the HPA axis in mouse hypothalamic neurons after bile acid treatment.

Development of an Operating Room Fire Prevention Device

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Background: Operating room (OR) fires present a real danger to surgical patients and occur at least as frequently as wrong-sided surgery. There is intense attention from the Anesthesia Patient Safety Foundation, the Joint Commission, the FDA, and others in reducing this risk. For fire to occur, the three points of the fire triad must be present: an oxygen source, ignition source, and fuel source. The electrosurgical unit (ESU) pencil triggers the vast majority of OR fires. Carbon dioxide (CO_2) is a gas proven to prevent ignition and suppress fire by displacing oxygen. We hypothesize that a device can be created that generates a protective cone of CO_2 gas around an ESU pencil tip and effectively mitigates OR fire risk.

Methods: A divergent nozzle with an inner diameter of 1.16 cm was fashioned from medical grade plastic. This portion of the device was then connected to a CO_2 source via silicon tubing, and then secured to an ESU pencil. Carbon dioxide was piped through the nozzle in order to create a fire prevention cone around the ESU pencil tip. The device was then tested in a flammability testing chamber in which the ESU pencil was activated for sustained current delivery to an aluminum test plate holding a laparotomy sponge. The ESU was activated at 50W cut mode for 30 seconds or until the sponge ignited. The tests were conducted in 21%, 50%, and 100% oxygen environments. Each test was performed five times with the device turned on (CO_2 flow at 8 L/min) and with the device turned off (control). Time to ignition was measured with high speed videography.

Results: The median $\pm$ SD [range] ignition time of the control group in 21% oxygen was 2.9s $\pm$ 0.44 [2.3 – 3.0], in 50% oxygen 0.58s $\pm$ 0.12 [0.47 – 0.73], and in 100% oxygen 0.48s $\pm$ 0.50 [0.03 – 1.27]. No fire was observed when CO_2 was applied in all concentrations of oxygen.

Discussion: Though recommendations, education, and regulation have raised awareness of OR fires in recent years, a recent study reported an increase in ESU-triggered OR fire claims over the last decade. Because the heightened awareness has not yet translated into the elimination of OR fires, there is a need to address the problem in another way. Most OR fires occur during operations on the head and neck due to the proximity to open oxygen sources. Our prototype device used an inexpensive, routinely available and medically safe gas - CO_2 - to displace this oxygen away from the active tip of the ESU electrode, removing one leg of the fire triad. By doing so, our device effectively prevented ignition by an ESU pencil in a highly flammable in-vitro model of fire. Further testing and development to optimize device shape and characteristics along with optimal CO_2 flow rates is justified. Future implementation of such a CO_2 fire prevention device may effectively reduce the risk of OR fires.

Evaluation of Models and New Treatments for Prostate Cancer

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Introduction: Traditional therapy for prostate cancer has utilized systemic agents, surgical removal of the prostate, and ultrasound guided injections of radioactive seeds or chemicals for tissue ablation. Alternative treatment of focal lesions limited to the prostate would be to utilize directed injections. Previous attempts, using an ex vivo human prostate model demonstrated heterogeneity of the aging prostate and diversion of infused solution through ducts to the urethra or back along the needle to the capsule, reducing potential efficacy. This project focused on evaluating the literature for technical improvements to the delivery method and consideration of in vivo models in nonhuman primates to add to the ex vivo evaluation using human prostates prior to conducting patient trials.

Hypothesis: In theory, a novel microporous hollow fiber catheter currently used experimentally for brain infusions could improve distribution of agents within the prostate, and certain species of nonhuman primates could serve for trials of effects of direct injections of prostate specific agents.

Methods: A literature review was performed using Medline, Google Scholar, and interviews, to obtain information about prostate specific agents of use for focal treatment, about imaging modalities that could guide injection devices, about local experience with a microporous injection and infusion system, and about nonhuman primate models, especially smaller species that could be easily handled and evaluated serially using imaging technologies

Results: The review identified several prostate cell specific toxins with potential for local administration. One used the enzymatic activity of prostate specific antigen, a serine protease, to release a pore for incorporation into cells leading to their demise. Others include agents that result in gene silencing and apoptosis or interference with mitochondria within prostate cells. These agents have the potential to act locally and focally. Because previous experience using ex vivo models with needles to deliver prostate cancer treatments resulted in heterogeneous distribution of agents with substantial leakage, an alternative device was tested. This novel microporous hollow fiber catheter in two pilot trials produced wide and even areas of diffusion with minimal reflux. The system could be monitored using magnetic resonance imaging, which suggests the possibility of using it to deliver focal therapy directly to the tumor sites. Males with potential for prostate cancer were evaluated using MR imaging procedures. Of 6 cases, 5 had sites identified by MRI that were potential tumors. In four of the these, the lesions appeared confined to the prostate, so that locally administered treatments could be of used. 3-D models developed from tracings of pelvic structures in these MR images were used to plan pathways for device passage to sites to show feasibility of this approach.

A literature search revealed that several large species of nonhuman primates (baboons, rhesus macaques, and Macaca fasicularis) have been used for prostate evaluations. These species are large and and present some challenges as an animal model. MRI has been used in the baboon for prostate measurements, but no work has been reported on smaller New World species. Literature from over three decades ago showed that squirrel monkey males developed both adenocarcinoma of the prostate and benign prostatic hypertrophy. However, no imaging information about this species was available. MRI using 3T Trios system with a wrist coil was conducted, and two independent reviewers traced prostate, seminal vesicles, and testes in older male monkeys. The tracings were used to generate 3-D models with 3-D Doctor software. Similar procedures were used for six humans undergoing similar imaging procedures. Squirrel monkey prostates measured 0.3705 +/- 0.0601 mL and 0.375+/-0.0566. Prostates of the six patients ranged in volume from 20.4 to 60.3 mL with CVs less than 10% for two reviewers.

Conclusions: MRI provides the potential to evaluate both animal models and patients serial during the course of treatment for prostate cancer. MRI methods allow measurement of prostate volume, tissue characteristics, and planning of pathways for placement of delivery devices to lesion sites. Future studies will further evaluate methods of prostate measure in the small nonhuman primate as well as potential for directing treatment devices to lesion sites in ex vivo prostates.

Mapping the Concentration of Carbon Dioxide To Prevent Operating Room Fires

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Introduction: Studies have estimated that operating room (OR) fires occur approximately 600 times a year. For a fire to occur in the operating room, an ignition source, fuel source, and oxygen source must be present. If one of these sources is displaced, a fire cannot occur. A nozzle device was created in order to combat OR fires by displacing the oxygen source with carbon dioxide. One test to see if the nozzle device will prevent OR fires is to map the CO_2 concentration as the gas is being propelled through the nozzle.

Hypothesis: Carbon dioxide (CO_2) mapping can be done to determine if a sufficient amount of CO_2 is being propelled through the nozzle device to displace enough oxygen to prevent OR fires.

Methods: The nozzle device was attached to an electrosurgical pencil and positioned in an upright position. A matrix was fashioned to mark points in 3D space. The nozzle device was positioned in the center of the matrix. Carbon dioxide was expelled through the device at a flow rate of 8 L/min. A high concentration carbon dioxide analyzer was used to obtain the data. The data points were then translated into a 4D-mapping program to obtain the carbon dioxide concentration graphs.

Results: Operating room fires have become a recent focus from many organizations such as the Joint Commission. Regardless of the articles published, OR fires have continued to occur. This prompts the need to address the problem in another way. Previous studies have found that if the oxygen concentration falls below 50%, the risk of fire will greatly decrease.[3] The three dimensional contour map demonstrates that enough CO_2 is being propelled through the nozzle device to displace oxygen, preventing operating room fires.

Dissecting the Stromal Signals in Pancreatic Ductal Adenocarcinoma

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Introduction: Pancreatic ductal adenocarcinoma (PDA) is an aggressive, often lethal exocrine cancer characterized by its high metastatic potential and lack of effective therapies. PDA is also characterized by the desmoplasia and predominant stromal fibroblast components. The contributions of stromal cells to PDA pathogenesis, metastases and drug resistance are, however, poorly understood. We recently identified a pro-metastasis, PDA-associated antigen, ANXA2. Our published study demonstrated that tyrosine 23 (Tyr23) phosphorylation-dependent cell-surface translocation of ANXA2 is critical for the invasion, metastasis, and epithelial to mesenchymal transition (EMT) process in PDA. The role of ANXA2 in metastasis is further supported by our recent unpublished data showing when ANXA2 is knocked out (KPCxANXA2-/- mice), a genetically engineered PDA model with knock-in alleles of both *Kras*G12D and *p53R172H* mutants (KPC mice) develop primary PDA but do not form metastasis.

Hypothesis: We hypothesize that paracrine stromal signals are critical for activating intracellular effectors in PDAs that culminate in Tyr23 phosphorylation of ANXA2. In particular, we hypothesize that two paracrine stromal-to-epithelial axes - the Hedgehog-IGF-1 receptor and hepatocyte growth factor (HGF)/c-met signaling pathways - form a functional link between PDA stromal and epithelial compartments, resulting in ANXA2 phosphorylation and EMT.

Methods: We have used two mouse PDA models to evaluate the role of the Hh and HGF/c-met signaling pathways in the development of spontaneous primary tumors and in mediating ANXA2-dependent metastases *in vivo,* respectively. The first is the KPC mice. The second is a liver metastases model, where PDA cells are injected into the splenic bed, followed by hemisplenectomy to establish liver metastases via a natural route. Small molecular inhibitors under clinical development for targeting Hh and HGF pathways were used in pilot experiments, where nine KPC and 12 liver metastasis mice were treated with either HGF/c-met inhibitor, Hh inhibitor, or combination of HGF and Hh inhibitors for 7 days via oral gavage. The sizes of the largest measurable tumors (index tumor) in the pancreas or liver were monitored by our Vevo770 ultrasound prior to the treatments, on day 4 of the treatment course, and at the end of the treatment course. Immunohistochemistry (IHC) staining to evaluate phosphorylated ANXA2, stromal fibroblast maker (alpha smooth muscle antigen, SMA), EMT markers (E-cadherin and vimentin) were developed for this study. Mice were euthanized 6 hours following the last treatment and tumors were harvested for anti-phosphorylated ANXA2, anti-E-cadherin, anti-vimentin and anti-SMA IHC.

Results: The results of the pilot experiments have supported the feasibility of testing our hypothesis in vivo in these animal models. More KPC and liver metastasis mice will be studied to further explore the optimal combination of clinical inhibitors targeting stromal signaling and standard chemotherapy targeting neoplastic cells for the treatment of pancreatic cancer.

Complementation of Escheria Coli Tat Pathway by Mycobacterium Tuberculosis Tat Pathway

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Introduction: Tuberculosis (Tb) is one of the leading causes of death in third world countries. Although there are significant improvements globally to control Tb, the burden still remains enormous. *Mycobacterium tuberculosis* (MTb) is the causative agent of Tb. To develop drugs and vaccines against Tb, it is important to understand the functionalities of various components of MTb. This is quite difficult to do, however, since MTb is infectious and grows extremely slowly. It has been shown that MTb **T**win **A**rginine **T**ranslocation (Tat) Pathway is essential for survival. Thus, we have chosen to express the MTb Tat genes in *E. coli* to determine if mechanistic studies can be performed using a more tractable model organism.

Methods: The incorporation of MTb Tat proteins into the *E. coli* inner membrane was determined by Western blot analysis against MTb-TatA, -TatB and -TatC inverted membrane vesicles (IMVs) derived from the inner membranes of *E. coli,* which were obtained as described (Bageshwar and Musser, 2007, J. Cell Biol. 179: 87-99). The morphology of *E. coli* stains in the absence and presence of the MTb Tat pathway was examined using light microscopy. SDS and EDTA sensitivity assays were used to determine the functional complementation of the *E. coli* Tat pathway by the MTb Tat pathway, as described earlier (Mickael et al, 2010, Infect. Immun. 79: 3495-505).

Results: The Western blot analyses show that the MTb TatA, TatB and TatC proteins were successfully incorporated into the *E. coli* inner membrane. The microscopy results reveal that the MTb Tat pathway is functional in *E. coli* because most of the *E. coli* cells (ΔTatABCDE) were converted to single or double cells upon expression of MTb Tat pathway indicating that the MTb Tat pathway at least partially complements the *E. coli* Tat system. Most control cells had a chain-like structure, characteristic of Tat^- strains. Additionally, in the presence of the MTb Tat pathway, *E. coli* was found to be tolerant to higher concentrations of SDS and EDTA. The IMVs obtained from *E. coli* expressing the MTb Tat pathway consistently bound a larger amount of an authentic Tat precursor pre-SufI. However, we were not successful in demonstrating in vitro translocation of pre-SufI using the IMVs obtained from *E. coli* expressing the MTb Tat pathway.

All-trans retinoic acid inhibits high glucose-induced fibrosis through regulation of NF-κB signaling

An Epidemiological Study of MS: A possible cluster

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Abatract: Multiple Sclerosis (MS) is an inflammatory, demyelinating, neurodegenerative disease affecting the central nervous system (CNS) [1]. MS is characterized by the presence of focal inflammatory lesions in the CNS, caused by the immune system targeting myelin related agents [2]. Environmental factors are thought to contribute to the development and progression of MS. A high prevalence of MS was noted in a small town in central Texas (9 cases out of a population of 1123). Interestingly, none of the patients had a family history of the disease, suggesting that an environmental factor may be an important contributing factor. Other studies have indicated that exposure to bovines may lead to the development of MS. This study focuses on environmental factors and the role cattle may play in the development of MS in rural Texas. Soil and water samples were taken from the area and shown to have high levels of Manganese (Mn). At low levels, Mn is known to cause neurological disorders, mainly through the immune response in the CNS. The high levels of Mn present in the samples obtained could cause toxicity via inhalation or ingestion.

B Cell Activity in Response to a Microbiota Metabolite

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Introduction and Hypothesis:

The intestinal tract is home to a vast number of commensal microbes that are collectively termed the microbiota. Under normal conditions the microbiota live in symbiosis with the host. We hypothesize that under these symbiotic conditions the microbiota produce beneficial compounds that promote homeostasis. Published work from our lab has shown indole to down regulate various TNF induced proinflammatory cytokines in intestinal epithelial cells while also increasing the integrity of the intestinal epithelial cell barrier upon antigen stimulation. However, given the natural abundance of indole in the gastrointestinal tract, multiple cell types are likely continuously exposed to indole in vivo. Here we propose that indole may modulate mucosal B-cell IgM and IgA secretion. To our knowledge the effect of indole on B cells has not been investigated. Signals found in the GI tract (dietary derived retinoic acid (RA), and TGFb) induce IgA - the most abundant immunoglobulin at mucosal surfaces and responsible for neutralizing toxins and pathogens at the mucosal barrier while keeping a sufficient binding affinity profile towards commensal bacteria to prevent dysbiosis.

Methods:

To test B-cell function, total splenocytes were harvested from C57BL/6 mice, and processed to single cell suspension.

To determine immunoglobulin production, cells were stimulated with LPS, conditioned ± indole, ± retinoic acid, and ± TGFβ. After seven days supernatants were harvested. ELISA procedure was performed to determine concentrations of secreted IgA and IgM.

To determine B-cell proliferation we used CFSE staining and flow cytometry analysis. First, cells were stained with CFSE then activated with LPS and conditioned ± indole for three days. Cells were then stained with a monoclonal antibody targeting B220, a surface marker found on B cells and flow cytometric analysis was performed.

Results:

Our data suggests that indole has an anti proliferative effect on LPS stimulated B cells after three days of culture. The effects of indole were dose responsive between 1mM down to 0.25mM. Interestingly, the supernatants of indole conditioned splenocytes had decreased concentrations of IgM and there was a synergistic effect with the addition of retinoic acid.

The Effects of Estradiol and nonylphenol on Breast Cancer Cells

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Introduction: Over the course of a lifetime, 1 in 8 women will be diagnosed with breast cancer. Estrogen receptor (ER) belongs to the nuclear receptor subfamily 3A (NR3A) and it binds to its natural ligand estrogens such as 17β-estradiol (E2) and ER-mediated responses play an important role in the development and growth of breast cancer cells and tumors. ER-negative breast cancers are difficult to treat, whereas ER-expressing breast tumors can be effectively treated with endocrine therapies using anti-estrogenic compounds such as tamoxifen or aromatase inhibitors. In this study we investigated the effects of natural E2 and a synthetic estrogenic compound (nonylphenol) in ER+ and ER- breast cancer cells and observed no significant growth promoting effects by these compounds in MDA-MB-231 and MCF-7 breast cancer cell lines. However, both E2 and nonylphenol activated ER in a transactivation assay in MCF-7 cells. The detailed mechanism of action of ER- mediated effects in breast cancer cells are currently being investigated.

Hypothesis: The purpose of this study is to investigate the effects of E2 and nonylphenol on the proliferation and estrogenic response in ER+ and ER- breast cancer cells.

Methods: Cell lines and maintenance: MDA-MB-231 and MCF-7 cell lines were purchase from American Type Culture Collection (ATCC) and maintained in Dulbecco's modified Eagle's medium (DMEM) nutrient mixture with Ham's F-12 (DMEM/Ham's F-12; Sigma–Aldrich, St Louis, MO) supplemented with 5% fetal bovine serum (FBS), 0.22% sodium bicarbonate and 10 mL/L 100× antibiotic antimycotic solution (Invitrogen, Carlsbad, CA). Cells were maintained at 37 °C in the presence of 5% CO_2 and the solvent [dimethyl sulfoxide (DMSO)] used in the experiments was ≤0.1%.

Cell proliferation assay: Breast cancer cells (2.5×10^4) were seeded on 12-well plates and allowed to attach for 24 hr. The cells were treated with the indicated compounds at different concentrations or solvent control (DMSO). Cells were then trypsinized and counted at 24, 48, and 72 hr using a Coulter Z1 particle counter. Results were expressed as means ± SD for at least three independent determinations for each treatment group.

Transfection and luciferase assay: Breast cancer cells were seeded on 12–well plates and allowed to attach for 16 hr. The cells were transfected with 800 ng ERE_{x3}-Luc and 80 ng hER plasmids using Lipofectamine 2000 agent according to the manufacturer's protocol. The transfection mix was replaced by treatments containing either the indicated compounds at different concentrations or DMSO after 5 hr. After 18 hr, cells were lysed and the cell extracts were used for luciferase and Bradford assays. Luciferase activity signals was normalized to protein concentrations and were expressed as means ± SD for at least three independent determinations for each treatment group.

Results: Both natural estrogen (E2) and synthetic (nonylphenol) estrogenic compounds were investigated in MDA-MB-231 and MCF-7 breast cancer cells. There was no significant growth promoting effect observed for either compound in this study, however there was considerable induction of ER-mediated transactivation in MCF-7 cells.

A Search for a Universal Fibrinogen-binding Motif

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Introduction: Staphylococcus aureus produces numerous fibrinogen-binding proteins to facilitate its pathogenesis. These extracellular proteins include Extracellular fibrinogen-binding protein (Efb) and Coagulase (Coa). Efb binds to fibrinogen to prevent neutrophils and other leukocytes from interacting with fibrinogen. Efb hinders the function of the innate immune system by binding to complement C3 convertase, which serves multiple functions including opsonization and cell lysis of the bacteria. Efb also binds to C5a and prevents it from recruiting neutrophils. Coa assists the virulence of S. *aureus* by binding to fibrinogen and forming an abscess that shields the bacteria from the host immune system. The Hook lab has recently identified a conserved amino acid sequence between Efb and Coa using the alanine scanning protocol. This consensus sequence presents the amino acids essential for the function of Efb and Coa. The segment of amino acids found, leads us to question whether there is a universal motif that identifies bacterial proteins that bind to fibrinogen. If there is a universal motif, scientists and clinicians could take a more proactive and preventative approach to dealing with S. aureus and predict infections from other bacteria with similar pathogenesis.

Hypothesis: The motif derived from the consensus sequence between Extracellular binding protein and Coagulase represents a universal motif that can identify bacteria that are capable of binding to fibrinogen.

Methods: The blast.ncbi.nlm.nih.gov database was used to search for proteins in other bacteria that have a match to the motif. Clustal Omega multiple sequence alignment tool was used to align the resulting proteins from the blast database to Efb and Coa to determine the similarities between the proteins. Afterwards, I evaluated significance of the proteins, including its function and conserved domain, and the number of identities between the motif and the proteins found using blast.

Results: The motif from Efb and Coa had a good alignment with 5 proteins: Coa from S. *pseudintermedius*; Ebh from S. *epidermidis*; Fbl from S. *lugdunensis*; LPXTG cell wall-anchored protein from S. *lugdunensis*; and ABC transporter permease from S. *pneumoniae*. Coa in S. *pseudintermedius* and Fbl are known to bind fibrinogen and Ebh binds to fibronectin. 8/19 (42%) similarities between the motif and Coa in S. *pseudintermedius*. 10/19 (53%) similarities between the motif and Ebh. 11/19 (58%) similarities between the motif and Fbl. 10/19 (53%) similarities between the motif and LPXTG. 11/19 (58%) similarities between the motif and ABC transporter permease. The search was also conducted in S. *intermedius*, S. *pyogenes*, Y. *pestis*, and C. *diphtheriae* but no significant hits were found at this time. I conclude that this motif or part of it is found in proteins produced by other staphylococcal and streptococcal species that may, therefore, be fibrinogen-binding proteins. Moving forward, I hope to evaluate the hypothesis with the identified proteins and see if they are experimentally capable of binding to fibrinogen, fibronectin or other ECM proteins.

The role of stem cell factor nucleostemin in hepatocellular carcinoma progression

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Introduction: Nucleostemin (NS) is a protein found within the nucleolus with enriched expression in stem cells and cancer cells. NS promotes the replication induced DNA damage repair system and is essential for maintaining stem cell self-renewal. Under specific damage conditions, hepatic stem/progenitor cells are recruited as a source of liver regeneration. During liver regeneration NS expression is upregulated and loss of NS results in increased DNA damage in proliferating cancer cells.

Hypothesis: We observe that NS plays an active role in protecting the genomic integrity of cancer cells and recognize that NS may also affect liver tumor development. We compared the level of NS in different samples of mouse liver cancer: 1. diethylnitrosamine (DEN) induced liver carcinoma and 2. Wnt signaling pathway activation (β-catenin overexpression) induced liver carcinoma. By comparing tumor progression in DEN-treated NS conditional knockout mice and control mice, we hypothesized that loss of NS would postpone normal to malignant cell transitions as a result of the cancer stem cells in the liver losing their ability to maintain self-renewal. Without the ability of NS to protect the cancer cell from replicative DNA damage, components of the tumor would lose essential cell function and induce a process of cell death known as apoptosis, thus impairing tumor progression. By studying the previously mentioned cancer models, we strive to gain a better understanding of the involvement of NS in tumor development.

Objectives:

1. To determine whether the expression of NS is found in liver tumors
2. To determine whether NS knockout (albumin Cre driven, in mature hepatocytes) in DEN treated mice affects the development of hepatocellular carcinoma.

Methods: DEN induced liver carcinoma: Tumors were induced in 15 day old male mice by injection of DEN. NS-flox/flox wild type and NS conditional knockout mice were killed 4, 6, 8, 10 and 14 months following the administration of DEN. Non-DEN treated wild type and knockout mouse livers were collected as a control. Liver tissues were embedded in paraffin and sections 4 μm thick were mounted onto slides. Separately prepared slides were stained with hematoxylin & eosin, NS, and Ki67. Livers were examined for the presence of nodular lesions and classified as hyperplasia, adenoma, early carcinoma, and late carcinoma. NS and Ki67 expression measured in cells per area were compared to areas of liver tumor development.

Wnt/β-catenin induced liver carcinoma: β-catenin liver samples were obtained from collaborators at MD Anderson. Liver sections were stained and tumors were classified as fetal hepatoblastoma, hepatoblastoma, and carcinoma. NS and Ki67 expression was observed.

Results: The data suggests that high levels of NS expression in areas identified as late hepatocellular carcinoma in both DEN-treated and β-catenin cancer models correlate to increased levels of Ki67.These findings confirm that NS is essential for tumor development. Furthermore, results show that NS knockout in DEN treated mice reduces adenoma and early carcinoma lesion areas compared to that of the DEN treated NS-flox/flox wild type mice. Reduction of these lesion areas indicates that loss of NS impedes the progression of malignant tumors in the liver. Results also show that there is not a significant difference of DEN induced tumor frequency in the NS knockout and WT control. The livers of the non-DEN treated NS knockout and NS flox/flox wild type mice yielded no indication of tumor formation and appeared to be NS negative. These results suggest that NS is present in the late stage of hepatocellular carcinoma and that NS plays a role in tumor development. Through this research we perceive that NS function is important in maintaining normal stem cell self-renewal and in protecting cancer cell proliferation during tumor progression in the liver.

TAMHSC SUMMER RESEARCH PROGRAM

August 7, 2013: 9:00 AM - 4:00 PM
Health Professions Education Building
Bryan, Texas

Novel Therapeutics Targeting Store-Operated Calcium Channels

Dedeepya Puvvada, Lian He[1], Guolin Ma[1], Ling Zhong[1,2], Goeun Bae[1,2], Adam T. Szafran[2,3], Michael A. Mancini[1,2,3], Clifford C. Stephan[1,2], Yubin Zhou[1]
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Introduction: Calcium signaling is significant to almost all biological processes. Important physiological roles of calcium signaling range widely from the cell's growth to motility to apoptosis. In the blood and the immune system, calcium signals participate in regulation of cell differentiation, gene transcription and effector functions. The depletion of ER calcium store in non-excitable cells activates calcium influx across the plasma membrane in mammalian cells and triggers nuclear translocation of NFAT proteins with calcineurin to activate T lymphocytes. The calcium enters the cells with the assistance of two major players: ORAI and STIM. This process is known as Store-Operated Calcium Entry (SOCE). SOCE is best exemplified in the immune system. Dysregulated STIM/ORAI pathway has implicated immunodeficiences, autoimmune disorders, tumor metastasis and even cardiovascular diseases. The purpose of this study is to screen small molecule inhibitors that specifically target the STIM-ORAI pathway to suppress immune function selectively and create a safety profile unlike the current immunosuppressive agents such as cyclosporine A and FK506.

Objectives: To determine whether the virtually screened small compounds can inhibit or promote the calcium ion channels and to further examine the top candidates selected from primary screening for calcium-influx testing.

Methods: Top fifty small compounds were screened and docked based on Structure-Activity Relationship (SAR) with the human ORAI1. The primary screening was used as readout with the following secondary screening. Here, the fifty candidates were screened for further testing in a cell-based assay using Green Fluorescence Protein-Nuclear Factor of Activated T lymphocytes (GFP-NFAT) HeLa cell line. The cells were plated in 384-wells with 25µl in each well and were incubated in a CO_2 chamber for 16-18 hours. The compounds with calcium
were prepared in a 96-well plate. The inhibitor thapsigargin (TG) was added to induce the depletion effect, so that there is calcium influx into the HeLa cells through the ORAI/STIM pathway. The transfer of compounds into the cells was processed by Biomek FX and the results were examined after fixing and staining of cells with 4', 6-diamidino-2-phenylindole (DAPI). The NFAT translocation was monitored by the high content In-cell Analyzer 6000.The images were captured with the Pipeline Pilot software for the selection of effective candidates. The top candidates were selected for testing of calcium-influx using HEK cell line and were evaluated with F_{340}/F_{380} fluorescence.

Results: The analysis depicted that the localization of GFP-NFAT protein was in the cytoplasm as seen in compound candidates 1, 4, 16, and 42. Compound 22 directly worked with TG to promote the localization in the nucleus. Drug-dose response was analyzed in each of the compounds and we were able to select two top compounds based on half maximal inhibitory concentration (IC_{50}). Further examinations showed that c ompound 42 inhibited calcium influx.

Conclusion: As the high-throughput screening of small molecules targeting calcium ion channels was established, we were able to selectively narrow down the candidates for analysis. The results will serve as a standard for further assessments in in-vivo testing with xenograft tumor models and auto-immunodeficiency models.

TAMHSC SUMMER RESEARCH PROGRAM

August 7, 2013: 9:00 AM - 4:00 PM
Health Professions Education Building
Bryan, Texas

Stereological Quantification of Neurodegeneration in a Rat Model of Epilepsy

Divya Raju, R. Ceiker, J. Brewer, R. Kuruba, X. Wu and D. Samba Reddy*
Department of Neuroscience and Experimental Therapeutics, COM, TAMU HSC, Bryan, Texas Texas A&M University Health Science Center College of Medicine, College Station

Introduction & Background: Epilepsy is a chronic neurological condition characterized by repeated unprovoked seizures. Novel therapies are desperately needed for status epilepticus (SE), an emergency neurological condition characterized by persistent seizures lasting more than 30 min. SE is associated with significant morbidity and mortality due to widespread neuronal damage in the brain.

Hypothesis & Objective: We hypothesized that neurosteroids that enhance GABAergic inhibition possess significant neuroprotection properties. In this study, we utilized the quantitative stereological approach for the analysis of neurodegeneration in a rat model of SE. We determined the effect of diazepam (a benzodiazepine), and THDOC (a neurosteroid) on SE-induced neurodegeneration in the rat pilocarpine SE model.

Materials and Methods: Persistent SE was induced chemically by lithium-pilocarpine in adult rats, and test drugs (THDOC or diazepam) were administered at 60 min after the onset of SE. The onset and termination of SE was monitored by behavioral seizures and EEG recordings. Animals were perfused at 72 h after SE induction for neuropathological studies. Neurodegeneration was determined by the NeuN (principal cells) and PV (interneurons) immunostaining of brain sections. The extent of neurodegeneration was by measuring three key parameters: (i) absolute neuron numbers; (ii) neuronal density; and (iii) tissue volume.

Results: Brain sections from control or untreated animals showed abundant loss of principal neurons and interneurons in various regions within the hippocampus and other areas. Neurodegeneration (principal and interneurons) was observed in CA1, CA3 pyramidal regions, and in the dentate gyrus hilus regions. There was also extensive neuronal damage in the amygdala. The benzodiazepine diazepam offered some neuroprotection, but the neurosteroid THDOC conferred significant neuroprotection by diminishing the neuronal cell death associated with SE. The extent of change in tissue volume in control and treatment groups was minimal. .

Conclusions: These studies indicate that stereological approach is an effective tool for quantitative characterization of neuroprotective ability of test drugs in epilepsy models. The pilot data shows that neuroprotection offered by neurosteroids appears to be much superior to benzodiazepines.

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Ethanol and Nicotine Suppress Expression of the Imprinted Dlk1-Dio3 Growth-Control Locus in Neural Stem Cells

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Introduction: Alcohol and nicotine are often co-abused and fetal exposure to each has been linked to growth retardation and neurodevelopmental and behavioral disorders. However, their combined effect on neural stem cells (NSCs), which are responsible for generating the majority of adult neurons mostly during the second trimester, is not well understood. It was previously shown that ethanol does influence the proliferation and maturation of NSCs, as well as the imprinting pattern of the gene Dio3, the product of which is a type 3 deiodinase that regulates thyroid hormone (TH) levels in the developing fetus. Dio3 is located at the end of a cluster of imprinted genes on the distal portion of chromosome 14 in humans (14q32) and chromosome 12 in mice (12F1). This locus contains a set of genes (Dlk1, Gtl2, Rtl1, Rtl1as, RIAN, Mirg, Dio3 and Dio3as) that are involved in an array of processes including angiogenesis, maintenance of the placenta, thyroid hormone regulation, tumor suppression and tissue differentiation.

Hypothesis: We sought to investigate the response of mRNA expression of this gene locus in NSCs in the presence of ethanol, nicotine, varenicline, 5'-azacytidine, mecamylamine, choline and folic acid in an effort to better understand the impact that the maternal environment plays on the neuronal development of the fetus. Based on recent literature, we predicted that alcohol would have an influence on the regulation of mRNA expression from the Dlk1-Dio3 locus.

Methods: Gestational day 12.5 fetal murine cortical-derived neurosphere cultures were exposed to EtOH, varenicline, choline and folic acid individually or in combination over a period of 5 days, to mimic exposure during neurogenesis of a fetus. These samples, along with previous samples exposed to nicotine, 5'-azacytidine and mecamylamine were analyzed for levels mRNA expression of the Dlk1-Dio3 locus using quantitative reverse transcription polymerase chain reaction.

Results: We found that both EtOH and nicotine cause suppression from the Dlk1-Dio3 locus**.** The effect of nicotine was partly prevented by mecamylamine, a nicotinic-receptor antagonist, and partly mimicked by varenicline, a nicotinic-receptor partial agonist. Additionally, DNA demethylation resulted in suppression, however methyl donors like choline antagonized the effect of EtOH and resulted in increased expression from the Dlk1-Dio3 locus.

TAMHSC SUMMER RESEARCH PROGRAM

August 7, 2013: 9:00 AM - 4:00 PM
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Contribution of *STM3602,* a transcriptional regulator, to intestinal colonization of *Salmonella enterica* serotype Typhimurium

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Introduction: *Salmonella enterica* is one of the leading causes of foodborne illness throughout the world. The disease is transmitted via the fecal-oral route and is characterized by diarrhea, fever, and cramps. In a prior study, we identified a putative transcriptional regulator, STM3602, necessary for intestinal colonization by *Salmonella enterica* serotype Typhimurium in calves. STM3602 is a putative regulatory protein that shares a conserved domain with PhnF, a known phosphonate regulator in *E. coli*.

Hypothesis: We hypothesize that the Δ*STM3602* mutant will fail to colonize the intestine of *Salmonella*-resistant CBA/J mice, confirming the phenotype in the calf model. We believe that the colonization defect of the Δ*STM3602* mutant is due to either involvement of STM3602 (1) in regulation of known virulence factors or (2) in regulation of phosphonate metabolism.

Methods: To determine whether the Δ*STM3602* mutant will colonize mice, groups of 5 CBA/J mice were infected by gavage with 10^8 CFU of an equivalent mixture of WT and Δ*STM3602* mutant either without treatment or 48 hours following treatment with 20 mg streptomycin. Feces were collected on the days 1, 3, 5, 7, and 10. Mice were euthanized 2 weeks post-infection, organs harvested, and CFU enumerated. Next, we determined the ability of a mutant in Δ*STM3602* to activate a SPI-1 promoter. A plasmid containing *lacZ* under the control of the *prgH* promoter (a part of the T3SS-1) was transduced into *⊿STM3602* mutant and wild type. Cells were grown to late-log phase at 37˚C with aeration to induce SPI-1. β- galactosidase activity was determined routinely. We then tested for motility, as motility and virulence are linked during infection. Normalized overnight cultures were spotted onto motility agar and incubated at 37˚C for 3 or 5 hours. *fliC* promoter activity was assessed by β-galactosidase activity using a promoter fusion. Expression of FliC in whole cells and on the cell surface was determined by western blot. Additionally, we examined whether STM3602 was involved in phosphonate utilization. Overnight cultures of Δ*STM3602* and wild type were subcultured 1:100 into a low-phosphorus modified minimal medium supplemented with inorganic phosphate or equivalent concentration of phosphonoacetic acid. CFU were enumerated every 2 hours.

Results: A mutant in Δ*STM3602* poorly colonizes the murine intestine of *Salmonella*-resistant CBA/J mice. This mutant fails to fully activate a SPI-1 promoter. It has poor motility, likely due to the failure to export the major flagellin to the cell surface. Additionally, the *⊿STM3602* mutant is unable to use phosphonoacetic acid as a sole phosphorus source.

Determining the IC_{50} of Palbociclib (PD0332991) on Osteosarcoma cell lines *in vitro*

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Osteosarcoma (OSA) is a devastating neoplasm of mesenchymal origin known to extensively damage the skeletal system and is most commonly seen in children and adolescents. The effects include but are not limited to osteolytic bone lesions, irreversible destruction of the bone, and lifelong complications through conditions such as osteoporosis. In some cases where the OSA cells differentiate, abnormal bone growth may also occur. These conditions decrease the quality of life of patients along with inducing constant pain and increased mortality. The majority of patients have a single lesion and have a good chance of survival after treatment. Unfortunately, patients with metastases or recurring disease have a poorer outcome so novel treatment options are needed.

Arresting cell cycle and preventing cell division is a key component in sensitizing cells to chemotherapy and radiation. Building on previous research that CDK 4/6 inhibitors are successful in arresting cell cycle in aggressive cancers such as ER-positive and HER2-amplified breast cancer cell lines, we focused on testing said inhibitor, Palbociclib (PD0332991), on various OSA cell lines. Since certain dog breeds are highly susceptible to developing OSA, canine OSA cells are considered a model system for the development and evaluation of novel therapeutic approaches.

Through tissue culture and cell cycle analysis, we have found that upon exposure to PD0332991, cell division was inhibited and cell cycle arrest occurred at the G_1 checkpoint in human and canine OSA cell lines. The results were confirmed through microscopy where drug-sensitive cell lines possessed multiple nuclei and increased in cell size. This suggests that PD0332991 prevented the cells from progressing into the S/G_2 phase and arrested cell cycle. The end result of such treatment translates to inhibiting tumor growth and probably metastasis. PD0332991 may therefore serve as adjunct treatment with other chemotherapeutic agents in inhibiting OSA tumor growth as well as sensitizing cancers to chemotherapy and/or radiation.

TAMHSC SUMMER RESEARCH PROGRAM

August 7, 2013: 9:00 AM - 4:00 PM
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BIOGENIC AMINES SECRETED BY CHOLANGIOCARCINOMA MODULATE MACROPHAGE ACTIVATION

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Introduction: Cholangiocarcinoma (CCA) is a devastating primary hepatic malignancy that originates from bile duct epithelium. Most CCAs are adenocarcinomas and with a poor prognosis (5-year survival is <5%). Because of this, determining the contributing factors to neoplastic and hyperplastic growth of this tumor could indicate possible treatment options for late stage disease. In addition to neoplastic cells, the tumor microenvironment consists of inflammatory cells, which support tumor growth. Most tumor-associated macrophages (TAMs) have an reduction in M1-like anti-tumor phenotype. CCA cells have been shown to secrete increased amounts of serotonin leading to increased proliferation, and THP-1 cell line macrophages express various serotonin receptors, suggesting a basis for interaction between CCA and macrophages.

Hypothesis: Cholangiocarcinoma secreted factors, particularly serotonin, causes macrophage phenotype to change from M1 (pro-inflamatory) to M2 (anti-inflammatory).

Methods: Immunohistochemical staining of CD68 and IL-10 on benign and CCA biopsy tissue was performed. THP-1 macrophages were pretreated with LPS or IFN-γ followed by various timepoint treatments with conditioned media from CCA cells or serotonin. MHCII expression was measured by flow cytometry and CIITA, IL-6, IL-10, TNF-α and GAPDH expression was measured by qPCR. Human THP-1 cells were differentiated and polarized into M1 or M2-like phenotypes for 3 days in the presence or absence of serotonin or CCA conditioned media. Stat1 activation was measured by immunoblotting and cellular localization by subcellular fractionation.

Results: Macrophages were found in both benign and CCA tumors as evidenced by CD68 immunoreactivity, while IL-10 (marker of M2 macrophages) was only found in CCA tissue. In a THP-1 monocytic cell line differentiated with IFN-γ or LPS to the pro-inflamatory M1 phenotype, the presence of serotonin or CCA conditioned media (CCA CM) lowered the expression of TNF-α, MHCII, and CIITA. Treatment with CCA CM or serotonin inhibited the nuclear translocation of Stat1 without altering the phosphorylation status. In the presence of CCA CM, differentiated macrophages acquire an M2-like morphology. mRNA expression profile of differentiated THP-1 macrophages demonstrates that serotonin dampens M1 cytokine expression (TNF-α, IL-6) and CCA CM induces partial M2-like cytokine profile (IL-10).

STUDIES ON PRORENIN AND ITS RECEPTOR ASSOCIATED NOVEL RENIN-ANGIOTENSIN SYSTEM IN PREGNANCY AND PREECLAMPSIA

Jessica Thomason, Darijana Horvat, Dean Leonard, Steven R. Allen, Thomas J. Kuehl, and Mohammad N. Uddin
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Introduction: Preeclampsia (preE) is a disorder affecting 3-10% of pregnancies and is frequently accompanied by reduced uteroplacental perfusion resulting in intrauterine growth restriction (IUGR) and release of vascular regulatory molecules from the placenta producing maternal systemic effects. Maternal circulating renin in human pregnancy is derived from a renal source which responds appropriately to renal-type physiological stimuli. However, the high prorenin levels in maternal circulation are likely of ovarian and/or placental origin. There are two renin angiotensin systems (RAS) operating at the placental boundary, one in the fetal placental tissue and the other in the maternal uterine vessels. Unpublished data from our lab demonstrated that preE patients have a higher level of plasma prorenin compared to normotensive patients. A postulated mechanism by which the placenta may influence maternal vascular tone is the placental production of prorenin and its receptor which locally activates the novel RAS and lead to vasoconstriction. Up regulation of the RAS in the placenta might be important for preE pathogenesis.

Hypothesis: It is hypothesized that differential expression of prorenin and its receptor in preeclamptic patients contributes to their disease via a novel renin-angiotensin pathway associated with prorenin and its receptor.

Methods: Human placentas (n=10) were collected from normotensive and preeclamptic patients after cesarean deliveries. Animal rat models utilized for this study were divided into three groups: control, nonpregnant animals (n = 10); normal pregnant (NP) animals were given tap water ad libitum (n = 10) and pregnant DOCA + saline (PDS) animals (n = 10) were injected initially with 12.5 mg of desoxycorticosterone acetate (DOCA) intraperitoneally in a depot form followed by 6.5 mg on a weekly basis. Their drinking water was replaced with 0.9% saline. The PDS animals developed hypertension, proteinuria, excessive weight gain, and intrauterine growth restriction. Placental samples from animal and human models were utilized to quantify prorenin and its receptor via Western blot. Protein levels were analyzed with Image J and protein of interest was compared to ß-actin protein levels. Immunohistochemistry was used to visualize prorenin and its receptor but was not used to quantify data. Normal squirrel monkey and owl monkey placentas were used to compare (P)RR expression to assess species specific expression of the receptor.

Results: It appears that increased expression of prorenin and its receptor in the placenta are related to the occurrence of preeclampsia in both animal models and human patients. Elevated levels of circulating prorenin in humans and rats with preE and increased placental levels of prorenin and (P)RR in humans and preE rats, provide evidence that prorenin and its receptor in the maternal circulation at the placental boundary and within the placenta play a role in preE pathogenesis.

3-D MRI Reconstructive Modeling and Spectroscopy Applications in the Diagnosis and Treatment of Prostate Cancer

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Introduction: Prostate cancer is currently the second most diagnosed form of cancer in men and the second leading cause of cancer deaths in men within the U.S (National Cancer Institute, American Cancer Society). Current standard treatments for prostate cancer include radical prostatectomy, chemotherapy, radiation therapy, and hormone therapy. Direct intraprostatic injection or infusion is a promising technique currently under research. This drug delivery method offers focal treatment for organ confined prostate cancer. However, current *ex-vivo* models indicate variable drug distribution and undesired heterogenic diffusion profiles. To that end, an alternative needle design employing a microporous hollow fiber catheter (miHFC) developed by Twin Star Medical, Inc. yields promising results. Success of the MiHFC system relies on the clinicians' ability to identify the lesion and guide the catheter projection into the prostate. Diagnostic MRI spectroscopy combined with standard MRI mapping has been proposed for this purpose. MRI spectroscopy allows for characterization of the lesion on a voxel by voxel basis, while the standard MRI offers visualization of anatomic landmarks not observed in other imaging modalities (e.g. ultrasound). 3-D reconstructive techniques of relevant patient cases demonstrate the feasibility of this guidance and injection system.

Hypothesis: A multi-parameter system utilizing a combination of spectroscopy and MRI imaging will yield a robust diagnostic and drug delivery system. 3-D modeling will demonstrate feasibility and implementation.

Methods: *MRI:* Patients with suspected prostate cancer were imaged utilizing a 3T Siemens Trios with body coil using standard volumetric series, a five minute dynamic series of scans while contrast agent is infused. Spectrometry of sites with perfusion defects is also performed.

MRI Tracings and 3-D Reconstruction: Prostate tracings of MRI images in the axial plane and 3-D renderings performed by two independent reviewers for six relevant patient cases.

MRI Spectroscopy: Metabolic profiles given as a choline+creatine/citrate ratio for suspected voxels of interest performed by radiology staff. Diagnosis confirmed with ultrasound directed biopsy by urologist and reported by pathologist.

Results: 3-D modeling of six patient cases demonstrates feasibility of MRI and spectroscopy for detection of focal cancer lesions and for the implementation of the MiHFC system. While markers such as PSA levels indicate at risk status, MRI techniques allowed documentation of specific tumor sites.

Conclusions: Literature demonstrates the availability of MR imaging techniques for detection of cancer sites limited to the prostate organ allowing for directed application of anti-tumor agents to these sites. Trials with images from a small patient cohort support the potential for this technique for prostate evaluation and size measurements. Future work includes use of human *ex-vivo* prostate infusion trials and *in-vivo* infusion studies in nonhuman primates (squirrel monkeys and baboons, respectively). These methods may lead to an option of directed local treatments with less morbidity than current options.

2013 College of Medicine
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Micah Newton	Dr. Warren Zimmer
Darren Samples	Dr. Rajesh Miranda
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Chevaun Johnson	Dr. Warren Zimmer
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Sarah Luna	Dr. William Culp
Irtiza Sheikh	Dr. Carl Gregory/Dr. Ulf Krause
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Francis Onyebuchi	Dr. Magnus Hook

Undergraduate Students	
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Kay Pham	Dr. Robert Tsai
Dedeepya Puvvada	Dr. Yubin Xhou

2013 College of Medicine
Summer Research Program Seminar Series

Date	Time	Topic	Presenter
5/31	9:00 AM	Record Keeping	Dr. Van Wilson
6/4	12:00 PM	CST*R Grand Rounds	---
6/7	9:00 AM	Commercialization in Research Medicine	Dr. Joe Jilka
6/11	12:00 PM	Scientific Method	Dr. David McMurray
6/18	12:00 PM	MD/PHD Program	Dr. Julian Leibowitz
6/21	9:00 AM	Translating Science into Clinical Medicine	Dr. David Huston
6/25	12:00 PM	Biotechnology/Ethics	Dr. James Samuel
6/28	9:00 AM	Science and the Microbe	Dr. Samuel Shelburne III
7/2	12:00 PM	Scientific Misconduct	Dr. Vernon Tesh
7/5	9:00 AM	Use of Animals in Biomedical Research: Why, Which Animal and How Many?	Dr. James Elliott
7/9	12:00 PM	Human Experimentation	Dr. John Quarles
7/12	9:00 AM	Research into the Contributors to Health Disparities	Dr. Robin Fuchs-Young
7/16	12:00 PM	Medical Research… Why Me?	Dr. William Culp
7/19	9:00 AM	Innovative Technologies	Dr. Peter Davies
7/23	12:00 PM	Role of the Basic Scientist in the Commercialization of Novel Therapies	Dr. Brett Mitchell
7/26	9:00 AM	Student Oral Presentations	---
7/30	12:00 PM	Genetic Basis of Thoracic Aortic Aneurysms and Dissections: Bedside to Bench and Back	Dr. Dianna Milewicz
8/2	9:00 AM	Student Oral Presentations and SRP Wrap-Up	Dr. Warren Zimmer
8/7	9:00 AM-2:00 PM	Poster Presentations and Reception	

Program Director
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