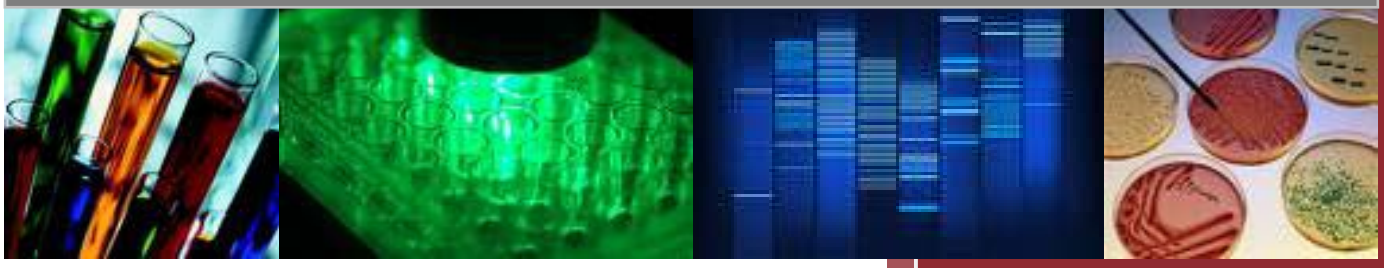
Poster Session and Reception

2010

Summer Research Program



August 18, 2010

9 a.m. – 2 p.m.

Reynolds Medical Building

TEXAS A&M
HEALTH SCIENCE CENTER
COLLEGE OF MEDICINE

Program

August 18, 2010

9 a.m.-2 p.m. **Poster Presentations**

Lobby, Reynolds Medical Building

11:30-12:15 p.m. **Lunch**

160 Reynolds Medical Building

12:30-1:00 p.m. **Speech**

Dr. James F. Martin, Professor and Interim Director Center for Molecular Development and Disease

Lecture Hall 1, Reynolds Medical Building

1:00-1:30 p.m. **Presentation of Certificates**

Dr. Warren Zimmer, Director of the Summer Research Program

1:30-1:45 p.m. **Presentation of Dean's Recognition Awards**

2 **p.m. Adjourn**

Speaker's Bio:

James F. Martin, MD, PhD
Professor and Interim Director Center for Molecular Development and Disease
Institutes of Biosciences and Technology
Texas A&M Health Science Center
Houston, Texas 77030

Dr Martin graduated magna cum laude in chemistry from Fordham University, Bronx, New York, received an M.D. from the University of Texas-Houston Medical School where he also did a residency in general surgery. He received a Ph.D. in molecular biology from the University of Texas-Houston Graduate School of Biomedical Sciences in 1995 and was a postdoctoral fellow in the Department of Biochemistry and Molecular Biology at the MD Anderson Cancer Center (1995-1996). He joined the Center for Cancer and Stem Cell Biology in the IBT, Texas A&M Health Science Center in 1996 as an Assistant Professor. He was promoted to tenured Associate Professor in 2002 and Professor in 2006. He is a member of the University of Texas Graduate School of Biomedical Sciences and holds a joint appointment in the Department of Molecular and Cellular Medicine, College of Medicine, Texas A&M Health Science Center. He is currently Interim Director of the Center for Molecular Disease & Development.

The focus of my lab is to understand the molecular mechanisms controlling cell growth and differentiation in the context of vertebrate embryogenesis and thus to advance our understanding of the causes of birth defects. Using the mouse as a model system, we study the role of homeobox genes in cell growth and differentiation within the craniofacial skeleton. Our experimental approaches include creating targeted gene mutations through "knock-out" technology, as well as other transgenic techniques to express genes of interest in the mouse. A related interest of our lab is to understand how environmental factors such as teratogens interact with the genome to generate congenital defects.

Acknowledgements

This is my third year as Director of the Texas A&M Health Science Center College of Medicine Summer Research Program. Each year the program has grown bigger and better. Even though we limited the number of participants to 30 this year, we had many more applications for the program (more than 150) and the competition for a spot in the program was especially keen (see pages 37 , 38 for a list of participants). I thank the committee for their work in reading and evaluating the applications and coming to a consensus on which students to offer a spot, it was a VERY difficult job. We have as a goal to provide students a foundation of the "how and why" of conducting a biomedical research project. We have two student populations, undergraduates and medical students. Undergraduate students from all over the country join the program to gain research experience and assess their desire to pursue graduate studies, perhaps in the combined MD/PhD program. Additionally, upon completion of their first year, our medical students have the opportunity to participate with the faculty in a research project. Last year we opened the program to three campus locations (College Station, Temple, and Houston) which we discovered enriched our program by giving the students many more research labs in which to work and learn and this also was a contributing factor to our success and growth.

All the program participants have worked extremely hard in the labs during the program. They have endured the pleasures of experiments working, and more often than they like they have experienced the "agony of defeat" by a test tube, mouse, or machine. On display at today's poster session/reception is the products of their hard work, and I invite you to come view the posters, ask questions and be prepared to lean.

The program would not be successful without the dedication of faculty, both as research advisors and for providing stimulating and informative lectures. Please see the content of these talks on page 39. In addition, 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. I also want to acknowledge Dr. Houston's contribution of involving faculty from several institutions in the Houston Medical Center (Methodist Research Institute and M. D. Anderson) which will enable us to offer an enriched program in the next years.

We obtained funding from a number of sources and would like to thank Dr. Sherwood, College Dean; Dr. Wesson, Vice Dean of the Temple Campus (Scott and White Research); and Dr. David Carlson, Vice President for Research and Graduate Studies for major contributions to our budget. In addition, we could not have had a successful year without the generous support of Dr. Roy Smythe, Chairman of Surgery; Dr. Alejandro Arroliga, Chairman of Medicine, and Dr. Harris Granger, Chairman of Systems Biology and Translational Medicine. Finally, I would like to thank Dr. Van Wilson and his staff in College Station, **Josephine Hernandez, Mary Ann Wolf, Courtney Mardis, and Katherine Gnadinger;** 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

Summer Research Program

TAMHSC SUMMER RESEARCH PROGRAM

August 18, 2010: 9:00 AM - 2:00 PM

Reynolds Medical Building, College of Medicine

Evaluation of Argininosuccinate Synthetase (ASS) and Myeloblasts Sensitivity to Arginine Depletion Therapy using Peripheral Blood from Subjects with Acute Myeloid Leukemia (AML)

Abram Albizo, Arthur Frankel M.D., Vaidehi Argrawal

Scott & White Department of Internal Medicine

Texas A & M University Health Science Center College of Medicine

Introduction

Current anti-cancer strategies work with a general lack of specificity, translating to a loss of both normal and cancerous cell populations. Cancer cell transformation involves creating altered metabolic pathways, which may enhance proliferation, but incapacitate a cell's ability to produce certain amino acids. The transformed cell becomes auxotrophic or dependent on the extracellular availability of an amino acid to continue growing; therefore, targeted amino acid depletion represents a selective anti-cancer treatment. The best example is the use of asparaginase, an enzyme able to deplete asparagine blood levels, to treat Acute Lymphoblastic Leukemia. Lympphoblasts are sensitive to asparagine depletion, while normal cells respond by synthesizing the non-essential amino acid. Similarly, arginine deprivation is effective against solid tumors that fail to express the ASS enzyme. Arginine is a semi-essential amino acid synthesized from argininosuccinate by Argininosuccinate lyase (ASL); however, ASS is the rate limiting step converting citrulline and aspartate to argininosuccinate. Depleting extracellular arginine is not a novel idea, but improvements in drug delivery mechanisms resuscitated interest, resulting in novel therapies. Phase I and II clinical trials of arginine deiminase, an extracellular arginine reducing enzyme, demonstrate improved outcomes in patients with hepatocellular carcinoma, malignant melanoma, and other tumors not expressing ASS. Loss of expression is associated with epigenetic methylation of the ASS gene promoter region.

Hypothesis

Argininosuccinate synthetase expression in hematologic malignancies, such as AML, is not well characterized. The goal of the project is to evaluate ASS and myeloblast sensitivity to arginine depletion therapy using samples obtained from subjects with a documented diagnosis of AML.

Methods

Heparinized peripheral blood samples will be taken from healthy subjects and subjects diagnosed with AML. The sample will be diluted 1:1 with RPMI 1640 and layered over Ficoll-Paque. Low density cells will be isolated using density gradient centrifugation, banded light density cells (<1.077 g/mL) will be diluted threefold with RPMI 1640 and centrifuged again at 1,300 rpm for 10 minutes. The band volume will be transferred to a 50 mL conical tube preloaded with 25 mL RPMI 1640 medium with 10% Fetal Bovine Serum (FBS), mixed, then transferred to a T75 flask for incubation at 37°C, 5% CO_2 for 1 hour. Non-adherent cells will be collected and centrifuged. The resulting pellet will be brought up in 2 mL of RPMI 1640 medium for immunoaffinity separation.

Cells are frozen in containers at -80ºC and transferred to liquid nitrogen. A small amount of the sample will be subjected to flow Cytometry using antibody labeling to characterize myeloblast isolation based on the presence of CD34+ signaling. After thawing or from fresh samples, cells will be reconstituted and processed for the presence of arginosuccinate synthetase (ASS) and β-actin by immunoblotting. Cell sensitivity to arginine depletion will be assessed by measuring inhibition (IC_{50}) of cell proliferation in the presence of different concentrations of arginase using ^{3}H-thymidine incorporation.

Results

The results are currently pending patient enrollment, optimization of the western blot, and further experimentation.

TAMHSC SUMMER RESEARCH PROGRAM

August 18, 2010: 9:00 AM - 2:00 PM

Reynolds Medical Building, College of Medicine

Sleep Related Factors Associated with Cervical Disc Degeneration

Brian Allgaier, Dr. Jonathan Friedman M.D.

Neurosurgery, Texas Brain and Spine Institute

Texas A & M University Health Science Center College of Medicine, College Station

Introduction and Hypothesis: Patients suffering from cervical disc degeneration (CDD) frequently seek guidance regarding suggested sleeping habits that might minimize the symptoms or progression of their condition. At the same time, no research has been published associating sleeping habits with the either the onset or management of symptomatic CDD. Some common sleep related factors which may relate to CDD are sleeping position (one's side, back, or stomach), sleep location (bed, couch, recliner), mattress firmness, number and softness of pillows etc… We are also interested in how obesity and other co-morbidities play a role in sleeping position habits, and how these factors may contribute to CDD. For instance, obese individuals tend to sleep on their side due to subclinical sleep apnea. We hypothesize that sleeping on ones side is a predisposition for CDD as it favors a forward position of the head, and may promote posterior ligament complex laxity over time. Integrity of the posterior ligament complex contributes to the stability of the spine. As our population becomes increasingly obese, side sleeping may result in a higher incidence of CDD.

Another related area of interest is how special sleeping devices marketed or implied to provide therapeutic benefits relate to CDD. While specialized pillows and mattresses are a multi-billion dollar industry, and many patients suffering from CDD purchase these expensive items, there is no published literature relating benefit or lack thereof received by CDD patients who use these devices.

Methods: Retrospective chart review with survey instrument. 606 patients at the Texas Brain and Spine Institute underwent one or two level anterior cervical discectomy with fusion (ACDF) for CDD from 2005-2009. A survey was constructed and mailed to all 606. This survey contained questions outlining the factors discussed above, as well as questions relating to traumatic injury from MVA and high-impact sports injuries which may have contributed to CDD. Questions regarding changes in sleep habits before and after the ACDF procedure are also contained to limit confounding factors. Once ample surveys are returned the responses will be correlated with factors from the patient's chart. These factors include, height, weight, BMI, level of CDD, diagnosis, location of symptoms, whether CDD was caused by trauma, ACDF level, whether symptoms were resolved by ACDF, what symptoms were not resolved, days from ACDF to last follow-up appointment, additional therapy if any etc… The results of the survey will also be compared to literature attempting to characterize the sleeping habits of average individuals in order to provide controls.

Results: We expect to have results showing a correlation between sleeping factors and CDD, or no correlation between the two. Either result is significant due to the lack of literature exploring this question, and will help physicians recommend sleeping habits to patients suffering from CDD.

TAMHSC SUMMER RESEARCH PROGRAM

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Reynolds Medical Building, College of Medicine

Endothelial Progenitor Cell Number and Function Following L-Arginine Supplementation of the Diet of Diabetic Rats.

Emelia N. Bittenbinder and Cynthia J. Meininger

Systems Biology/Translational Medicine

Texas A & M University Health Science Center College of Medicine, Temple

Introduction: Endothelial Progenitor Cells (EPCs) are bone marrow-derived circulating cells that have the potential to undergo postnatal vasculogenesis and are important for vascular repair. EPC recruitment from the bone marrow as well as their function in the peripheral circulation is dependent on nitric oxide-mediated signaling pathways. Endothelial nitric oxide synthase (eNOS) produces nitric oxide but becomes uncoupled from its cofactor tetrahydrobiopterin (BH_4) in diabetes, resulting in reduced nitric oxide bioavailability. The result is a decrease in the number and function of EPCs in circulation. This is likely to contribute to the pathogenesis of cardiovascular disease common in diabetic patients. BH_4 could be supplemented in an effort to re-couple the eNOS pathway; however BH_4 is readily oxidized in solution, making it an inefficient and costly option. L-arginine can increase the expression/activity of GTP cyclohydrolase I (GTPCH) activity, the rate controlling enzyme for BH_4 synthesis. Modulating this enzyme may prove a more practical means of increasing BH_4 levels and re-coupling the eNOS/BH_4 pathway in order to increase EPC function and number.

Objective: To determine whether raising BH4 levels in EPCs can restore circulating levels of these progenitor cells and improve their function in animal models of type 1 diabetes mellitus.

Specific Aims: To demonstrate increased numbers and improved function of EPCs in diabetic rats following L-arginine supplementation of the diet.

Methods: Sixteen male Sprague Dawley rats were injected with streptozotocin to induce type I diabetes. These rats were randomized to receive either 1.55% L-arginine HCl or 2.55% L-alanine [isonitrogenous control] in their drinking water for 21 days and then sacrificed. Peripheral blood was collected via cardiac puncture and the mononuclear cell layer was collected using Ficoll Hypaque 1077 density gradients, and plated onto fibronectin-coated dishes with EGM-MV media. EPCs were cultured until distinct colonies were apparent. Bone marrow was aspirated from rat femurs, isolated on the same density gradients, and plated onto fibronectin-coated dishes with 20% FBS in EBM. The non-adherent cell population was replated at 24 and 48 hours. At 72 hours the non-adherent portion was removed and cells were given EGM-MV and were cultured until EPC colonies became apparent. EPCs from both sources will be characterized by ac-LDL uptake, migration assays, tube formation assays, and nitrate and BH4 analysis with HPLC.

Results: This pilot study was initiated later than expected due to a parvovirus outbreak in the animal facility. We are in the process of culturing EPC colonies. Thus far we see the beginnings of colonies and are hopeful that the results will reflect our predictions. Experiments will continue as detailed above.

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Reynolds Medical Building, College of Medicine

The use of Total Truncal Adipose Volume measured on CT to estimate BMI

Bryce J. Busenlehner[2], David F. Ferguson[1], Christopher D. Chaput[1,2], Sachin M. Mehta[2], Mark D. Rahm[1,2], Juhee Song[3]

[1] *Department of Orthopaedic Surgery, Scott & White Hospital, Temple, Texas*; [2] *College of Medicine, Texas A&M Health Science Center, College Station, Texas;* [3] *Department of Biostatistics, Scott & White Hospital, Temple, Texas*

Texas A & M University Health Science Center, College of Medicine, Temple, Texas

Introduction: The prevalence of obesity is reaching epidemic proportions in the United States, this is also reflected in the increasing numbers of trauma patients seen with Class I-III obesity . This obesity classification score is defined by BMI as: Class I, 30.00-34.99; Class II, 35.00-39.99; and Class III, ≥40.00. In 2007-2008, the age-adjusted prevalence of obesity was 33.8% overall, 32.2% among men, and 35.5% among women. BMI is a good indicator of excess adiposity, but differences in BMI of relatively thin individuals can be largely due to fat-free mass. Thus, the accuracy of BMI varies according to the degree of adiposity. Obesity has been linked to an increasing number of co-morbidities and medical complications. In relating to trauma, obese patients have been seen to have a higher incidence of multiple organ failure and mortality. Moreover, calculating an accurate body mass index is difficult in the polytraumatized patient, who is generally supine, intubated, and secured in spinal precautions. The need for weight measurement becomes important when the polytrauma patient is first encountered in the ED as some literature states a positive weight gain between 4.37% - 13.9% after fluid resuscitation. If there were a simple method to calculate Body Mass Index (BMI) from existing trauma scans, the study of patient obesity in the trauma setting could be facilitated. Thus, the true prevalence of obesity might be strikingly higher than that estimated by BMI. Consequently, establishing this true prevalence of obesity is especially important since current research shows that obesity has induced lipid/lipoprotein alterations and oxidative stress, irrespective of age, even in the absence of diabetes, hypertension, renal or liver diseases. The purpose of this study is to utilize data from routine CT scans to quantify obesity in polytrauma patients without the need to obtain a height and weight.

Hypothesis: We hypothesize that using CT scans to calculate BMI will have a strong association with traditionally calculated BMI and potentially lead to a more accurate calculation of adiposity.

Methods: Analysis of a comprehensive radiographic and clinical polytrauma database including digitally archived multidetector CT images obtained in the emergency department (ED) on all polytrauma patients. One thousand one hundred seventy-four patients were reviewed from 2006 to 2008 and, of these, 280 were found that had a documented height, weight, or BMI as an outpatient within 6 months of trauma activation. Age, gender, height, weight, and radiographic measurements were recorded and the Truncal Adiposity Volume (TAV) was calculated from 3D reconstructions of the CT scans of the thorax and abdomen obtained in the ED.

Results: Comparison of BMI to TAV demonstrated a linear distribution of increasing TAV to BMI (Correlation Coefficient=0.77; p-value<0.0001). This can determine TAV from a previously known or patient-stated BMI and thus, assist in WHO classification of obesity, defined as: underweight, <18.50; normal, 18.5-24.99; overweight, ≥25.00; and obese, ≥30.00. Within the obese class lies a subgroup, defined as: Class I, 30.00-34.99; Class II, 35.00-39.99; and Class III≥40.00. A good correlation (Correlation Coefficient=0.78; p -value<0.0001) was seen when comparing traditional BMI calculation to the BMI calculated by the 3D reconstructed equation using TAV.

TAMHSC SUMMER RESEARCH PROGRAM

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Role of PSGR Overexpression in a Murine Prostate Cancer Model

Nicole Canon, Melissa Rodriguez- Villanueva, Zhishi Chen, Mingyao Liu

Center for Cancer and Stem Cell Biology, Texas A&M Health Science Center Institute of Biosciences and Technology, Texas A&M College of Medicine Houston Campus, Houston, Texas, 77030, USA.

Introduction: PSGR, a new prostate-tissue specific gene has been discovered. A recent addition to the GPCR family, investigations have postulated the gene's homology with the G-protein coupled odorant receptor family. PSGR is specifically expressed in prostate tissue and overexpressed in prostate cancer. Its localization in prostate epithelial cells as well as its particular expression suggests that it may play a role in prostate cancer tumorigenesis. In this study, we analyzed the differences in morphology and cell marker expression patterns of PSGR wild type vs. transgenic mice of FVB background. Studies showing a 93% comparison between the PSGR amino acid sequences for the human and the murine make the mouse a reasonable model for our experiment.

Methods: PSGR transgenic mice were generated from young FVB mice through the excision of the hPSGR gene, and its insertion into the pBSK-SSI vector, which contains a rat probasin promoter, a PolyA site and a chicken insulator. Genomic DNA was extracted from the mice through the purification of mice toes followed by its subjection to a genotyping PCR and Southern Blot for identification of the transgene. Subsequently, RNA was extracted to measure for PSGR expression between wild type and transgenic littermates through an RT-PCR. Moreover, prostates were excised from mice at different age intervals, fixed under standard histological procedures and stained with HE staining for differences in morphology of the tissues expressing the PSGR transgene versus wild type littermates. We also analyzed, through immunohistochemistry, the overall effects of the PSGR transgene on tissue homeostasis, particularly on the androgen receptor. Antibodies such as c-myc, PTEN, p-AKT, E-cadherin, CK8, α-syn, α-SMA, P63, AR, and KI67 were utilized to observe the course of basal cells, luminal cells, endogenous receptors, and its proliferation potential upon overexpression of PSGR.

Results and Discussion: Results show that the transgenic mice exhibited PIN lesions after 12 months of age in contrast to their wild type counterparts. Analysis of three different age intervals shows no apparent progression of PIN lesions to a higher grade or carcinoma. Furthermore, immunohistochemical analysis shows an increase in cell proliferation in transgenic mice, as well as an increase in androgen receptor and c-Myc expression, suggesting PSGR may play a role in cell proliferation during the initiating phases of prostate cancer progression. Due to its high specificity in expression, and possible effects on the androgen receptor, understanding the molecular mechanisms underlying PSGR expression may offer new treatment opportunities for patients with prostate cancer.

TAMHSC SUMMER RESEARCH PROGRAM

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Reynolds Medical Building, College of Medicine

Identification of Dominant Modifiers of Position Effect Variegation in Drosophila

Soumili Chatterjee, P.I: Dr. Jun-Yuan Ji

Department of Molecular and Cellular Medicine

Texas A&M Health Science Center, College of Medicine, (Campus: College Station)

Introduction & Background: Chromatin structure and dynamics play fundamental roles in regulating gene expression, and deregulated gene expression can result in abnormal development and a wide-variety of diseases such as cancer. To indentify novel factors that regulate chromatin dynamics, we performed a dominant modifier genetic screen based on the bristle phenotypes caused by Position Effect Variegation (PEV) of the *yellow* (*y*) locus. The PEV of the *yellow* locus (y^{3P} allele) is caused by chromatin rearrangements of the *y* locus, which presumably disrupted the normal boundaries between euchromatin and heterochromatin. Differential spreading of heterochromatin is thought to contribute the variegated expression of *y* locus. Screen for modifiers of the PEV of the *y* locus allows us to quantify the severity of modifications. We screened a collection of *Drosophila* deficiency lines for dominant modifiers, i.e., suppressors and enhancers, of the bristle phenotypes caused by PEV of the *y* locus.

Specific Aims: Perform a dominant modifier genetic screen to identify genes when reduced in their dosages can either enhance or suppress the PEV phenotype of the *y* locus.

Hypothesis: If an unknown gene encodes an enzyme or a protein that are involved in regulating hetero-chromatin spreading or chromatin dynamics, then reducing their dosage by half will affect the chromatin structure *in vivo*. As the result, the expression of *y* gene will be affected, which can be assayed by analyzing the color of bristles on the wing blade. Thus, PEV can be used to genetically identify the novel factors in regulating chromatin dynamics and transcription.

Methods: As a proof of principal, we genetically crossed males from 215 deficiency (*Df*) lines from Bloomington deficiency kit with the y^{3P} females. In each batch of crosses, we used wild type males (w^{1118}) as a control. We then sorted males (with the following genotype: y^{3P}/Y; *Df/+; +*), mounted >15 wings for each genotype, and then counted the frequency of yellow bristles under microscope. If reduction of genes uncovered by these deficiencies can increase the frequency of yellow bristles, then we call this *Df* line as an enhancer. On the contrary, if reduction of genes uncovered by these deficiencies can decrease the frequency of yellow bristles, then we call this *Df* line as a suppressor.

Result: In the control (genotype: y^{3P}/Y; *+; +*), the frequency of yellow bristle is 25~30% (N=). Of 215 deficiency lines tested, we identified X lines of strong enhancers, which show more than 40%(?) of the yellow bristles, and Y lines of strong suppressors, which show less than 10%(?) of the yellow bristles. These results show that this genetic system can be successfully used to identify factors that modulate chromatin dynamics and transcription.

Future plan: First, we will expand this analysis to the 200 Df lines on the third chromosome. Second, we will identify the specific genes that are uncovered by these suppressor and enhancer Df lines by testing partially overlapping Df lines and the mutants of specific genes. Third, we will verify whether the genes that we identified can also modify the PEV of other genes. Finally, we will analyze how the novel modifiers from this screen regulate chromatin dynamics and gene expression.

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Reynolds Medical Building, College of Medicine

Differential effects of IL-3 and IL-33 on TSLPR and IL-7R□ activation in human basophils

Cortez Angela N, Henson Cory L, Thombare A, Tavana A, Lei JT, Huston DP

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Basophils are one of the least understood granulocytes in human circulation, but increasing evidence has revealed a crucial, nonredundant role in immune response. These cells express the high-affinity receptor for immunoglobulin E (IgE), Fc□RI, and crosslinking of this receptor by IgE leads to their activation and degranulation. Basophils can also be activated by the hematopoietic cytokine, IL-3, a known mediator of allergic inflammatory response. IL-33 is another basophil-activating cytokine that belongs to the IL-1 superfamily and drives the production of IgE and Th2 cytokines. Thymic stromal lymphopoietin (TSLP) – a potential therapeutic target – is a hematopoietic cytokine with a potential role in Th2 inflammatory response. TSLP binds TSLP receptor, a low affinity receptor, and interleukin 7 receptor alpha chain, a high affinity receptor, for cytokine on myeloid dendritic cells (mDC). TSLP-treated dendritic cells are capable of priming naïve CD4+ helper T-cells to differentiate into proinflammatory Th2 T-cells producing IL-4, IL-5, IL-14, and tumor necrosis factor alpha (TNF-□), but its role in basophil mediated Th2 allergic inflammation is not yet clear.

Objectives: We investigated the potential for IL-3 and IL-33 promote the capacity of basophils to modulate immune response through TSLPR upregulation. We also investigated the potential of IgE receptor crosslinking to induce basophil activation and upregulate TSLP receptor. **Methods**: Purified human basophils were examined with flow cytometry for the expression of TSLPR and IL-7Ralpha, activation surface markers CD203c and CD69 and degranulation marker CD63 after crosslinking IgE receptor in the presence and absence of IL-3 and/or IL-33. **Results**: IL-3 & IL-33 stimulation results in increased expression of cell surface markers CD203c and CD69 on human basophils. IL-3 & IL-33 overnight stimulation results in TSLP receptor□ upregulation□on human basophils. IL -3 & IL-33 overnight stimulation with IgE-dependent (anti-IgE antibody) crosslinking led to increased surface expression of CD63 on basophils. Conclusions: IL-3 and IL-33 are potent modulators of prolonged basophil upregulation of TSLP and therefore, basophil activation and immune function. Moreover, IL-3 & IL-33 may be important targets in the regulation and duration of prolonged allergic response or host defense.

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August 18, 2010: 9:00 AM - 2:00 PM

Reynolds Medical Building, College of Medicine

A TGF-β Pathway May Mediate the Temperature-Size Correlation in *C. elegans*

Matt Evans and Tina L. Gumienny

Molecular and Cellular Medicine

Texas A & M Health Science Center College of Medicine, College Station

Introduction: The N2 wild-type worms, and most mutant derivatives, follow the temperature-size rule, which is an inverse relationship between temperature and body size widely seen in ectotherms. The molecular determinants of this response are largely unknown in any system. The transforming growth factor beta (TGF-β) pathway is involved in the determination of body size in *C. elegans*. In addition, some genes that are required to sense other environmental cues may play a role in this TGF-β pathway (Gumienny lab, unpublished). The goal of my study was to determine if TGF-β mediates the animal's body size response to temperature. A preliminary study performed in our lab by Holly Doebbler showed that the TGF-β pathway is required for temperature-dependent body size changes. Normal animals or animals with higher than normal levels of a TGF-β showed reduced body lengths at higher temperatures than at lower temperatures. However, animals lacking this TGF-β were the same size at three temperatures tested (15° C, 20° C, and 25°C).

Hypothesis: The *C. elegans* DBL-1/TGF-β pathway controls the temperature-size phenomenon. Animals lacking the DBL-1/TGF-β or positive regulators of this pathway will not show a body size difference when grown at different temperatures. Animals expressing normal or increased levels of DBL-1/TGF-β signaling will show an inverse temperature-size correlation.

Methods: We chose 6 strains that had different levels of TGF-β signaling. Each strain was grown at 15° C, 20° C, and 25°C for at least three generations without starvation before measuring. 30 late larval hermaphrodites were imaged for each strain and temperature. The wild type was measured with each set as a control. Imaging and measuring were performed with a Leica SMZ 1600 dissecting microscope using a Retiga 2000R CCD color camera and BioVision IVision software. Statistical analyses were performed to test significance.

Results: Animals with low or no TGF-β signaling showed no body size response to temperature changes. Initial results suggest that body size in animals with normal or overactive DBL-1 pathway signaling is responsive to temperature. Some data sets will be resampled to increase confidence levels.

TAMHSC SUMMER RESEARCH PROGRAM

August 18, 2010: 9:00 AM - 2:00 PM

Reynolds Medical Building, College of Medicine

The effects of chlorotoxin on cholangiocarcinoma

Eric Golden[1,3], Gabriel Frampton[2], Sharon DeMorrow[1,2,3]

Department of Research and Education[1]; Digestive Disease Research Center[2],

Scott and White Memorial Hospital, Temple, Texas.

Texas A&M Health Science Center College of Medicine[3], Temple, Texas.

Introduction: Cholangiocarcinoma (CCA) is a very deadly tumor affecting cells of the bile duct epithelium (cholangiocytes). This disease carries a dismal prognosis due to the fact that very few early-detection measures exist, with most patients receiving a diagnosis when it is too late to resect the cancerous cells. Furthermore, CCA has historically shown a high resistance to traditional chemotherapeutic agents. Chlorotoxin, a component of venom isolated from the deathstalker scorpion (Leiurus quinquestriatus), has anti-tumoral effects in gliomas, predominantly by inhibiting matrix metalloprotease-2 (MMP-2), a gene involved in the tissue remodeling that occurs during tumor development.

Hypothesis: Because CCA also displays high levels of MMP-2 expression, the aim of these studies was to explore the effects of chlorotoxin on different measures of cancer severity including proliferation, induction of apoptosis, migration, invasiveness and angiogenesis.

Methods: Two human CCA cell lines (HuCCT-1 and CCLP-1) and one human non-malignant cholangiocyte cell line (HIBEC) were used in these studies. Cell lines were treated with various concentrations of chlorotoxin (30-500 nM) for 24 hours and the effects on MMP-2 activity were assessed using a commercially available chemoluminescent kit. In parallel, cells were treated with chlorotoxin (30-300 nM) for 48 hours and cell viability was assessed by MTS proliferation assays and Annexin V immunofluorescence staining as an indication of apoptosis. The effects of chlorotoxin on cholangiocarcinoma cell migration were assessed using a Boyden invasion chamber assay and a wound-healing scratch assay. HUVEC cells were used to assess the effects of chlorotoxin on angiogenesis and features such as branching and tube formation were measured. In addition, HuCCT-1 and CCLP-1 cholangiocarcinoma cells and HIBEC cholangiocytes were treated with chlorotoxin (30-300 nM) for 24 hours and the expression of angiogenic signaling molecules VEGF A and VEGF C were assessed by real time PCR.

Results: We have demonstrated that chlorotoxin treatment inhibits MMP-2 activity in a similar manner to that observed in glioma cells. Furthermore, chlorotoxin inhibited cell proliferation and increased apoptosis in cholangiocarcinoma cells, whereas non-malignant cholangiocytes were relatively resistant. Chlorotoxin treatment also increased the time required to close the scratch wound and decreased the number of cells that migrated to the lower surface of the Boyden invasion chamber, indicating that chlorotoxin may inhibit cholangiocarcinoma migration and invasion. Lastly, chlorotoxin inhibited angiogenesis as demonstrated by decreased branching and tube formation, and decreased VEGF A and C mRNA expression in CCA cells, but not non-malignant HIBEC cells.

Conclusions: These promising results suggest that chlorotoxin-derived treatments could be used to improve the prognosis of patients with cholangiocarcinoma.

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Reynolds Medical Building, College of Medicine

EFFECT OF ENDOGENOUS NEUROSTEROIDS ON SEIZURE EXPRESSION IN THE HIPPOCAMPUS KINDLING MODEL OF EPILEPSY

Jordan Gould and D. Samba Reddy*

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Introduction: Epilepsy is a debilitating neurological disorder characterized by the repeated unpredictable occurrence of seizures. Neurosteroids are steroids that are synthesized locally in the brain, which play an important role in the modulation of neuronal excitability through interactions with inhibitory GABA-A receptors. Neurosteroids such as allopregnanolone, which is derived from the ovarian hormone progesterone, exhibit anticonvulsant properties. Cyclical changes in progesterone levels during the menstrual cycle can lead to changes in seizure susceptibility. This leads to a disorder in women known as catamenial epilepsy (CE). It is therefore critical to understand the effects of endogenous neurosteroids on seizure expression to develop better treatment strategies for CE. The main objective of this study was to determine the effects of neurosteroid exposure and withdrawal on seizure susceptibility, using the hippocampus kindling model of partial complex seizures.

Hypothesis: It is hypothesized that seizure susceptibility decreases when neurosteroid levels are high (mid-luteal phase), and increases during their withdrawal (perimenstrual periods).

Methods: A chronic seizure condition was created using the hippocampus kindling model in adult female mice. Kindling stimulations were delivered via a bipolar electrode surgically implanted into the hippocampus, at 125% of the afterdischarge (AD) threshold until stage 5 seizures were elicited on 3 days consecutively ("fully kindled" state). Seizure activity was rated according to the Racine scale (stage 1–5). The effect of neurosteroids, induced endogenously by gonadotropin treatment (GNT), was assessed by recording of (i) seizure stage; (ii) seizure duration; and (iii) AD duration. Neurosteroid withdrawal was induced by blocking its synthesis by treatment with finasteride.

Results: Daily kindling stimulation was associated with a steady progression of behavioral and electrographic seizure activity. Mice reached the fully kindled state with consistent stage 5 seizures after ~14 stimulations. The overall mean seizure duration and AD duration were ~40 and ~35 sec, respectively. The severity of generalized (stage 4/5) seizures was reduced during the period of GNT-induced elevation in neurosteroids. Neurosteroid withdrawal was associated with exacerbation of seizure activity as evident by increase in seizure severity, AD duration and generalized seizure duration. The ADT for evoking generalized seizures was markedly decreased 24 h after withdrawal, suggesting vulnerability to seizures.

Conclusions: These results suggest that endogenous neurosteroids protect against seizures and that their withdrawal, such as that which occurs during menstruation, may exacerbate seizure activity.

TAMHSC SUMMER RESEARCH PROGRAM

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Reynolds Medical Building, College of Medicine

Optimization and Development of a Diagnostic Assay for Anti-IgE FcεR1α Receptor Antibody

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FcεR1α is the alpha chain of the high affinity IgE Fc receptor (FcεR1) and is expressed on mast cells, basophils, and activated eosinophils. Activation of this receptor results in varied immune responses including allergies and asthma, which are TH2 cell driven diseases. Recent clinical evidence has revealed that patients who suffer from severe allergy (i.e. chronic uticaria) have autoantibodies to the high affinity receptor for IgE (FcεR1). However, diagnostic assays to test for the presence of these autoantibodies are currently unavailable. Our primary goal in this study was to develop a diagnostic assay capable of detecting the presence of anti-IgE FcεR1α receptor antibodies in patient serum using primed human basophils. We hypothesized that if receptor autoantibodies were present in our samples, then incubation with primed human basophils should result in the up-regulation of activation markers on the basophil cell surface. Our first objective was to identify a useful biomarker on activated human basophils after anti-FcεR1 antibody (CRA-1) cross-linking.

To this end, freshly-isolated basophils from peripheral human blood were examined by flow cytometry for the upregulation of activation markers CD203c, CD69 and CD63 after incubation with various combinations of IL-3, IL-33, and CRA-1. Although CRA-1 alone up-regulated the expression of CD203c and CD63 on basophils, pretreatment with IL-3 + IL-33 prior to cross-linking resulted in a ten-fold increase. This trend was even more apparent for CD63 as pre-treatment with the cytokines resulted in a 26-fold increase. In aggregate, our data suggests that CD63 can prove to be a useful biomarker in assay assessment. Future experiments should examine the synergistic properties (if any) of IL-3 and IL-33 cytokine as well as optimization of assay using patient serum.

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Shiga Toxin Involvement in Apoptotic and Autophagic Signaling in HK-2 and Vero Cells

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Introduction: Shiga toxins continue to play a major role in food poisoning in the United States. Gaining a better understanding of the mechanisms that these toxins utilize will allow better treatments and prevention. Apoptosis is a well-studied mechanism of cell death. Autophagy refers to self-eating. In this process, a double layered membrane envelopes a volume of intracellular space. Traditionally, autophagy has been thought of as a means whereby cells survive in a nutrient deficient environment by consuming and recycling portions of it's own cellular machinery. More recently, autophagy has been implicated in substantive roles in other processes such as induction of apoptosis. Through a variety of mechanisms, autophagy has been shown to work with apoptosis to promote cell death, and also to antagonize apoptosis to promote cell survival.

Hypothesis: Our hypothesis is that both autophagy and apoptosis are involved in the Shiga toxin related cell death in HK-2 and Vero cells.

Methods: Numerous methods were used to try to prove our hypothesis. First we utilized MTT protocols to show that shiga toxins cause cell death. Then we used western blots to test for caspase-8 and PARP activation to show evidence of apoptosis. Then we used western blots to test for LC3 cleavage to show evidence of autophagy. By using confocal microscopy we confirmed the western blot results to demonstrate LC3 lipidation as evidence of autophagosome formation.

Conclusion: Our data shows that Shiga toxins are cytolethal to HK-2 and Vero cells and they activate both apoptotic and autophagic pathways.

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Use of Adult Human Mesenchymal Stem Cells to Recondition Decellularized Cadaveric Bone for Allograft.

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Introduction: A major hurdle of bone grafting techniques is obtaining adequate amounts of graft material. Decellularized cadaveric cancellous bone (DCCB) can be used to repair defects in bone without the additional costs (surgical incisions, donor site destruction, or the post-surgery morbidity) associated with autografting. However, allograft complications including delayed union, fatigue fractures, and resorption of graft material, occur in part due to the absence of osteogenic cells on the graft surface as well as lack of an appropriate niche for cellular adhesion. Our group is currently developing a procedure to "condition" DCCB with human mesenchymal stem cells (hMSCs) with the hope that such material could serve as a superior alternative to autograft. This poster presents the preliminary work done in an attempt to determine: a) whether hMSCs could be cultivated on the DCCB surface, b) if the hMSCs could be induced to differentiate and deposit extracellular matrix (ECM) to form a niche, and c) if adding dexamethasone to the culture media increased the rate of ECM deposition on the bone surface.

Hypothesis: Human MSCs will grow readily on the surface of DCCB and, in the presence of the appropriate osteogenic media, will secrete ECM that will serve as a niche for invading osteogenic and angiogenic progenitors upon implantation. In addition, we expect that the presence of the synthetic hydrocortisone, dexamethasone, will increase the rate of ECM deposition.

Methods: DCCB chips were sterilized and soaked in fetal bovine serum (FBS) for 24 h. Green fluorescent protein expressing (GFP+) hMSCs were incubated in the presence of DCCB chips, with complete culture media (CCM) consisting of αMEM containing 20% (v/v) FBS, 8 mM L-glutamine and antibiotics. When the cells reached confluency on the surface of the DCCB, the media was changed to an osteogenic base media (OBM), which consisted of CCM enriched with β-glycerol phosphate, ascorbate-2-phosphate and, in half of the samples, dexamethasone. Samples were biochemically, histologically, and microscopically at 8 and 21 days.

Results: At the time of writing, our data is still being compiled and analyzed. However, preliminary results indicate that GFP+ hMSCs readily grow on the DCCB surface, and that osteogenic differentiation and ECM deposition does occur in the presence of both OBM and OBM with dexamethasone. Whether dexamethasone accelerates or improves ECM deposition has yet to be determined.

Impact/summary: These data suggest that hMSCs have the capacity to substantially enhance the cellular compatibility of cadaveric bone used for allograft. The co-culture process is simple, effective, and if necessary, it can be employed using autologous sources of hMSCs.

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Neurofunctional effects of exogenous cortisol on memory: A functional magnetic resonance imaging study

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Introduction: Cortisol is released by the hypothalamic-pituitary-adrenal (HPA) axis during stressful events, and its ability to cross the blood brain barrier has variable effects on the brain. The effects of exogenous cortisol on the limbic system and the medial temporal lobe are not well understood, despite both systems having glucocorticoid and mineralocorticoid receptors. In the present study, we examined the differences in blood-oxygen-level-dependent (BOLD) signal of the limbic system and the medial temporal lobe in two different functional tasks before and after injection of either cortisol or saline. The two tasks were chosen based on previously established activation patterns. One task, the affective face matching task, activates the limbic system by presenting fearful and angry face matching and labeling with a sensorimotor control task. The second task, a paired-associate, face-naming task involves the encoding and recall phases of memory. Since the limbic lobe processes emotions and has connections to the medial temporal lobe, which is responsible for memory, we hypothesized cortisol administration will feedback on the HPA axis to dampen the response in these areas.

Methods: In a double blind study, 24 healthy volunteers were presented with the face-matching and face-naming tasks before the administration of cortisol or saline. In the post-administration phase, the subjects received an intravenous injection of hydrocortisone or placebo after which they were presented with the same two functional tasks. A fMRI expert analysis tool (FEAT) was used to evaluate the individual and group data.

Results: In the face-matching task, the fusiform gyrus, posterior cingulate, and parahippocampus were hypoactivated, while the cingulate was hyperactivated in the cortisol group compared to the placebo group. For the face-naming task, the hippocampus and parahippocampus were hypoactivated in the encoding and recall phase. The cingulate and insula were the only two regions to show hyperactivation in the encoding phase and hypoactivation in the recall phase.

Conclusions: We found cortisol had widespread effects throughout the medial temporal lobe and limbic system, demonstrating the neurofunctional effects of exogenous cortisol.

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The functional connectivity of the human orbitofrontal cortex: A meta-analytic connectivity modeling (MACM) approach

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The localization of functions and processes of the orbitofrontal cortex (OFC) are largely unknown. The OFC is implicated in a range of pathologies such as post traumatic stress disorder, obsessive compulsive disorder, and drug addiction. It is the focus of many psychiatric tests and has held the interest of researchers for decades. Many of its wide ranging functions have been studied and are already included as parts of emotion, cognition and perception related pathways in the brain. We aim to identify a comprehensive model of the functional connectivity of the OFC, using a novel approach called Meta Analytic Connectivity Modeling (MACM). This method capitalizes on years of functional MRI (fMRI) and PET research to provide valuable information on how the OFC is functionally connected. Through comparisons with diffusion tensor imaging as well as data from current literature, we concurrently test the robustness MACM. In MACM, an anatomically defined outline of the OFC was used to search the BrainMap Database for coordinates of regions that have been co-activated with OFC activation. The data returned from the database was thresholded statistically and organized by behavioral domain. Results showed a surprising dominance of perception-based coactivations between the OFC and other brain regions. This supports Rolls et al. in further confirming the OFC as a sensory integration center. Results also revealed the functional connectivity of a vast array of regions with other behavior specific functions and processes. A Pubmed keyword search returned 37 recent fMRI studies of the OFC, which were not included in the BrainMap Database. The coactivation regions mentioned throughout these papers agreed with those taken from MACM and confirmed MACM's utility to map functional connectivity. Diffusion Tensor Imaging on 49 subjects was also used to find regions of coactivation and also confirm MACM's utility.

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A genetic test of a proposed model for the secondary structure of the coronavirus 3' untranslated region

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Mouse Hepatitis Virus (MHV) is a group two coronavirus of the *Coronaviradae* family. It is a positive sense, single-stranded RNA virus that is 32 kb long. MHV provides a good animal model virus for a plethora of human diseases, the most well known to be Severe Acute Respiratory Syndrome (SARS) which is caused by a related group two coronavirus. A detailed understanding of viral replication is needed both to understand viral pathogenesis in molecular terms and to develop antiviral drugs. It is well known from previous experiments that required cis-acting elements necessary for replication are located in the 3' untranslated region (3'UTR). A detailed understanding of the RNA secondary structures in this region of the virus must be gained to learn of its means of replication. Previous work by Zust et al. in 2007 proposed a secondary structure model in which the most 3' portion of the 3'UTR folds into a cruciform structure. Based on this model and our results with a previous set of mutants that should affect two arms of this structure, we hypothesized that at least one or the other stem is essential to viral replication. In this study, further mutants were created in the 3' UTR to confirm or reject the proposed model. We created complementary mutants to those created by Johnson et al. in 2005 as well as restorative mutants and a quadruple restorative mutant which restored all base pairing in both stems. The quadruple mutant is predicted to result in a structure identical to that of wild type MHV-A59. If the model and our hypothesis are correct, mutations that disrupt both stems will be lethal; mutants that disrupt one stem will moderately impair replication, as judged by plaque size and titer; and the quadruple mutant that maintains the structure will replicate as well, or nearly as well, as wild type virus. The results from our assays and growth curve suggest that the mutants that disrupt one side of the stem structure are viable but weaker in phenotype to that of wild type. The mutants that disrupt both stem structures result in nonviable virus, and the quadruple mutant is not statistically different from that of the wild type MHV-A59 in terms of titer. These results confirm the proposed model for the 3' UTR in MHV. Further studies are being done to analyze negative strand subgenomic mRNA production for the nonviable mutant.

Reference:

Zust, R., T. B. Miller, et al. (2008). "Genetic Interactions between an Essential 3' cis-Acting RNA Pseudoknot, Replicase Gene Products, and the Extreme 3' End of the Mouse Coronavirus Genome." J. Virol. **82**(3): 1214-1228.

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Comparison of Endotracheal Tube Cuff Pressures with Tracheal or Esophageal Intubation

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Introduction: While several techniques are available to detect proper placement of an endotracheal tube during surgery, none of them alone is sensitive or specific enough to guarantee proper placement, especially in an emergency department or out-of-hospital setting where CO_2 capnography may not be available. Due to the structural differences between the esophagus and the trachea, this study hopes to develop an easy, safe, and effective way to detect proper placement of the endotracheal tube using compliance differences between the trachea and the esophagus.

Hypothesis: Upon incremental increases in the endotracheal cuff volume, we expect the cuff pressure to be higher in esophageal intubations when compared to endotracheal intubations.

Methods: Approximately 50 patients at Scott & White Hospital in Temple, TX undergoing elective surgery requiring intubation will be anesthetized and both esophageally and endotracheally intubated in series. The cuff pressure changes will be compared at 1 mL increments as air is injected into the cuff.

Outcome Measures: The respective compliances in both the esophagus and the trachea as a function of cuff volume will be analyzed to determine if the cuff pressure can be used to potentially help identify inadvertent placement of endotracheal tubes into the esophagus.

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Ossification of Spinal Ligaments: A CT Investigation of a Proposed Link between Obesity and Calcification of the Osteoligamentous Structures of the Spine

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Introduction: Obesity is surging to epidemic levels within the United States, with nearly 40% of the population being described as obese or morbidly obese, according to CDC measures. Concurrently, the U.S. is seeing a rise in the diagnosis of type II Diabetes Mellitus (DM), as well as an alarming increase in the incidence of diabetes and obesity in children and young adults. Recent studies have linked excess adipose tissue with increased levels of circulating pro-inflammatory cytokines, which may predispose obese patients to a higher rate of developing an inflammatory disease. Pro-inflammatory cytokines and other mediators are known to influence bone formation, healing, and resorption. Hyperostotic conditions like DISH and OPLL, where spinal ligaments abnormally calcify, have been linked to an insulin resistance state and obesity. Given that excess adipose tissue and insulin-resistance may cause a systemic increase in serum levels of pro-inflammatory cytokines and other mediators, it is possible that spinal ligaments may have receptors for these signaling molecules that cause soft tissue to calcify over time. These calcifications can grow into the spinal canal, which may cause severe back pain, immobility, and paralysis.

Hypothesis: Clinically, Drs. Chaput and Rahm have noticed an increasing number of young, obese adults with unusual calcifications in the spine. Our hypothesis is that the increasing rate of obesity and associated co-morbidities such as type II diabetes and hypertension might play a role in this observation.

Methods: Of the one-thousand one-hundred seventy-four (1174) polytrauma patients in a Trauma 911/922 database from years 2006 through 2008, two-hundred fourteen (214) were ultimately included for the purpose of this study. Electronic medical records were searched for patients with documented age, gender, type II diabetes, and hypertension, and the data was recorded for patients aged 29 to 50 years, accordingly. Additionally, BMI was obtained and recorded using a novel method which assesses Truncal Adipose Volume (TAV) from usable CT reconstructions. Then, we viewed previously obtained CT images of the cervical, thoracic, and lumbar spine, taken in these polytrauma cases. At each intervertebral space, a score indicating the degree of calcification in the Anterior Longitudinal Ligament (ALL), Posterior Longitudinal Ligament (PLL), and the Yellow Ligament (LF) was assigned. Scores were added from the 21 levels for each of the three ligaments to assess the amount of calcification. Appropriate statistical evaluations were made to correlate abnormal spinal calcifications with: age, gender, insulin resistance state, obesity (BMI) determined by TAV, and hypertension.

Results: R-squared is a statistic associated with how much variation in the response variable is explained by explanatory variables. Age, Gender, BMI by TAV, and Type II were significantly associated with the calcification score total and the ALL calcification score; 31% of variation is explained by these models. Age and HTN were significantly associated with PLL calcifications; 9% of variation is explained by this model. BMI was significantly associated with LF calcifications; 16% of variation is explained by this model. Additional regression models are pending, but positive correlations are expected.

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Thymic-stromal Lymphopoietin Receptor Expression is Upregulated by IL3 in Eosinophils

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Thymic stromal lymphopoietin (TSLP) is an IL-7 like cytokine that serves as a master switch for activation of dendritic cells, Th2 cells, basophils and other cell types, resulting in varied immune responses such as allergies and asthma. Moreover, the multifunctional eosinophil is another critical cell type involved in the pathogenesis of allergic asthma. However, although TSLP and EOS are key players in the pathogenesis of this disease, the interplay between them has yet to be defined. In this study, we examined whether freshly-isolated EOS from peripheral blood expressed a functional TSLP receptor (TSLPR), and whether IL-3, IL-5, and GM-CSF could up-regulate its expression. Flow cytometry analysis revealed that the TSLPR is expressed constitutively on the cell surface of freshly-isolated EOS and is up-regulated in the presence of IL-3 in overnight cultures. IL-5 and GM-CSF, on the other hand, had marginal effects on the cell surface expression of the receptor under the same conditions. Furthermore, deconvolution fluorescence microscopy was used to confirm TSLPR expression, and revealed slight differences in trafficking patterns in response to all three cytokines. In sum, our data clearly demonstrate the presence of a functional TSLPR in EOS, and provide a biological connection between two important regulators of allergic inflammation.

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Preventing Diabetic Cardiomyopathy Through Inhibition of the Renin-Angiotensin System.

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Introduction: The renin-angiotensin system (RAS) is a hormone system that regulates blood pressure and fluid balance. Activation of the RAS has been strongly implicated in the etiology of the cardiovascular complications of diabetes. Angiotensin II, the primary effector of the RAS, generated either in the circulation (endocrine) or locally at tissue sites (autocrine/paracrine), mediates pathological effects primarily through the AT_1 receptor. Recent reports have found intracellular production and actions (intracrine) of angiotensin II, which are not blocked by commonly used RAS inhibitors, angiotensin converting enzyme (ACE) inhibitors and AT_1 blockers (ARBs). Aliskiren, a new drug released in 2007, is a direct renin inhibitor that blocks the circulatory, local, as well as the intracellular RAS. Intracellular angiotensin II production in the heart is up-regulated in diabetes. ACE inhibitors and ARBs have a diminished effect on blood pressure in diabetic patients. Intracellular angiotensin II induces cell growth and cardiac hypertrophy in mice that is not blocked by ARBs. These observations suggest that blocking of intracellular angiotensin II by a renin inhibitor may have better clinical benefits compared to ACE inhibitors and ARBs. The goal of this study is to determine which drug therapy would provide the best cardiac protection in diabetes.

Hypothesis: Aliskiren, which inhibits both extra- and intracellular angiotensin II synthesis, would provide better cardiac protection in diabetes, in contrast to ACE inhibitors or ARBs, which block only the extracellular renin-angiotensin system.

Methods: Diabetes was induced in mice by multiple intraperitoneal streptozotocin injections. Diabetic mice were treated with insulin, valsartan (ARB), benazepril (ACE inhibitor), or aliskiren (direct renin inhibitor). Hemodynamic and morphometric changes were measured using echocardiography and left ventricular catheterization. Fibrosis was quantified from tissue sections stained with picrosirius red and analyzed using image analysis software.

Results: Echocardiography data showed that diabetic mice developed significant cardiac dysfunction, as measured by mitral valve E/A fraction, isovolumetric relaxation time, left ventricle posterior wall diameter, ejection fraction, and fraction shortening. Aliskiren completely prevented the cardiac dysfunction and showed better performance in some parameters compared to ACE inhibitors and ARBs. The interstitial fibrosis was slightly increased in diabetic animals over controls (2.17 vs. 1.81 % area), with aliskiren and valsartan able to prevent the increase in fibrosis (1.75 and 1.73 % area, respectively). Data from benazapril treated mice are pending. While all the data have not been gathered, aliskiren shows promise as a better therapeutic agent in this animal model of diabetic cardiomyopathy.

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GENDER-RELATED DIFFERENCES IN THE GABA-A RECEPTOR SUBUNIT EXPRESSION IN THE HIPPOCAMPUS

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Introduction: Epilepsy is a debilitating neurological condition with recurrent unpredictable seizures that causes significant morbidity in men and women. Gender differences in seizure susceptibility are one of the longstanding issues in epilepsy research, at both the basic and clinical levels. Clinical evidence shows that the incidence of epilepsy is generally higher in men than in women. However, the precise mechanisms underlying the gender differences in seizure susceptibility are not clear. Gender differences in the development of seizure suppressing neuronal networks and steroid hormones and endogenous neurosteroids may partly account for sex related susceptibility to seizures. GABA-A receptors play a critical role in control of epileptic seizures. The subunit composition of GABA-A receptor determines its function in the hippocampus, a critical region for epilepsy.

Hypothesis: It is hypothesized that differential expression of GABA-A receptor subunits in the hippocampus contributes to gender differences in seizure susceptibility.

Methods: The TaqMan real-time PCR assay was used for quantification of GABA-A receptor (α1-4, γ2 and δ) subunit mRNA expression. Hippocampus was dissected from adult male and female mice. Total RNA was extracted from the hippocampus and cDNA was prepared for TaqMan PCR analysis. For each subunit gene, a set of primers and TaqMan fluorogenic probe were used to selectively amplify the target template. The threshold cycle (Ct), the point at which the fluorescence exceeds a threshold limit was used as key parameter for calculating input cDNA for relative quantification of the target subunit expression. The TaqMan PCR data was normalized in every assay using GAPDH as an internal standard. The data was analyzed by the Student's *t*-test.

Results: Gel electrophoresis of PCR products revealed bands at the expected range for each target subunit, confirming the specificity of primers. In the amplification plots, fluorescence intensity increased as the PCR cycles increased. A standard curve for GAPDH and one of the target genes, constructed using the cDNA, revealed slopes around−3.3 (r2 = 0.992), reflecti ng similar optimum PCR efficiencies. The δ-subunit expression was significantly (4-folds) increased in females when compared to males. There was a marked increase in the expression of α1 and γ2 subunits in females. In contrast, no changes in levels of α2 and α4 subunit expression were observed between genders.

Conclusion: These results suggest that GABA-A receptor subunits are expressed in a gender-dependent fashion.

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Analysis of the cellular distribution of mitochondria in cholinergic basal forebrain neurons during aging using electron microscopy

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Introduction: Previous results from our laboratory have demonstrated disruption in calcium (Ca^{2+}) signaling in cholinergic neurons of the rat basal forebrain during aging (Murchison et al., 2009). One mechanism that could contribute to these age-related changes is the ability of mitochondria to buffer intracellular Ca^{2+} concentrations. The purpose of this study is to utilize electron microscopy to examine the size and location of mitochondria within cholinergic neurons in the basal forebrain in mice of different ages. These neurons play an important role in memory and cognition, and in humans, may contribute to the cognitive decline associated with age-related disorders such as Alzheimer's disease. A better understanding how these neurons change with age will be an important step in developing potential therapeutics for this disease.

Hypothesis: We hypothesize that mitochondria size, location and number will increase during aging and this increase will be greatest in cholinergic versus non-cholinergic neurons.

Methods: BAC transgenic mice expressing enhanced green fluorescent protein (eGFP) were used and divided into three groups based on age: young (3-6 mo), middle-aged (9-12 mo) and aged (>20 mo). Each mouse was perfused with 4% paraformaldehyde/0.25% glutaraldehyde and the brain was removed. 175 um thick sections cross sections of whole brain were cut using a vibratome and cholinergic cells were identified using diaminobenzidine (DAB) immunohistochemistry. Slices were flat imbedded in araldite plastic and the basal forebrain was removed and imbedded in bullets of araldite. Ultra thin sections were cut on the surface of the tissue containing DAB-stained cells and were subsequently stained with lead nitrate. Both DAB-stained (cholinergic) and non-stained (non-cholinergic) neurons were photographed using a FEI Morgagni 268 Transmission Electron Microscope. Neurons were identified as cells with well defined nuclear and plasma membranes and containing organelles. Image J was used to measure mitochondrial parameters such as percent area of cytoplasm occupied by mitochondria, number of mitochondria, and distance of mitochondria to plasma and nuclear membrane.

Results: A total of 24 mice of different ages have been perfused and processed for detailed EM analysis. Preliminary results demonstrate DAB staining successfully distinguishes cholinergic and non-cholinergic neurons at different ages at this EM level. Analyses of mitochondrial parameters are in progress. A future study will investigate these same parameters in behaviorally-characterized mice.

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Metaanalytic Connectivity Modeling of the Human Thalamus: Developing a Comprehensive Model of Structural and Functional Connectivity

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Introduction: The thalamus is a large complex structure composed of many nuclei which function as important relay and integrative centers for information processing between areas of the brain. The thalamus is also implicated in many neuropathological as well as neurodegenerative diseases. Because of its important role in normal function and disease states of the brain, the thalamus is well studied as are its functional and structural connections. We've replicated a robust and comprehensive technique called Metaanalytic Connectivity Modeling (MACM) in the current study to explore these connections.

Hypothesis: The robust nature of MACM affords a precise understanding of the brain's global functional connectivity with the thalamus and a particular understanding of these connections specific to behavioral domains, setting up a comprehensive foundation from which further exploration of thalamic connectivity can progress.

Methods: We used the Harvard-Oxford structural probability atlas to define the thalamus as an anatomically defined region of interest (ROI). We then seeded the BrainMap database with the designated ROI and downloaded standardized coordinates from functional neuroimaging studies reporting activation of the thalamus. Coactivation with other brain structures was determined via an activation likelihood estimation (ALE) metaanalysis. We were also able to distinguish functional activation of the thalamus within behavioral domains using a similar process by further restricting the studies downloaded to those of the domain specified. Finally, we used Diffusion Tensor Imaging (DTI) to explore the structural connectivity of the thalamus.

Results: Our results of the functional and structural connectivity of the thalamus were consistent with the literature, and have the advantage of providing comprehensive connectivity data based on an anatomically defined ROI. We hope that these results can provide a foundation from which further studies of thalamic connectivity can expand.

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Perlecan Domain V Effects on Alzheimer's Disease

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Texas A&M Health Science Center College of Medicine-College Station

Alzheimer's Disease is an irreversible and progressive disease that causes patients to have memory loss and a slow decline in thinking and reasoning skills. It affects over 5 million people worldwide usually 65 years, and older although inherited familial mutations can result in earlier onset of the disease. At the cellular level, the amyloid beta (Aβ) peptide in amyloid plaques is a key pathological feature of Alzheimer's disease. Aβ plaque deposition and neuronal toxicity can be modeled *in vitro* by treating cortical neuronal cultures with Aβ and demonstrating robust Aβ deposition and neurotoxicity. We have recently demonstrated that this Aβ neurotoxicity is mediated by the neuronal α2β1 integrin receptor. We now hypothesize that other ligands for these receptors, such as the extracellular matrix component Perlecan Domain V (DV) which binds to α2β1, could interfere with Aβ neurotoxicity and thereby represent a novel therapeutic approach to Alzheimer's. Furthermore, such a naturally derived therapy should be well tolerated. In this study, we used a two-pronged approach to investigate DV's potential as a therapy in Alzheimer's disease. First, we determined whether DV was able to cross the blood-brain barrier in an Alzheimer's disease mouse model and how long DV remained in the brain. Secondly, we examined the effect of DV on the Aβ signaling pathway in mouse fetal cortical neurons. Several techniques were utilized including small animal imaging, protein concentration assays, and western blots. We determined that DV was able to cross the blood-brain barrier and remain in the brain for up to 7 days. Additionally, DV inhibited downstream steps in the Aβ signaling cascade preventing the activation of molecules involved in neurotoxicity.

TAMHSC SUMMER RESEARCH PROGRAM

August 18, 2010: 9:00 AM - 2:00 PM

Reynolds Medical Building, College of Medicine

A Retrospective Analysis of TemozolomideTreatment for Glioblastoma Multiforme Patients in Brazos County

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Texas A & M University Health Science Center College of Medicine

Introduction and Background: Glioblastoma multiforme is the most lethal primary brain tumor in adults, with a median survival time of 10 months after surgical tumor resection and radiation therapy up to 60 Gy. In 2005, a paper published in the New England Journal of Medicine asserted that treating patients with both temozolomide and radiation therapy increased survival time by a median value of 2.5 months after surgical resection. Currently, temozolomide plus radiotherapy is the standard of care for patients who have already undergone tumor resection surgery. However, it has been five years since the original study using temozolomide for glioblastomas (GBM) was published and little research describes how temozolomide affects survival time with older patients, those who have worse performance statuses, or who have not had tumor resection surgery. The aim of this study is to determine how temozolomide has impacted the survival times of GBM patients in a regionally defined population such as those patients who were treated for GBMs at St. Joseph's Regional Health Center in Bryan, Texas.

Hypothesis: Temozolomide will increase median survival time by 1-2 months. A lower survival benefit was expected compared to the study published in the New England Journal of Medicine due to patient population differences.

Methods: At the St. Joseph's Cancer Center (Bryan, TX) between 1996 and 2010, 13 GBM patients who completed a treatment course of radiation therapy alone and 24 GBM patients who completed a course of radiation therapy with concurrent temozolomide were analyzed. Patient survival time, demographic data, tumor size, tumor location, Karnofsky performance status, surgical resection status, neurological deficits before treatment, duration of neurologic symptoms, radiation dose received, and co-morbidities were recorded. The survival time was calculated from the time the GBM was first diagnosed to the date that the patient expired. Life tables for the two groups were formed to determine the median survival times. Kaplan-Meier survival curves were constructed with death being the endpoint. A Cox proportional hazards analysis was conducted to measure the impact of patient age, performance status, temozolomide use, and surgical resection on survival.

Results: The death dates for 13 (100%) patients in the radiotherapy-only group and 20 (84%) patients in the radiotherapy-plus-temozolomide group were recorded. The median survival times were 7.0 and 7.1 months for the radiation-only group and the radiotherapy-plus-temozolomide group, respectively. The adjusted hazard ratio for death in the radation-plus-temozolomide group was 1.0 (95% confidence interval, 0.94 to 1.06; $P < 0.88$ by Cox proportional hazards analysis).

Conclusion: Although the results of this study are preliminary, there was no statistically significant survival benefit for GBM patients who were given radiation plus temozolomide as opposed to radiation alone. The sample size will need to be increased in order to improve the statistical power of this study. The ultimate goal is to create a recursive partitioning analysis that will stratify GBM patients into different groups based on their prognostic factors. The recursive partitioning analysis will then help physicians determine which GBM patients are likely to benefit from radiotherapy plus temozolomide and which patients are not.

TAMHSC SUMMER RESEARCH PROGRAM

August 18, 2010: 9:00 AM - 2:00 PM

Reynolds Medical Building, College of Medicine

Determination of Cardiovascular Function in the Normal Developing Mouse Heart Using High Frequency Ultrasound Biomicroscopy

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Introduction: Mice are proven to be an excellent model system for studying human heart diseases. Many of the major congenital heart anomalies seen clinically can be reproduced using transgenic mouse models. Echocardiography has become invaluable for cardiovascular assessments in mouse models. However, the small size of the fetal mouse heart has made echocardiography of fetal mice technically challenging.

Specific Aim and Relevance: We used high resolution echocardiography to establish a developmental profile of cardiac functions of *in utero* C57BL/6J fetal mice from embryonic day 8.5 (E8.5) to 18.5 (term). These included cardiac anatomy as well as systolic and diastolic functions. Knowledge of baseline functions in wild type C57Bl/6J mice will help us to identify diastolic and systolic dysfunction in transgenic mice.

Methods: This study used the Vevo 2100 system with color Doppler capability with a linear phased array MS400 transducer (18-38 Mhz) (Visual Sonics Inc., Toronto, Ontario, Canada). Changes in cardiac geometry and function were obtained serially in C57BL/6J mice from E8.5-E18.5 (term). The gestational age of the fetal mice was calculated postnatally based on the date of delivery. All measurements were taken during systole and diastole. The parasternal long-axis view (PSLAX) was used to evaluate the thickness of the inter-ventricular septum (IVS), left ventricular internal diameter (LVID) and left ventricular posterior wall thickness (LVPW). Ejection fraction percentage (EF %) was derived from the B-mode images using Simpsons rule. The diameter of the left ventricular outflow tract (LVOT) was measured most often on PSLAX. M-mode was used to calculate left ventricular mass (LVMASS), and left ventricular volume (LVVOL). LVOT velocity time integral (LVOT VTI) was subsequently calculated using the PW Doppler close to the aortic valves. RVOT VTI was calculated by measuring the flow across the pulmonary trunk. The CO was determined from the formula 0.785 * (LVOT diameter) ^2) * LVOT VTI * HR. The early diastolic (E) and late diastolic atrial (A) waves were evaluated using PW Doppler on the mitral annulus. Aortic ejection time (AET), isovolumic relaxation time (IVRT), no flow time (NFT), E wave deceleration (Edecel) and deceleration time (decelT) were evaluated at the tip of the mitral leaflets.

Results: Our analysis showed that average heart rate increased from 38.875 beats/min (E8.5) to 142.666 beats/min (E18.5). Concurrent peak outflow velocity, E-wave, E/A ratio and ventricular dimensions also increased from E8.5 to E18.5. E-waves in embryos increased from 39.976 mm/sec (E8.5) to 118.701 mm/sec by term. Conversely, E/A ratio followed a sinusoidal pattern with high ratios (0.66) at E8.5, lower ratios at E12.5 (0.216) and higher ratios by term (0.470). Physical dimensions of the heart such as LVVOLD (0.092-2.754), LVVOLS (0.038-0.947), LVMASS (0.224-3.848), LVIDD (0.288-1.040), and LVIDS (0.225-0.679) all increased from E8.5 to E18.5. The A-waves also followed a sinusoidal pattern (236.975 at E9.5, 584.884 at E13.5 and 256.605 at E18.5). Systolic function remained stable. Ejection fraction and fractional shortening remained steady around 71.14% and 43.22%, respectively.

Conclusion: These results could serve as a standard for evaluating cardiovascular pathophysiology in transgenic mice models. The ability to correlate cardiovascular physiology phenotype with corresponding genotype should provide insight into mice embryogenesis, disease and death. The use of ~~a~~ non-invasive echocardiography can reveal physical cardiac defects that may precede structural damage.

TAMHSC SUMMER RESEARCH PROGRAM

August 18, 2010: 9:00 AM - 2:00 PM

Reynolds Medical Building, College of Medicine

Alcohol and/or Nicotine Exposure: A Risk Factor for Autism Spectrum Disorder?

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Texas A & M Health Science Center College of Medicine, College Station, TX 77843

Introduction: Women continue to consume alcohol and use tobacco products during pregnancy despite obvious warnings from various government agencies. While not all offspring show uniform deformities in physical morphology when exposed prenatally to alcohol or nicotine, neurodevelopment may be severely affected by exposure to these drugs leading to a predisposition to neurodevelopmental disorders. To demonstrate this link, we will investigate the genes that are suggested to be involved in Autism spectrum disorder (ASD). ASD affects thousands of children each year and while the exact causes are still unknown, a number of genes, such as FoxP2 and MecP2, may play a role in the manifestation of ASD. Foxp2 is involved in specific language impairment which is a characteristic of ASD; and Mecp2 gene duplication has been found in individuals with autism. Therefore, the expression of these genes, Foxp2 and Mecp2, were evaluated following exposure of embryos to alcohol and/or nicotine.

Hypothesis: We hypothesize that exposure to alcohol and/or nicotine during gastrulation may predispose the offspring to neurodevelopmental disorders by altering the expression of genes associated with the disorder. We will use genes identified with defects related to ASD as a measurement to study the link between early prenatal alcohol and/or nicotine exposure and ASD.

Methods: Zebrafish embryos were exposed to 2% (w/v) alcohol, 40 µM nicotine, the combination of alcohol/nicotine or artificial salt water (control) beginning at 3.5 hours post-fertilization (hpf) and the treatment lasted for 4 hours during the gastrulation stage of the zebrafish embryo development. The embryos were sacrificed at 48 hpf and collected for RNA quantification using Real-time PCR.

Results: Analysis showed marginal changes in gene expression when comparing both alcohol and nicotine groups to the control group. Although the differences were not significant statistically, the physiological impact of such changes on embryonic and neuro-development may not be overlooked. It is important to note that timing may be critical in revealing the differences in the expression of these genes due to the transient nature in the expression of specific genes during development. Moreover, the degree of severity affected by drugs varied among embryos in this study; thus, future RNA analysis performed on embryos categorized by the severity of morphological deformities may offer more insights regarding how alcohol or nicotine interferes with the expression of genes associated with ASD.

TAMHSC SUMMER RESEARCH PROGRAM

August 18, 2010: 9:00 AM - 2:00 PM

Reynolds Medical Building, College of Medicine

Effect of Survival of Motor Neuron gene on Spinal Muscular Atrophy

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Texas A & M University Health Science Center College of Medicine, College Station

Introduction: Spinal Muscular Atrophy (SMA) is an autosomal recessive disease that targets motor neurons. The Survival of Motor Neuron (SMN) gene produces protein that is essential to all cells but that appears integral to the survival of motor neurons. Reduced levels of SMN cause the SMA disease. The death of motor neurons adversely affects the activity of the muscles, causing difficulty in physical movements. This disease affects approximately 1 in 10,000 people, and approximately 1 in 50 people are carriers for it. In the human genome, two SMN genes exist, SMN1 and SMN2. Mutations in SMN1 lead to SMA. The two genes slightly differ on position six of exon seven. This variation causes the SMN2 gene to produce a different version of the protein, SMN- 7, which is unstable and almost non-functional. The production of the protein from the slightly different SMN2 gene, therefore, mainly decreases the severity of the phenotypic effects of SMA. While the physical effects of SMA can be partially alleviated by various means, no direct and accepted cure for SMA exists at this point in time.

Hypothesis: Would expression of the SMN gene only in motor neurons be sufficient to rescue SMA model mice? The expectation is that it would be.

Methods: For this project, transgenic mice that express human SMN under the control of the motor neuron-specific promoter Hb9 were generated in the Rimer lab. These animals also carry a GFP cDNA cloned downstream of the hSMN transgene that is expressed under the same Hb9 promoter. The transgenic mice are being crossed with SMA carrier mice, which are heterozygous for SMN1 and homozygous for SMN2. DNA extraction was used for obtaining DNA samples from the tails of the mice. The presence of the SMN1 gene, of the SMN2 gene, and of the hSMN transgene was tested for via the polymerase chain reaction (PCR) and agarose gel electrophoresis. Also, the Western blot was used for protein level analysis. Animal crosses were set up to manipulate the mouse genotypes so that the hypothesis could be tested. In this project, other various methods and tools were used to help with the data accumulation of the mice we worked with. For example, using a cryostat, tissue sections of the mice were made. Following this, the slides were immunostained using antibodies.

Results: The project is currently ongoing and as we come closer to reaching the desired genotype of the mice, more detailed results will be expected. To test the hypothesis, the following genotypes are needed: GFP+/-; Smn -/- ; SMN2 +/+ and GFP-/-; Smn-/-; SMN2+/+. The first organism is expected to survive and not be affected by SMA. The second organism is expected to suffer from SMA and will therefore be used for comparison purposes.

References:

Lunn MR, Wang CH (2008) Spinal muscular atrophy. The Lancet 371:2120-2133.

Wilson R, Ogino S (2008) Carrier frequency of spinal muscular atrophy. The Lancet 372:1542.

TAMHSC SUMMER RESEARCH PROGRAM

August 18, 2010: 9:00 AM - 2:00 PM

Reynolds Medical Building, College of Medicine

The Role of Double-stranded RNA Receptors in Hypertensive Disorders of Pregnancy

Weaver LE, Chatterjee P, Mitchell BM

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Introduction: Hypertensive disorders of pregnancy (HDPs) affect approximately 15% of pregnancies worldwide. The maternal immune system has been found to play an important role in the pathogenesis of HDPs. Double-stranded (ds) RNA receptors, including TLR3, RIG-I, and MDA-5, play a major role in the innate immune system by recognizing dsRNA expressed by viruses or released from necrotic tissue which then initiates a pro-inflammatory immune response. Whether or not these three dsRNA receptors play a role in the development of HDPs has yet to be fully investigated.

Specific Aims: The first aim was to determine differential changes in gene and protein expression related to dsRNA receptor activation in human CTB cells stimulated with poly I:C, a synthetic dsRNA mimetic. The second aim was to determine changes in gene and protein expression of dsRNA receptors in placentas of mice with gestational hypertension (GH). The final aim was to examine changes in gene and protein expression of dsRNA receptors in placentas from women with HDPs.

Methods: CTB cells were cultured in monolayers and treated with 10 μg of poly I:C. Mice were given intraperitoneal injections of poly I:C and sacrificed on either gestational day 14 or gestational day 18. Protein and mRNA were isolated and analyzed by immunoblotting and qRT-PCR, respectively. Human placentas in paraffin blocks were obtained from Scott & White Department of Pathology. Histological samples were prepared and immunohistochemistry performed.

Results: Poly I:C increased RIG-I, MDA-5, and TLR3 expression in CTB cells. In pregnant mice, poly I:C treatment caused a significant increase in systolic blood pressure as well as increased placental protein and mRNA expression of RIG-I, MDA-5, and TLR3. Finally, 4/5 human placental samples from women with HDPs demonstrated an increased expression of all three dsRNA receptors compared to controls.

Conclusions: Taken together, data from cells, mice, and humans suggest that dsRNA receptors play a role in HDPs. Future studies will determine the individual role of each dsRNA receptor in the development of HDPs. This is the first study to demonstrate a relationship between activation of RIG-I, MDA-5, and TLR3 and HDPs and may provide a novel therapeutic target for the treatment of HDPs.

TAMHSC SUMMER RESEARCH PROGRAM

August 18, 2010: 9:00 AM - 2:00 PM

Reynolds Medical Building, College of Medicine

Horizontal Gene Transfer of an Integrative and Conjugative Element (ICE*Bs*1) from *Bacillus subtilis* to *Mycobacterium smegmatis*

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Microbial and Molecular Pathogenesis
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Introduction: Mobile genetic elements contribute to bacterial evolution. One such element in *Bacillus subtilis*, ICE*Bs*1, has been shown to be a functional integrative and conjugative element capable of transferring not only between *Bacillus* species but also across genera to *Listeria monocytogenes*. ICE*Bs*1 is regulated by quorum sensing, with expression induced by an excess of bacteria that do not carry the element.

Hypothesis: We investigated whether ICE*Bs*1 from *B. subtilis* can be transferred to *Mycobacterium smegmatis* by conjugation. If successful, this method will provide a valuable tool for genetically manipulating *Mycobacterium tuberculosis,* the causative agent of tuberculosis, which currently afflicts 1/3rd of the world's population.

Methods: All organisms were cultured in LB media or on LB plates. Tests were carried out to establish the antibiotic susceptibility of the *B. subtilis* donor and the *M. smegmatis* recipient strains by plating on varying concentrations of antibiotics. Based upon these results, the following antibiotic concentrations were used to select for the donor, recipient, and any *M. smegmatis* transconjugants containing ICE*Bs*1: kanamycin at 10 μg ml^{-1} for the donor strain, ampicillin at 200 or 500 μg ml^{-1} for the recipient strain, and kanamycin+ampicillin for any transconjugants. Growth curves were used to determine the number of colony forming units corresponding to a given optical density (OD, λ = 600 nm). At the predetermined OD_{600}'s to give 1:100 donor:recipient, the bacteria were mixed and placed on a 0.45 nm pore filter and incubated on a defined mycobacterial minimal media, Proskauer-Beck, or on LB for 24 hours to allow conjugation to occur. Bacteria were resuspended in liquid media and plated on the above antibiotic plates to select for transconjugants.

Results: We have successfully observed transconjugants with Bacillus, but have not observed transconjugants in *M. smegmatis*. These observations are most likely due to the low efficiency of intergenera conjugation. The presence of ICE*Bs*1 in any colonies of *M. smegmatis* arising on the transconjugant plates will ultimately be confirmed by PCR, Southern analysis, and sequencing.

Summer Research Program Participants (M1s) 2010

College Station -4
Temple – 14
Houston - 2

M1 Student	Mentor	Department/Affiliation
Abram Albizo	Frankel	Internal Medicine/Temple
Brian Allgaier	Toussaint & Friedman	NEXT/Inst Brain & Spine
Emelia Bittenbinder	Meininger	Systems Biology/Temple
Bryce Busenlehner	Chaput	Orthro/Temple
Angela Cortez	Huston	Medicine/Micro Houston
Lessie Eric Golden	DeMorrow	Medicine/Temple
Cory Henson	Huston	Medicine/Micro Houston
Matthew Jenson	Tesh	MMPA
Sandy Lee	Robinson	Neuroscience Inst S & W/Temple
Rishi Malla	Robinson	Neuroscience Inst S & W/Temple
Varghese Mathukcutty	Culp	Medicine/Anesthesiology/Temple
Sachin Mahta	Chaput	Orthro/Temple
Preston Milburn	Baker	Medicine/Temple
Samantha Otokunrin	Asea	Pathology/Temple
Jonathan Seale	Sanghera	Neuroscience Inst S&W/Temple
Alan Sheydwasser	Toussaint & Friedman	NEXT Inst Brain and Spine
Suraj Sunder	Dostal	Medicine/Temple
Alan Sutak	Chen	NEXT
Laura Weaver	Mitchell	Medicine/Temple
Brandon Williamson	Cirillo	MMPA

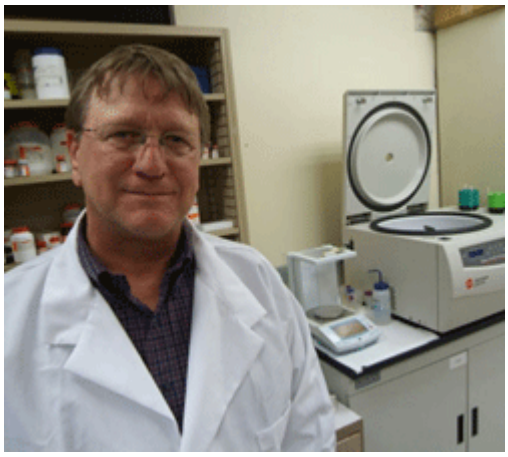
Summer Research Program Participants (Undergrads) - 2010

Student	School	Mentor	Depart
Nicole Canon	UT Dallas	Mingyao Liu	IBT-HOU
Soumili Chatterjee	University of Houston	Jun-yuan Ji	MCMD
Matt Evans	St. Olaf College	Gumienny	MCMD
Jordan Gould	TAMU	Reddy	NEXT
Faryal Masud	TAMU	Leibowitz	MMPA
Andrew Menezes	St. Mary's University	Huston	IBT-HOU
Varsha Pawate	TAMU	Reddy	NEXT
Matthew Schilling	TAMU	Griffith	NEXT
Akanksha Sarma	TX Acad. of Math & Sci	Bix	MCMD
Trivedi Akash	Lamar University	Rimer	NEXT

2010 Summer Research Program

Sites: College Station: 160 Reynolds , TPL: R213, Houston IBT: 1105 ***(updated: 5/27/10)***

DATE	TOPIC	PRESENTER
June 8; noon	Record Keeping	Dr. Van Wilson
June 11; 9:00 am	Scientific Method	Dr. David McMurray
June 15; noon	Graduate program	Dr. Emily Wilson and grad students
June 18; 9:00 am	Biotechnology/ethics	Dr. James Samuel
June 22,; noon	Scientific Misconduct	Dr. Vernon Tesh
June 25; 9:00 am	Medical Research…Why Me?	Dr. W.C. Culp, Jr., M.D.
June 29;	Acromegaly/Endocrine Clinical Research	Dr. Robert Gagel, Head, UT-MDA
July 2; 9:00 am	Human Infectious Disease Research	Dr. Edward A. Graviss, MRI
July 6; noon	MD/PhD Program	Dr. Leibowitz and MD/PhD students
	Molecular Pathogenesis	Dr. James Samuel
July 9; 9:00 am	Animal Research	Dr. Andrews-Polymenis
July 13; noon	Neuroscience	Dr. Rajesh Miranda
July 16; 9:00 am	Commercialization in Medicine	Bruce Leander, Retired President, Ambion, Inc.
July 20; noon	Translational Research	Dr. David Huston
July 23; 9:00 am	Human Experimentation	Dr. John Quarles
July 27; noon	Cardiovascular Research	Dr. David Dostal
July 30; 9:00 am	Cell and Mol Biol	Dr. Kayla Bayless
	Biochem and Structural Biol	Dr. Sarah Bondos
Aug 3; noon	Student Oral Presentations	
Aug 6; 9:00 am	Student Oral Presentations	
Aug 10; noon	Student Oral Presentations	
Aug 13; 9:00 am	Student Oral Presentations	
Aug 18; 9:00 am – 2:00 pm	ALL - Poster Presentations and Reception - Reynolds Medical Building	



Program Director
Dr. Warren E. Zimmer, Scott Exter Professor

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