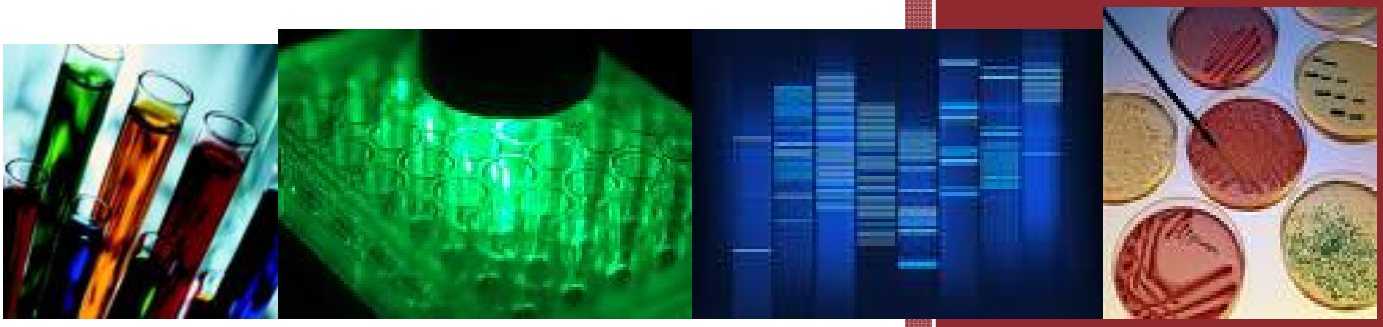
Poster Session and Reception

2012

Summer Research Program



August 08, 2012
9:00am-2:00pm
Health Professions Education Building
Bryan, TX

TEXAS A&M
HEALTH SCIENCE CENTER
COLLEGE OF MEDICINE

Schedule of Events

August 08, 2012

8:30am	**Registration Table Opens** HPEB L1 Lobby
9:00am-2:00pm	**Poster Presentations** HPEB LL43 A&B
11:30am	**Lunch** HPEB L1 Lobby
12:15-1:00pm	**Keynote Speaker** T. Samuel Shoemaker, M.D., J.D. *Research and You: Why you should care about randomized controlled trials* HPEB LL46
1:00-1:30pm	**Presentation of Certificates** Dr. Warren Zimmer, Summer Research Program Director HPEB LL46
1:30-1:45pm	**Presentation of Dean's Recognition Awards** HPEB LL46
2:00pm	**Adjourn**

Speaker's Biography

T. Samuel Shomaker, M.D., J.D.
The Jean and Thomas McMullin Dean of Medicine and Vice President for Clinical Affairs
Texas A&M Health Science Center College of Medicine
Bryan, TX

Dr. Sam Shomaker currently serves as The Jean and Thomas McMullin Dean of Medicine and Vice President for Clinical Affairs with the Texas A&M Health Science Center College of Medicine. In addition, Dr. Shomaker has an appointment as tenured professor in the department of Anesthesiology.

Previously, Dr. Shomaker was professor of anesthesiology at the University of Texas Medical Branch and Chancellor's Health Fellow at the University of Texas System. He served as Dean of Austin Programs for UTMB, Vice Dean and Interim Dean of the University of Hawaii School of Medicine and Interim Dean and Senior Associate Dean of the University of Utah College of Medicine.

He holds a Bachelor's degree from St. Louis University, a law degree from Georgetown University and an MD from the University of Hawaii. He completed residency training in anesthesiology at the University of Utah and the University of Florida. His research interest is health policy.

Dr. Shomaker is married to Dr. Suzanne Yandow, a pediatric orthopaedic surgeon. They have three children; Simone, Dylan and Garrett.

Acknowledgements

The Summer Research Program continues to improve each year. This year we have truly "world wide" attendance with participants from Scotland, Virgin Islands, and Puerto Rico. In addition, with input from Dr. Sam Shomaker, Dean COM, and Mr. Jack Heart, Temple Health and Bioscience District, we have created two new programs, the Prairie View Scholars and the THBD Scholars to expand our student population. These changes gave us the strongest application pool in my 5 year tenure as Director, with well over 100 applications for the 30 summer positions (20 medical students and 10 undergraduate students). I am totally thankful for the faculty that give graciously of their time to help the program through serving on the committee that reads and evaluates each application to get the strongest students each summer; presenting stimulating and informative lectures; and allowing students to work in their labs. These latter interactions have also provided the strong mentoring that has allowed the program to operate at the highest levels. Again, the faculty should get the loudest "round of applause" for their continued strong commitment and involvement in the program; The program would not be able to sustain its quality and existence without them.

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

We obtained funding from a number of sources and would like to thank Dr. Shomaker, College Dean; Dr. Wesson, Vice Dean of the Temple Campus (Scott and White Research); Mr. Jack Heart and the Temple Health and Bioscience District Board; 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 many department chairs, both clinical and basic science. It is difficult, but not impossible, to provide content simultaneously to three locations. The work of Drs. Murray (College Station), Mitchell (Temple) and Huston (Houston) as site coordinators keeping things running efficiently is greatly appreciated. Finally, I would like to thank Dr. Van Wilson and his staff in College Station, **Rachel Levins, Tarah Kennedy, Kaelan Henze, Mary Ann Wolff, and Peggy Hazelwood;** Dr. Huston's staff in Houston, **Anna Wirt and Karol Franks;** 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

Abstracts

TAMHSC SUMMER RESEARCH PROGRAM

August 8, 2012: 9:00 AM - 2:00 PM
Health Professions Education Building
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Effect of Neurosteroid Treatment on Spontaneous Seizures in a Rat Model of Temporal Lobe Epilepsy

Pydi Akella, Ramkumar Kuruba and D. Samba Reddy*
Department of Neuroscience and Experimental Therapeutics,
Texas A & M University Health Science Center College of Medicine, Bryan, Texas

Introduction & Background: Temporal lobe epilepsy (TLE) is one of the most common forms of intractable epilepsy. TLE is characterized by an initial injury followed by chronic epileptic state displaying as spontaneous seizures after a latent period of few months to years. These features are expressed in pilocarpine-treated rats, which represent a suitable model of TLE. Neurosteroids are endogenous modulators of seizure susceptibility. Allopregnanolone-like neurosteroids are synthesized in the brain and activate GABA-A receptors with powerful anticonvulsant properties. However, the therapeutic potential of neurosteroids has not been established in animal models of TLE.

Hypothesis & Objective: It is hypothesized that neurosteroids that enhance GABAergic inhibition possess significant antiepileptic properties. In this study, we tested the efficacy of ganaxolone in the rat pilocarpine model of TLE with spontaneous seizures. Ganaxolone is a synthetic analog of allopregnanolone and a positive modulator of GABA-A receptors and could be new treatment option for chronic epilepsy.

Materials and Methods: Epilepsy was induced in rats by treatment with pilocarpine (20 mg/kg, ip). Rats were monitored for the occurrence of seizures during 4 months post pilocarpine treatment. EEG seizure activity was verified by recording of EEG from hippocampus and frontal cortex. The efficacy of ganaxolone (5 mg/kg, s.c. for 7 days) was evaluated in rats with frequent seizures. Timm staining of brain sections was performed to assess the extent of aberrant mossy fiber sprouting in the hippocampus.

Results: Pilocarpine treatment was associated with progressively increased seizures and high-amplitude EEG spikes. Spontaneous seizures were evident after latency of 30 to 50 days post pilocarpine. At three months post pilocarpine, majority of rats exhibited bilateral neurodegeneration and a robust aberrant mossy fiber sprouting, an index of epileptogenesis. Electrographic seizure discharges were well correlated with behavioral seizures. Ganaxolone therapy significantly decreased the severity (duration) of seizures, but it has no effect on the frequency of spontaneous seizures. It suppressed seizures in 2 of 4 rats tested in the study.

Conclusions: In conclusion, pilocarpine model of epilepsy in rats is associated with frequent spontaneous seizures, EEG spikes and mossy fiber sprouting, which are clinical features of TLE in humans. Pilocarpine model was selected because it replicates several clinical features of human epilepsy and allows testing new drugs on the frequency and severity of spontaneous seizures. Pilot data shows that ganaxolone treatment reduced the severity of seizures without diminishing the frequency of seizures in epilepsy rats. Further studies in large cohort group are needed to establish the efficacy of neurosteroid therapy in TLE.

** *Supported by NIH Grants NS052158 & NS071597 (to Dr. Reddy)***

TAMHSC SUMMER RESEARCH PROGRAM

August 8, 2012: 9:00 AM - 2:00 PM
Health Professions Education Building
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Age-related differences in blood microRNA expression profile in an ischemic stroke model

A. Baker, A. Selvamani, F. Sohrabji
Department of Neuroscience and Experimental Therapeutics
Texas A & M University Health Science Center College of Medicine
Bryan, TX

Introduction: MicroRNAs (miRNAs) are small, non-coding RNA molecules of 18-25 nucleotides that function as translational repressors by binding to complementary messenger RNAs (mRNAs), thus inhibiting translation and/or degradation of mRNA. Several studies show that their expression levels in plasma or serum are indicative of disease state, which makes them an attractive candidate as a diagnostic marker. Several studies have recently found identified stroke-induced miRNAs in blood and plasma in both experimental stroke models and in stroke patients.

Previous work from our lab indicates that following focal cerebral ischemia, older females sustain a larger infarct as compared to younger females (Selvamani and Sohrabji, 2010), however this difference is not observed in age-matched males. Ischemia results in the induction or suppression of a large number of genes, and since a single miRNA has the potential to regulate hundreds of target genes, specific miRNAs could be differentially regulated in younger versus aging females.

Hypothesis: This study aimed to compare the unique miRNA profiles and regulation differences in 2 age groups, thereby evaluating which candidate miRNAs could serve as biomarkers.

Methods: We examined the profiles of miRNA extracted from 2 groups of rats that model the pre- and post-menopausal female. Females were purchased as proven breeders 6-7 months old (young adults) and as retired breeders 9-11 months old (middle-aged). All animals were subject to stroke and allowed to survive for 5d post stroke. At termination, plasma was obtained from intact young adults and middle-aged females (n=6 in each group). Qiagen miRNeasy Mini Kit was used for total RNA isolation from plasma. Total RNA was quantified using NanoDrop 2000 Spectrophotometer, and Exiqon Universal cDNA Synthesis Kit was used to synthesize cDNA. For RT-PCR amplification, Exiqon SYBR Green master mix Universal RT was used in conjunction with Exiqon focus panels containing miRCURY LNA Universal RT microRNA PCR assays for microRNAs found in serum and plasma. Each focus panel contained 168 LNA microRNA primer sets that focus on serum/plasma relevant human microRNAs and 7 reference microRNAs. The focus panels were used with ABI 7900HT for amplification. Candidate miRNA were normalized to reference miRNA (dCT) and group differences were analyzed by t-test using the Benjamini-Hochberg correction for multiple comparisons.

Results: The data indicated that a small subset of these miRNA had significantly different expression patterns in the young and middle-aged groups. Specifically, miRNAs -1974, -885-5p, -95, -374a, -144, -151-3p, -363, -106b, -182, -338-3p, -26a, -33a, -17, -101 were upregulated in the young female group, while miRNAs -92b, -205, -29a, -200c, -99a, -126 were upregulated in RS. Some of these miRNA (-374a, -144, -363, -106b, -182, -17, -101, 29a) have been previously implicated in stroke and cardiovascular disease. MiRNA 1974, 885-5p and 95 were virtually undetectable in the middle-aged group, indicating that these miRNAs could potentially serve as biomarkers for stroke severity.

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August 8, 2012: 9:00 AM - 2:00 PM
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Sex Differences in Aortic Aneurysm Formation

Cecilia Benz, David Howell, Emily Wilson
Department off Systems Biology and Translational Medicine
Texas A & M University Health Science Center College of Medicine,
College Station

Introduction: Epidemiological studies show that males have a higher incidence of aortic aneurysm formation than females. The pathogenesis of an aneurysm begins by the initial loss of homeostasis within the wall of the vessel. This may be caused by a change in the levels of Transforming Growth Factor-β (TGF-β), which regulates the amount of connective tissue that maintains the integrity of the aorta. With a change in the elasticity or contractility of the vessel, it is possible that the smooth muscle cells in the wall of the aorta become weak, leading to a widening of the vessel, called an aneurysm. This change predisposes patients to aortic dissections, which can lead to a rapid loss of blood, and a quick death.

Literature indicates that Insulin-Like Growth Factor-1 (IGF-1) stimulates anti-apoptotic effects in cells, and increase in contractile proteins in blood vessels. This increase in collagen formation can enhance the stability of a vessel, and potentially, stabilize an aneurysm.

Due to the increased incidence in aneurysm in women, estradiol (a sex steroid known to be at high levels in females) can potentially be protective against formation of aneurysm. In turn, the male sex steroid, androgen, has the potential to do the opposite. This is what we plan to explore in our experiments.

In our lab, we draw parallels between mouse smooth muscle and the smooth muscle of humans. By culturing the smooth muscle from the aorta of a pre-pubescent mouse, we are able to draw inferences about the potential risk factors and causes of aortic aneurysm.

Hypothesis: Due to the known effects of TGF-β, changes in the levels of TGF-β will lead to the formation of an aortic aneurysm. IGF-1 has been shown to have anti-apoptotic effects in vascular smooth muscle, and cause increases in collagen formation. In females, estrogen produced during the post-pubescent years will induce IGF-1, which will stabilize vascular walls, and stop aneurysm formation, as well as aortic dissection. Androgen, however, will not induce IGF-1, allowing for more male deaths due to aneurysm dissection.

Methods:

Cell Culture: Mouse Aortic Smooth Muscle cells (passage 11) were collected and grown in 6 well plates (30mm wells) using 10% Charcoal Stripped FBS Phenol-Free DMEM until reaching 80% confluency. The cells were then changed to 1% Charcoal Stripped FBS Phenol-Free DMEM for 24 hours before treatment. The cells were put back into 10% FBS DMEM, and were treated with 10ng/uL TGF-β, 10-5 ug/mL Androgen, or 30nM Estradiol 17B for 24 hours before collecting the cells.

RNA was collected by washing the cells in PBS, then lysing the cells in RLT buffer, and spinning the cells down in a separating tube.

Protein Analysis: Cell Media was collected and concentrated using a protein concentrator tube kit. BCA analysis was conducted in order to determine the concentration of protein in each sample.

Cells were collected for protein by first washing the cells in PBS, then by lysing the cells in RIPA buffer. BCA analysis was also done for these samples.

Standard western blot analysis of vascular smooth muscle cells treated with TGF-β, Estradiol 17B, or Androgen. Blot was transferred overnight at 12V, blocked 1 hour in 1x TBST plus 5% milk, primary antibody at 1:200 at 4° overnight. After three washes in TBST, secondary (1:20000) was applied for 1 hour, and the blots were visualized using an imager and SuperSignal West Dura Substrate.

Results:

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August 8, 2012: 9:00 AM - 2:00 PM
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H. pylori affects gastric epithelial expression of novel receptors involved in effector T cell regulation

Keegan Bradley, Taslima T. Lina, Iryna V. Pinchuck, Victor E. Reyes,
Department of Pediatrics, Clinical and Experimental Immunology and Infectious Diseases
The University of Texas Medical Branch, Galveston, TX

Introduction and Background: *Helicobacter pylori (H. pylori)* is a bacterium that has been directly linked with instigating gastric and duodenal ulcers, chronic gastritis, and gastric cancer. During H. pylori infection there is an upregulation of CD4+ T cell induction, but those T cells are hyporesponsive. *H. pylori* uses several mechanisms to subvert the host immune response. Our group has already demonstrated that there is an upregulation of B7-H1, a T-cell inhibitory molecule, on GECs upon *H. pylori* infection. It is hypothesized that there will also be an upregulation of other members of the B7 homologue family in GEC following infection by *H. pylori*. In this study we looked at the effect of *H. pylori* to other B7 family molecules which also can work as T cell co-inhibitory molecules e.g. B7-H3 and B7-H4. By using flow cytometry assay we showed here that *H. pylori* increases both B7-H3 and B7-H4 molecule on GEC upon infection. To see whether *H. pylori* uses it's virulence factor, Cag pathogenicity island (PAI), for this upregulation we used two different mutant strains Hp cag PAI$^-$ and cagA$^-$ strain along with the wild type strain to infect GEC. This study showed the function *H. pylori* Cag pathogenicity island (PAI) elements and CagA virulence factor play in regulation of B7-H3 and B7-H4 molecule expression on GEC. This was demonstrated by *H. pylori* WT strain showing upregulation of B7-H3 and B7-H4 molecule in GEC. Multiple GEC lines were infected with different *H. pylori* strains (WT and mutants: *cagA*$^-$ and Cag *PAI*). Following infection, the level of expression of B7-H3 and B7-H4 were determined via flow cytometry. It has also been demonstrated that Notch signaling play's a direct role in gastric cancer development. Our lab predicted that *H. pylori* infection will cause an increase in specific notch signaling which has been tied to gastric cancer. "Delta like" ligand-1 (DLL1) and its receptor Notch 1 have already been shown to be up regulated in gastric epithelial cancer. In this study we looked at the expression of notch ligands (DLL1, DLL4, and Jagged 2) in differing GEC lines upon infection by using different *H. pylori* strains. GECs were infected with WT strains of *H. pylori*, and the change in the level of notch expression was then quantified by means of flow cytometry. While all notch ligands showed an increase upon *H. pylroi* infection, Jagged 2 notch ligand showed the most significant increase in expression. Jagged 2 and its receptor Notch 3 have been strongly linked with development of gastric cancer

Materials and Methods: Goal was to determine if CagA or Cag PAI in different *H. pylori* strains contribute to upregulation and/or down regulation of expression Notch, B7-H3, and B7-H4 in different GEC lines.

1) Infected gastric epithelial cells with *H. pylori* and allow to incubate for 24 hours.
2) Analyze cells, and also collect supernatant and store at 80°C (for future experiments with just supernatant).
3) Quantify the degree of expression of Notch and/or B7 homologues on infected GECs relative to non infected control GEC.
    a. Flow cytometry (surface expression)
    b. Alternative methods: qRT-PCR & Western Blotting

Cell lines used: N87 and AGS cells
H. pylori strains: 51B, 26695, and *16639
Conditions used: Control (GEC only), GEC+ Hp WT, *GEC+ Hp CagA$^-$, *GEC+ Cag PAI$^-$
(*) was only used for quantifying B7 homologues

Conclusion: As the results demonstrate, *H. pylori* infection has an effect on the levels of expression of both the B7 homologue family and notch ligand Jagged 2. The data demonstrated that in all cases there was an up regulation in these cell proteins. An important difference that seemed to surface was the dependence of B7-H4 expression on the presence of CagPAI [figure 2], and the alternate dependence of B7-H3 expression on the presence of the effector protein CagA [figure 3]. The differing percentages of increase in B7-H3 expression between different Hp strains demonstrated that the amount of B7-H3 upregulation in GEC upon infection is dependent on which certain strain is used. Jagged 2 had a significant increase in expression upon *H. pylori* infection, demonstrating that Hp upregulates more than a single notch ligand.

The B7 homologue data could help to demonstrate why, although there is a host T cell response, the immune reaction is insufficient to clear the infection. These up regulated B7 homologues prevent the propagation of CD4+ T cells, and could be a direct factor in immune hyporesponsiveness with regards to Hp infection. The data from the Jagged 2 expression would be important in cancer treatment as well as prevention. Jagged 2 and its receptor notch 3 have been demonstrated to be upregulated in gastric cancer. Screening for upregulation of Jagged 2 and notch 3 in Hp infections could be used as early detection prognostic tools for gastric cancer progression. Future research down the road would involve studying the effect of this up regulation of notch signaling on T cell development. Th17 cells specifically, which scientists have also began to discover are upregulated in certain cancers.

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Comparison of squirrel monkey EAS volumes obtained by MRI with volumes obtained histologically

Jordan T. Buess, B.S., Thomas J. Kuehl, Ph.D., Michelle Reyes, B.A.,
Christopher Chung, M.D., Jil Gendron, Veronica Miller
Departments of Obstetrics & Gynecology, Radiology, Pediatrics and Molecular & Cellular Medicine
Scott & White Healthcare and Texas A&M University Health Science Center College of Medicine
Temple, Texas

Introduction: The squirrel monkey female is relatively unique among animal species in spontaneously developing pelvic floor dysfunction in response to pregnancy, parturition and aging that resembles that of women in terms of degree and frequency. Previously, pelvic floor muscles, their innervation, and connective tissue elements of the vaginal wall have been described using magnetic resonance imaging and histology of fixed tissues. In humans, parturition, aging, and pelvic organ prolapse are associated with injury to the external anal sphincter. This has not been evaluated in the squirrel monkey.

The squirrel monkey was first described as a model species for the development of spontaneous pelvic organ prolapse (POP) in the mid-1990s (Coates, 1995). Subsequently the unique capabilities of the NIH sponsored Squirrel Monkey Breeding and Research Resource (SMBRR) to have breeding animals with obstetrical histories were used to demonstrate an association of POP with parity and age (Coates, 1995). In 2003, Pierce et al., using animals obtained from the SMBRR and NIH R01 funding, went on to demonstrate the innervation of the key pelvic floor muscle in this species (Pierce, 2003).

Building on these observations, the group at Scott & White/Texas A&M University Health Science Center College of Medicine has gone on to describe the anatomy, histology, and detailed nervous connections of pelvic floor muscles and connective tissues in unique, characterized animals from the SMBRR (Pierce, 2005; Kramer, 2006; Pierce, 2006; Pierce, 2007; Pierce, 2008). The techniques adapted to these studies have included nerve tracer trials and magnetic resonance imaging (MRI). The latter technique has been important to document POP using anatomic endpoints not easily visualized in a perineal exam due to the small size of the species (Kramer, 2006; Pierce, 2008; Bracken, 2011). MRI has also become critical as it allows serial observations to track the impact of life experiences including pregnancy, parturition, and aging on these animals.

Hypothesis: We anticipate that MRI and histology based measures will correlate and show reliability, validating the MRI technique.

Methods: *Squirrel monkeys.* This study uses a cohort of animals that are housed at the Scott & White Animal Facility in Temple, Texas. The group includes 3 parous young females without pelvic organ prolapse (POP), 3 parous old females without POP, 3 parous old females with POP, 2 males, 1 young female with a C-section in labor and 1 old nulliparous female. Animals undergo MRI and then are euthanatized for tissue collection

MRI and histology. The validated procedure used for MRI has previously been described in the literature by Kramer et al. (2006). Magnetic resonance images of the external anal sphincters are obtained immediately prior to euthanizing the squirrel monkeys. Immediately following euthanasia, a tissue section containing the anal sphincter is frozen and stained with a succinic dehydrogenase stain for striated muscle to allow for determinations of external anal sphincter volumes. The volumes obtained through histological staining are compared to the volumes obtained through magnetic resonance images, in order to establish the degree of reliability between the two methods of obtaining muscle volumes.

Results:

TAMHSC SUMMER RESEARCH PROGRAM

August 8, 2012: 9:00 AM – 2:00 PM
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Anti-TEM8 antibody drug conjugate as a potential therapy in colorectal cancer metastases

Peter Chen, Carol Carter, Keri Kleypas, Lindsey Green, Matthew Cho, Arthur Frankel
Scott & White Cancer Research Institute, Texas A &M Health Science Center College of Medicine

Abstract: Tumor endothelial marker 8 (TEM8) has been demonstrated to show selective expression on colorectal cancer tumor endothelia in comparison to normal colonic endothelia. We have previously isolated a monocolonal antibody that recognizes TEM8 from a mouse immunized with cells expressing TEM8 and created a chimeric mouse-human antibody. We worked to create a novel single chain antibody (scAb) to demonstrate affinity to TEM8 and to act as a template for future affinity maturation as well as humanization.

Background: More than one million people develop colon carcinoma annually and it is the second most common cause of cancer in women and the third most common cause of cancer in men (Cunningham, 2010). Colorectal cancer cells display uncontrolled cell growth and eventually outgrow their initial blood supply. They gain access to the host's blood supply through angiogenesis. A potential therapeutic has been suggested: to attack the tumor vessels directly. TEM8 serves as a good target as it has been found to be expressed in tumor stromal and embryonic endothelia but not adult tissue endothelia (Carson-Watkins, 2001).

Methods: A plasmid was designed containing an insert comprised of the Vh and Vl sequences of the anti-TEM8 antibody cAF334 combined with a G4S linker. Two orientations were designed: Vh-Vl and Vl-Vh and the inserts were subcloned into pMOPAC16 vector (Hayhurst, 2003). Two cell lines of *Escherichia coli*, ABEL-C and BL21, were induced with IPTG and grown to an optical density of 0.5. The protein was purified through a nickel column and ran on a Western blot with an anti-mycHRP antibody (Figure 1). A Coomassie and Silver Stain was done to confirm for purity (Figure 2). An ELISA was done on the scAb with TEM8 attached to GST (Figure 3).

Specific aim: The overall objective of this project was to create a potential antibody drug conjugate reactive with TEM8. The initial aim was to engineer and purify an anti-TEM8 scAb and measure its affinity via ELISA.

Conclusion: The Western blot (Figure 1) shows the presence of the scAb protein but the results of the ELISA suggest little to no affinity to TEM8 (Figure 3). The scAb protein is likely to have not folded correctly, the concentration of the purified scAb was too low, or the purity of the sample was not high enough for detection.

References:

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Cunningham D, Atkin, W, Lenz, HJ, Lynch, HT, Minsky, B, Nordlinger, B, Starling, N (2010). "Colorectal cancer.". *Lancet***375** (9719): 1030–47. doi:10.1016/S0140-6736(10)60353-4.

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TAMHSC SUMMER RESEARCH PROGRAM

August 8, 2012: 9:00 AM – 2:00 PM
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Bryan, Texas

d-Ruby: A Mycobacteria Novel Imaging Tool

Gilbert A. Encarnación1, Jeffrey Cirillo2, Suat Cirillo2, and Ying Kong2
1Department of Microbial and Molecular Pathogenesis, Texas A&M Health Science Center,
College of Medicine, College Station.

Introduction and Background: Tuberculosis (TB) is a bacterial infection caused by the etiologic agent Mycobacterium tuberculosis that most often affect the lungs. TB is curable and preventable. Even so about one-third of the world's population has latent TB, which means people have been infected by TB bacteria but are not (yet) ill with disease and cannot transmit the disease. This organism normally about 10 days to growth in liquid culture, and about 10 to 4 weeks in agar plates, so an immediate readout for bacterial detection and numbers is extremely important.

Specific Aims: Our goal is to develop a new approach in fluorescence imaging to help us better understand the "behavior" of these bacteria in the laboratory as well in animal infection. This work describes a strategy for the development of fluorescence imaging that can be used to study bacterial infection in mice.

Materials and Methods: The method consists of the expression of d-Ruby gene in the bacteria. For this strategy, fluorescence signals are imaged using an IVIS live animal imaging system. The protein is to be expressed in the bacteria from E.Coli-Mycobacteria shuttle-plasmid vectors containing the appropriate transcriptional promoters for Mycobacterium as well as the d-Ruby gene cloned through a combination of the polymerase chain reaction and appropriate restriction enzyme digestion and ligation. Two different plasmid combinations, with same antibiotic resistance, Kanamycin and 2 transcriptional promoters, L5 and Hsp60, were used.

Results: With the appropriate vector plasmids constructed, the plasmid can be transformed into mycobacteria to evaluate the presence of dRuby through the use of a spectrometer or IVIS, an in vivo animal imager. If the presence of dRuby is detected, the plasmid can be transformed into M.bovis BCG strain.

Conclusion: With this dRuby labeled bacterium, we could infect mice and image them with IVIS to determine bacterial loads and location in live mice. We have successfully cloned dRuby into the plasmid with L5 promoter, and are characterizing its fluorescence in the plasmid with Hsp60 promoter. We will compare and select the strain having the highest fluorescence per CFU for animal infection experiments. These constructs should have broad utility in the study of tuberculosis pathogenesis as well as development of novel therapeutics and vaccines.

TAMHSC SUMMER RESEARCH PROGRAM

August 8, 2012: 9:00 AM - 2:00 PM
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The Uncontrollable Characteristics of Patients that affect Satisfaction in the Emergency Department

Shannon Essler and David Morgan, M.D.
Department of Emergency Medicine, Scott & White Hospital
Texas A&M Health Science Center College of Medicine
Temple

Introduction: Patient-centered care has become the focus of efforts to optimize health and health care delivery. Patient satisfaction with care is one of the pillars of patient-centered care. Results from patient satisfaction surveys can be a driving force behind changes in health care delivery. PressGaney is the largest patient satisfaction measurement company in the world. Hospital emergency departments (EDs) have increased their use of patient satisfaction data to track data over time. Previous studies have found factors that correlate with increased satisfaction include: empathic attitude of the ED staff, an acceptable waiting time, pain management and receiving medical information, which can be somewhat controlled. However, some characteristics of the patient must also determine the degree of satisfaction of that patient.

Hypothesis: There may be uncontrollable characteristics of the patient that affect patient satisfaction.

Methods: In a prospective study, patients (or family/friend) discharged from the ED at Scott and White Memorial Hospital, Temple, Texas, were consented and enrolled. Seven hour time blocks, during the ten week study period, were semi-randomly selected for surveying patients. Blocks were weighted favoring those with most patients discharged. Four questions from a Press Ganey survey addressed the patient's satisfaction with the doctors treating them. The questions were answered on a scale of one to five (1= very poor, 5 = very good). Patients were asked about the: courtesy of the doctor, degree to which the doctor took the time to listen to you, doctor's concern to keep you informed about your treatment and doctor's concern for your comfort while treating you. From subject's medical records, the number of ED visits since December of 2009, age, acuity, turn around time, gender, chronic versus acute pain and trauma versus non-trauma status of the research subjects were gathered. Research subjects were divided into two groups: patients who gave their doctors perfect scores (5 out of 5 on all four questions) and those who gave less than perfect scores. Differences between the two groups with regard to the uncontrollable patient characteristics were analyzed using chi-square analyses and t-tests.

Results: There were 1,248 responses to the 4 questions by 321 patients. The majority of the responses were scored as "5" (88%). All "1","2", and "3" responses totaled only 7%. There was a significant difference between (non)-trauma vistis and patient satisfaction ($p = 0.0002$). Of the sample, 84% were non-trauma patients. Trauma patients are more likely to be satisfied with their doctors than non-trauma patients. There was a significant difference in the age of the perfect scorers ($M= 48$, $SD = 22$) and the non-perfect scorers ($M = 40$, $SD= 20$), ($p = 0.0014$). A significant difference also was found between the turn around time of the perfect scorers ($N = 174$ min, $SD = 113$) and the non-perfect scorers ($N = 209$ min, $SD = 145$), ($p = 0.0321$). Thus, increased age and shorter turn around time lead to increased patient satisfaction. No significant differences were found with regard to gender, acuity, number of visits to the ED and chronic versus acute (duration less than one month) pain.

Conclusion: Many factors influence patient's satisfaction with a medical encounter. Ironically, the actual quality of medical care does not usually have much of an impact on patient satisfaction. Factors that do determine satisfaction with care include the cleanliness of the facility, the personalities and demeanor of the staff and medical information patients received. Understanding the characteristics that affect patient satisfaction is critical for the optimization of health care delivery. Satisfied patients are more likely to be compliant with their medications and return for continuing medical care. Previous studies have analyzed controllable factors of the patient encounter that impact satisfaction scores. This is the first study demonstrating the chief complaint (trauma/non-trauma), an uncontrollable patient characteristic, also affects satisfaction. Additional studies are needed to determine other significant uncontrollable factors which would be valuable when comparing satisfaction scores between different groups of patients.

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Hepatic Alterations following Traumatic Brain Injury

Cassandra Harris, Suzanne Zeitouni, Jason Justice, Gabriel Frampton, Sanjib Mukherjee, Lee A. Shapiro
Scott & White Hospital; VA Central Texas Health Care System;
Texas A & M University Health Science Center; Texas Bioscience Institute

Background: Traumatic brain injury (TBI) results in approximately 60,000 deaths a year in the U.S. Moreover, an estimated 5.3 million Americans are living with disabilities from TBI. The leading causes of TBI are falls, motor vehicle accidents, impacts from assaults and battlefield injuries. Studies have shown that it is not only the initial impact of the injury that can be fatal, it is also the cascade of the inflammatory response produced by the body that worsens the condition. A condition known as multi organ dysfunction syndrome (MODS) has been observed to occur after TBI and contributes to morbidity[1]. In MODS, inflammatory response results in peripheral organ damage or non-neurologic organ dysfunction. It is also possible that peripheral inflammatory molecules can enter the brain, as the blood brain barrier is compromised in TBI. The aim of this study is to examine the role TBI and the resulting inflammation and in particular its effect, if any, in hepatic inflammation.

Hypothesis: Our hypothesis is that TBI results in hepatic alterations, with a specific focus on alterations to inflammatory molecules.

Methods: TBI was carried out in mice using the fluid percussion injury (FPI) model. Tissue was collected at 2, 6, and 24 hours post injury and flash frozen. This was to allow for examination of immediate and early effects of TBI on liver alterations. The tissue was kept in -80º C until homogenization in protein extraction buffer for protein isolation. The homogenate was next centrifuged at 14,000 rpm for 10 minutes at 4º C. The supernatant containing soluble protein was taken off and stored in -20º C. After washing the leftover pellet with buffer, it was resuspended and dissolved in extraction buffer solution that contained SDS and centrifuged again at 14,000 rpm. The supernatant containing membrane fraction of the protein was collected and stored in -20º C. Protein levels were estimated using Coomassie R-250 to stain 12% Tris-Glycine SDS-PAGE gel. This allowed calculation of appropriate load for each gel.
RT PCR: RT PCR was used for mRNA analysis and observing the level of Shh, Ihh, Gli1, Gli2, Gli3, and TGR5 at time points 2 and 6 hour after TBI using ΔΔCT analysis as described in previous research paper[2].
Western Blot: Western blots were carried out using a standard method to analyze calcineurin levels. Briefly, the samples were run on gels and transferred to a nitrocellulose membrane using the Bio-Rad Transblot system. Membranes were washed in tris buffered saline containing Triton X-100 and blocked for one hour at RT in LI-COR blocking buffer. 5mL of primary antibody diluted in LI-COR blocking buffer was added to the membrane and incubated at 4º C overnight. The membranes were then washed with TBST, and incubated in the secondary antibody that was diluted in 2.5mL LI-COR blocking buffer and 2.5mL of TBST for 1 hour, then washed again. Following that, the blot was imaged on LI-COR machine.

Results: Qualitative RT PCR revealed reduced levels of mRNA were found at 2 hours after FPI in Shh, Ihh, Gli1, Gli2, Gli3, and TGR5. At the 6 hour time point there was a significant reduction in the mRNA levels of Gli2.

Western blot analysis showed a slight increase in the levels of the protein Calcineurin at the 24 hr time point following TBI. GAPDH was used as the loading control.

1. https://www.braintrauma.org/tbi-faqs/tbi-statistics/
2. http://www.biomedcentral.com/1472-6793/7/6#sec5 "Prolactin stimulates the proliferation of normal female cholangiocytes by differential regulation of Ca2+-dependent PKC isoforms"

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Stroke induced blood-brain barrier permeability in middle-aged females: role of insulin-like growth factor 1

Jessica Heibel, Shameena Bake and Farida Sohrabji
Department of Neuroscience and Experimental Therapeutics
Texas A & M University Health Science Center College of Medicine
Bryan Campus

Introduction: Stroke is the leading cause of disability and third leading cause of death in the U.S., with higher morbidity and mortality in the older female population. Uncovering the underlying mechanisms that explain this variance between age and sex is the primary focus of our lab. Previous studies indicate that stroke, or middle cerebral artery occlusion, results in larger infarct volume in middle-aged (9-11 month old) female rats. This population also has a more permeable blood brain barrier compared to their younger counterparts. Insulin-like growth factor 1 (IGF-1), a peptide hormone, is down regulated in postmenopausal women and reproductive senescent rats. IGF-1 has also been shown to have neuroprotective actions in animal models of stroke. Previous studies from this laboratory have shown that IGF-1 treatment reduced infarct volumes in middle-aged female rats. The present study will test whether the neuroprotective actions of IGF-1 are due to improving the integrity of the blood brain barrier after stroke in middle-aged, reproductive senescent rats.

Hypothesis: Intracerebroventricular (ICV) infusion of IGF-1 will decrease blood-brain barrier permeability following stroke/ MCAo in female middle-aged rats, leading to better stroke outcome in these animals.

Methods: Female rats were characterized by daily vaginal smears for acyclicity, and were used in the study only if they were in constant diestrus for at least 2 weeks. A 28-gauge cannula is placed into the right lateral ventricle of each rat using stereotaxic coordinates one week prior to MCA occlusion. For occlusion of the MCA, we used a suture stroke model, where an intraluminal suture is placed into the internal carotid artery through the exposed external carotid artery. From there, the suture was advanced until it reached the origin of the MCA and was secured in place. After 90 minutes, the suture was withdrawn to allow reperfusion. ICV infusion was started 45 minutes prior to reperfusion by connecting a pre-primed osmotic mini pump to the cannula. The pump was loaded with either IGF-1 or artificial CSF (as control) and inserted subcutaneously once connected.

The blood-brain barrier permeability was determined by the concentration of extravasated Evan's blue dye. Evan's blue dye (normalized to body weight) was injected into the rat's jugular veins 4 hours post-reperfusion. After 30 minutes, the animals were decapitated, brains removed from the cranium, and cortex and striatum dissected from both ischemic and non-ischemic hemispheres. Tissues were either 1) frozen immediately in liquid nitrogen and kept at -80°C or 2) weighed, dried in an incubator, reweighed, homogenized in an organic buffer and centrifuged. The supernatant was collected, loaded onto a micro plate, and read at 620 nm and 680 nm. Concentrations of Evan's dye were determined from standard curve using Magellan software.

Results and Conclusion: Our results show that IGF-1 treatment after stroke in middle-aged females significantly reduced concentration of Evan's blue dye, which indicates decreased BBB permeability compared to control animals. This data supports the hypothesis that IGF-1 may act at the blood brain barrier to maintain brain homeostasis and neuroprotection.

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The Role of GPR124 in Endothelial Cell Migration and Tumor Angiogenesis

Zach Henderson, Ying Wang, Mingyao Liu, Ph.D
Texas A & M University Health Science Center College of Medicine,
Center for Cancer & Stem Cell Biology, Institute of Biosciences & Technology, Houston, TX

Introduction: Angiogenesis, the process of creating new blood vessels, provides oxygen and essential nutrients to tumor cells. Therefore, angiogenesis supports tumor growth and is the key element that allows tumor cells to metastasize. Understanding the complex and still widely unknown mechanisms underlying tumor angiogenesis is essential to developing new therapeutics that could halt the growth and metastasis of cancer.

Vascular endothelial growth factor (VEGF) secreted from tumor cells activates VEGF receptor-mediated angiogenic signaling in endothelial cells. This activation induces endothelial cell proliferation, migration, and invasion, facilitates vascular permeability, and induces the formation of new vessels. In tumor angiogenesis, signals are mainly directed toward VEGFR2 and leads to an activation of downstream factors such as Src, AKT, FAK, ERK, and JNK, ultimately giving endothelial cells these properties. Studies have suggested that VEGF-induced angiogenesis requires both Src and FAK phosphorylation, specifying that FAK phosphorylation at FAK Y397 is an integrin-mediated autophosphorylation, and phosphorylation at Y576/577 is Src-dependent. Studies have additionally shown that $pFAK^{Y397}$ is associated with an inhibition of angiogenesis and metastasis while $pFAK^{Y576/577}$ has shown to promote tumor angiogenesis.

G-protein-coupled receptor 124 (GPR124; also known as tumor endothelial marker 5 - TEM5), has been implicated in regulating the angiogenic ability of endothelial cells through mediating cell-extracellular matrix and cell-cell interactions. Prior research performed on GPR124 has shown that deletion of GPR124 causes embryonic lethality while GPR124 overepxression leads to abnormal vascular formation.

GPR124, which has been shown to be both highly and specifically expressed in endothelial cells of tumor vasculature, is thought to be a vital part of VEGF-induced tumor angiogenesis, and could serve as a therapeutic target for the development of new treatments for patients with metastatic cancers.

Hypothesis: GPR124 plays an important role in the signaling mechanisms of VEGF-induced tumor angiogenesis by causing upregulation of pro-angiogenic transcription factors and expression of angiogenic-related genes downstream. Limiting GPR124 expression in endothelial cells could inhibit tumor angiogenesis and ultimately reduce tumor growth and metastasis.

Methods: Human Umbilical Vein Endothlial Cells (HUVEC) were infected with either lentivirus containing a short hairpin RNA (shRNA) to knockdown the expression of GPR124, or with lentivirus control.

***In vitro* studies**:

- Endothelial cell migration, invasion and tube formation assays were performed on the two cell lines as described in the Humtsoe et al. study Lipid Phosphate Phosphatase 3 Stabilization of β-Catenin Induces Endothelial Cell Migration and Formation of Branching Point Structures (2010).
- VE-cadherin expression and cell-cell interaction using immunofluoresence was measured as described in the same Humtsoe et al. study stated above.

***In vivo* studies:**

- MDA-MB-231 breast cancer tumor cells were mixed with HUVEC control or HUVEC with GPR124 shRNA and injected into mice. Tumor volume was measured daily and harvested at 24 days. After harvest, vessel number of each tumor was counted.

Expression studies:

- Western blot, using protein-specific antibodies, was used to determine intracellular signaling components associated with the VEGF signaling pathway.
- QPCR, using gene-specific primers, was used to determine the expression of angiogenesis related genes and transcription factors

Results: GPR124 silencing in HUVEC cells leads to a decrease in tube formation, cell invasion, cell migration, and vascular permeability - inhibiting tumor angiogenesis. GPR124 silencing showed to increase phosphorylation at FAK Y397 while decreasing pFAK Y576/577 and showed that GPR124 plays a role in the dynamics of VEGFR2/Src/FAK complex. This role causes a downregulation in the expression of pro-angiogenic transcription factors Id1, COUP-TFII, HOXD3, HOXA9 and RUNX2, while upregulating anti-angiogenic transcription factor HEX. This leads to a decrease in expression of downstream genes needed for angiogenesis such as VEGFR2, Tie-2, Ang1, uPA, Survivin, TACE, and IL-6. *In vivo*, studies showed GPR124 silencing in endothelial cells decreased breast cancer tumor volume and vessel number. Overall, GPR124 is thought to be a potential new target to limit tumor angiogenesis and, through new treatment options, give a better prognosis to patients with metastatic cancer.

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Effect of Exercise Training on Myocardial Remodeling in a Porcine Model of Chronic Occlusion

Gabrielle C. Henslee, Sanjukta Chakraborty[1], Cristine L. Heaps[2] and Mariappan Muthuchamy[1]
[1]Systems Biology and Translational Medicine
Texas A&M University Health Science Center College of Medicine, College Station
[2]Department of Veterinary Physiology and Pharmacology
Veterinary College of Medicine, Texas A&M University, College Station

Introduction: Exercise training improves myocardial function in healthy adults and patients with ischemic heart disease (or patients with coronary artery disease) as well as in animal models of chronic coronary artery occlusion (CCO). Our laboratory uses the highly clinically relevant porcine model of CCO and exercise training because of its similarities to humans in the cardiac anatomy, collateral density following arterial occlusion, maximal aerobic capacity and in the response to exercise. The porcine model of CCO results in decreased left ventricular ejection fraction, myocardial ischemia, and decreased left ventricular wall thickness during systolic phase. In addition, during stress, such as exercise, myocardial perfusion is insufficient to support normal myocardial function distal to occlusion in the porcine model and diseased patients. Results from our laboratory demonstrate that twenty-two weeks following ameroid placement, sedentary pigs manifest impaired myocardial function. Cardiac tissue remodeling occurs in the ischemic myocardium during the development of coronary artery disease or other pathological mechanical loads such as pressure or volume overload. It is also worth noting that the cardiac myocytes undergo dramatic changes in cell shape during the development of ischemic processes in the myocardium. Thus, an abnormal strain pattern during contraction is evident while the heart grows and remodels aberrantly. This is due to the altered hemodynamic loading that leads to an acute change in the strain pattern that is outside a physiological dynamic range. The resulting growth and remodeling is aberrant because the myocytes are unable to re-establish a homeostatic state. Alternately, the abnormal strain pattern is the result of other tissue processes (such as apoptosis, Ca^{2+} deregulation, or myocarditis) that are causes or co-contributors to the aberrant cardiac growth and remodeling. In this remodeling process, the characteristics of integrin-ECM interaction also change and increasing evidence shows that ECM proteins and integrin expression are altered during the development of heart diseases. Integrins are an important class of receptors for the ECM proteins, which have been proposed to play a central role as mechanotransducers during normal development and in response to mechanical forces associated with physiological and pathophysiological states.

Hypothesis: The expression of integrin subunits and extracellular matrix proteins, such as fibronectin, will differ between sedentary and exercise-trained animals and between the occluded and nonoccluded sections of the hearts.

Methods: The left circumflex (LCX) coronary arteries of adult female Yucatan miniature swine were surgically occluded using ameroid occluders and the pigs separated into sedentary and exercise-trained groups. The exercise-trained group completed a fourteen-week progressive treadmill exercise protocol beginning eight weeks post-operatively. The sedentary group remained confined in pens as a control. Twenty-two weeks after the operation, the hearts were removed and the left ventricular myocardial wall isolated from both occluded (collateral dependent, previously LCX dependent) and nonoccluded (left anterior descending (LAD) artery dependent) regions. The tissues were snap frozen in liquid nitrogen. Proteins were isolated and Western blot analyses were performed to compare the protein expression of tissue lysates from both sedentary and exercise trained animals and from the LAD and LCX dependent regions of the heart. Samples were loaded into a 4-20% Tris-HCl gel (BioRad) and then transferred to a membrane for further analysis. Depending on the comparison being made (sedentary vs. exercise, LAD vs. LCX, sedentary LCX vs. exercise LCX), the lysates were loaded in different groupings and orders. The blots were probed for multiple proteins using specific antibodies (fibronectin, GAPDH, integrin β1, and integrin β3).

Results: While exercise-trained pigs showed an increase in β1 integrin expression, β3 expression was detected at an increased level in the sedentary myocardium. An increase in the amount of total FN and its fragments were found in the myocardium from collateral-dependent regions of exercise-trained and sedentary pigs when compared to the non-occluded myocardium regions. The fibronectin antibody detects the 277 kDa band along with other lower molecular weight fragments, as others have shown with differences observed in their expression between occluded and non-occluded regions. Thus our data suggest that changes in integrins and ECM proteins during the remodeling of myocardium that occurs under both ischemic (pathological) and exercise-trained (physiological) conditions will alter the downstream mechanotransduction signals, which subsequently will modulate the cardiac muscle dynamics.

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Exploring Mechanisms of Leucine-Induced Vascular Dysfunction

Hai Hoang, Sam Padgham, Katherine Kelly, Guoyao Wu, Cynthia Meininger
Department of Systems Biology and Translational Medicine
Texas A & M University Health Science Center College of Medicine – Temple

Introduction: Obesity and diabetes are growing public health crises worldwide and leading risk factors for vascular insulin resistance. Because insulin exerts an important vasodilatory action via the stimulation of nitric oxide (NO) production by endothelial cells (ECs), vascular insulin resistance may play a critical role in obesity-associated cardiovascular disease. However, the mechanism by which vascular insulin resistance occurs in obesity is unknown. It was discovered that glucose and glutamine are potent inhibitors of NO synthesis in ECs. In addition, it was demonstrated that leucine concentrations in plasma were markedly increased in diet-induced obese rats in association with vascular insulin resistance. Furthermore, it was shown that leucine activated glutamine:fructose-6-phosphate amidotransferase (GFAT) and inhibited NO synthesis in cultured ECs.

Leucine is a branched-chain amino acid (BCAA). Concentrations of BCAAs and glutamine are markedly elevated in plasma of obese patients and rodents. Published work indicates that increased concentrations of leucine in plasma are associated with insulin resistance in obese humans and rodents. Evidence suggests that L-leucine may be a novel regulator of GFAT activity by 1) enhancing GFAT protein expression via the mTOR signaling pathway; and 2) activating the enzyme in EC.

In skeletal muscle, physiological levels of L-leucine stimulate the phosphorylation of S6K1 and 4EBP1, leading to increased synthesis of proteins. Leucine may act upon mTOR, which phosphorylates eIF4E-binding protein-1 (4EBP1) and ribosomal protein S6 kinase-1 (S6K1), resulting in initiation of polypeptide synthesis in ECs. L-leucine may stimulate the synthesis (i.e., expression) of GFAT in ECs via the mTOR signaling, thereby increasing glucosamine production, leading to vascular insulin resistance.

Reduction of GFAT activity should block the effect of hyperglycemia on glucosamine synthesis and superoxide production in EC. Thus, reducing circulating levels of leucine may provide a potentially novel strategy for preventing and treating cardiovascular disease in obese subjects.

Hypothesis: Increasing leucine concentrations stimulate GFAT activity by enhancing GFAT protein expression via the upstream mTOR signaling pathway.

Methods: Bovine coronary venular endothelial cells (CVEC) grown under hyperglycemic conditions were utilized as an in vitro model of diabetes. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with high glucose (4.5 g/L) and supplemented with 1% Fetal Bovine Serum for 48 hours to downregulate transcription factors and protein synthesis. Cells were then incubated in a customized DMEM containing 0, 0.1, 0.25, 0.5, or 2 mM L-leucine. These concentrations of leucine were chosen because they increased GFAT activity, increased glucosamine synthesis, and inhibited nitric oxide synthesis in freshly isolated rat coronary endothelial cells and are close to plasma concentrations in obese and L-leucine-supplemented rats. After 48 hours of incubation with medium containing various concentrations of L-leucine, cell proteins were collected in lysis buffer containing protease inhibitor cocktail. The concentrations of the proteins were quantified using a BioTek Synergy H1 Microplate Reader. Proteins (10 ug/lane) were loaded on and run and separated on a sodium dodecyl sulfide-polyacrylamide gel. Afterwards, the gel was transferred for western blot analysis of phosphorylated and total levels of mTOR, 4EBP1, S6K1, eNOS, as well as succinate dehydrogenase complex subunit A (SDHA) as a loading control. The membranes were blocked for at least 1 hour at room temperature with rocking using 0.1% Tween-20 in Tris Buffered Saline (TTBS, pH 7.5) contained either 5% Carnation evaporated non-fat milk (for non-phosphorylated proteins) or 3% bovine serum albumin (BSA, for phosphorylated proteins). The primary antibodies were used at 1:1000 in the corresponding blocking buffer and the membranes were incubated overnight at 4 degrees Celsius with rocking. The antibodies were obtained from commercial sources (4EBP1 and S6K1 antibodies from Cell Signaling, Danvers, MA; and eNOS antibodies from BD Biosciences, San Jose, CA). The membranes were washed with TTBS 3 times (5 minutes per wash) with rocking. Secondary antibodies were used at 1:25000 in the corresponding blocking buffer for 1 hour at room temperature with rocking. The secondary antibodies were obtained from Jackson ImmunoResearch (West Grove, PA). The membranes were washed 6 times (5 minutes per wash) with rocking. SuperSignal West Dura Extended Duration Substrate (Thermo Scientific, Rockford, IL) was added for 5 minutes. Chemiluminescence was detected using Fuji Film LAS4000 Chemiluminescence Imager. Density of bands was determined using MultiGauge 3.0-Software included with the imager.

Conclusion: From the density graphs depicting the level of protein expression, leucine-induced eNOS expression is upregulated in a dose-dependent manner. Although eNOS expression is increased, previous data has shown that superoxide is produced rather than nitric oxide, leading to vascular dysfunction. There appears to no significant difference between the level of expression for S6K1 and EBP1 in the phosphorylated and unphosphorylated state. Thus we do not believe that with these specific experimental conditions that l-leucine is driving S6K1 or EBP1 activation.

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IGF-1 Contributes to Stabilization of TAA by Inducing Pro-Collagen and Influencing VSM Survival

Charlie Howard
Systems Biology and Translational Medicine
Texas A & M University Health Science Center College of Medicine,
College Station

Introduction: Aneurysms and dissections of the thoracic aorta are the fifteenth leading cause of death in the United States. Rupture and dissection of an aneurysm are associated with a high degree of morbidity and mortality. The aorta is composed of three layers: a thin inner layer, the tunica intima; a thick middle layer, the tunica media; and a thin outer layer, the tunica adventitia. The pathological hallmark of TAD (thoracic aortic dissection) is medial degeneration, which is characterized by loss and fragmentation of elastic fibers and accumulation of proteoglycans in the aortic media. The first genes identified to cause TAD were FBN1, TGFBR2, and TGFBR1. The identification and characterization of these genes along with preliminary data suggest TGF-B pathways are involved in pathogenesis. A sex-based comparison of the demographics and history of patients with TAD suggests that women are older than men at the onset of TAD. We hypothesize this is do to increased insulin-like growth factor-1 (IGF-1) expression in females. IGF-1 is an endocrine and autocrine/paracrine growth factor that circulates at high levels in the plasma and is expressed in most cell types. IGF-1 has major effects on development, cell growth, differentiation and tissue repair. Evidence shows that IGF-1 increases atherosclerotic plaque stability in animal models. We hypothesize that IGF-1 may also have a significant stabilization role in aneurysms and dissections accounting for the differences seen between men and women. Because evidence shows that the TGF-B pathway is upregulated in TAD, we will be looking at the possible beneficial effects of IGF-1 in conjunction with TGF-B.
Hypothesis: Does increased IGF-1 expression stabilize aneurysms by increasing structural stability and altering survival factors in vascular smooth muscle cells.

Methods:

Cell Culture: Mouse aortic vascular smooth muscle cells (VSMC) were maintained in 10% Dulbecco's Modified Eagle's Medium (DMEM) until growth at 80% confluency and then serum starved in 1% DMEM. Cells were split with trypsin into 3 6-well plates and grown to 80% confluency prior to treatment. Cells were treated with IGF-1 (50ng/mL), TGF-B (10ng/mL), and IGF-1 (50ng/mL) + TGF-B (10ng/mL).

RNA Extraction and RT-PCR: 24 hours after treatment RNA was extracted using Quaigen RNeasy mini kit. RNA levels were determined by spectrophotometry. Sets of primers for the mouse Collagen-1, Collagen-3, Elastin, Platelet derived growth factor beta, bcl-2, Caspase-8 and Caspase-9 were designed using Primer Express Software. The RT reaction was performed using Invitrogen cDNA Synthesis Kit according to the manufacture's instructions. RT PCR was performed with Applied Biosystems SYBR Green master mix according to the manufacturer's instructions. Expression levels were calculated using the delta Ct method, and normalized to the expression of GAPDH. Measurements were carried out in quadruplicate.

Results:

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Determining the presence of putative *Clostridium difficile* genes in clinical isolate strains

Sheryl Jacob, Caná Ross, Magnus Höök
Center of Extracellular Matrix Biology, Institute of Biosciences and Technology,
Texas A&M University Health Science Center College of Medicine, Houston, TX

Introduction and Background: The prevalence of *Clostridium difficile* infections have been increasing over the past few years. The population most at risk are individuals with advanced age, individuals who are immunocompromised, and individuals who have been recently exposed to antibiotics. As a nosocomial infection, *Clostridium difficile* has lengthened the stay in hospitals for individuals contributing to increased health care costs in the US.[2] Rates of infection by this bacterium in the United States have increased with the increased use of antibiotics leading to increased contamination in hospitals.[4] Given its importance in public health, it is vital to understand how *C. difficile* colonizes the host.

Clostridium difficile is a Gram-positive, spore forming, anaerobic bacteria of the genus *Clostridium* that produces two exotoxins: toxin A and toxin B. It is a pathogen that causes antibiotic associated diarrhea and pseudomembranous colitis. It is thought that exposure to antibiotics allows for *C. difficile* growth by killing the normal intestinal flora, resulting in toxin production. Toxin A is an enterotoxin and toxin B is a cytotoxin. Both are high molecular weight proteins capable of binding to specific receptors on the intestinal mucosal cells. Both toxins play an important role in the pathogenesis of colitis.

Adhesion to the host tissue is the first critical step for microbial colonization and infection. Gram-positive and Gram-negative bacteria utilize cell wall anchored proteins and protein assemblies for adhesion. A subfamily of surface proteins known as MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) are covalently linked to the cell wall peptidoglycans of many Gram-positive pathogens and mediate attachment to host extracellular matrix proteins (ECM), such as fibrinogen (Fg), fibronectin (Fn), and collagen.[5, 6] Little is known about how *C. difficile* mediates attachment and colonizes the host. We have previously identified genes that encode putative MSCRAMMs in the genomes of several *C. difficile* isolates. However, the distribution of these genes is unknown.

Hypothesis: The putative MSCRAMM-encoding genes (*pmg*1-6) identified within the genomes of five sequenced *C. difficile* strains (630, R20291, 63q42, NAP7 and NAP8) are present in clinical isolates.

Methods:

Polymerase Chain Reaction (PCR): We conducted PCR analyses of the expression of genes *pmg*1-6 in 94 clinical isolate strains. Briefly, primers specific to genes *pmg*2-6 were amplified from DNA using Apex Taq Master Mix (Genosee) according to the manufacturer's instructions. Primers specific to gene *pmg*1 were amplified using the Herculase II (Agilent Technologies) according to the manufacturer's instructions. The annealing temperatures were 50 C or 52 C. All denaturing steps were at 95 C and extension steps were at 72 C and included 30 cycles on the Veriti Thermal cycler (Applied Biosystems). PCR products were separated on a 1% agarose gel and visualized with ethidium bromide and UV light.

Clinical Isolates DNA: DNA was kindly provided by the Zhi-Dong lab at the University of Texas School of Public Health. DNA was extracted from *C. difficile* isolates obtained from St. Luke's Hospital in the Texas Medical Center. All DNA was given a numeric identification and no patient data was associated with the DNA sample.

Results: Results of the PCR showed genes *pmg*1, *pmg*2, *pmg*3, and *pmg*4 as being prevalent in most clinical strains. 64% of the strains were positive for gene *pmg*1, 71% were positive for *pmg*2, 82% were positive for *pmg*3, 78% were positive for *pmg*4. These data indicate that these genes may be important for colonization. Finally, *pmg*5 and *pmg*6 were less conserved; 15% of the strains were positive for *pmg*5, and 6% were positive for *pmg*6, indicating that these genes may be less important. It is also possible that these are newly acquired genes. Identifying the strains types will allow us to better understand the results described here.

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The Role of Hedgehog in Rescuing Radiation-induced Hyposalivation

Lai Jiang, Bo Hai D.D.S., M.D., Fei Liu, M.D., Ph.D.
Institute for Regenerative Medicine
Texas A & M University Health Science Center College of Medicine

Introduction: Many head and neck cancer survivors treated with radiotherapy suffer from impairment of their salivary gland function. Hyposalivation exacerbates dental and periodontal disease and impairs the quality of life of patients significantly by causing mastication, swallowing problems, and a burning sensation of the mouth.. This condition is caused by lack of regeneration of functional stem/progenitor cells following radiotherapy. Current treatments such as artificial saliva can only temporarily relieve the symptoms. Because previous data has shown that human salivary stem cells are dormant after radiation, activation of these dormant stem cells by molecular cues could serves as a novel approach to restore salivary gland function. One of which is the activation of Hedgehog (Hh) pathway because of its role in regulating regeneration or repair of various types of tissue after injury.

Hypothesis: Transient activation of Hh pathway after radiation can rescue the loss of salivary function in mice by upregulation of molecular signaling activities related to functional differentiation and maintenance of salivary stem/progenitor cell.

Methods:

1. All mice were treated with 15 Gy single dose irradiation in head and neck region (IR)
2. Submandibular gland (SMG) samples of Ptch1-LacZ Hh reporter transgenic mice were the collected.
3. Keratin5-rtTA/tetO-Shh transgenic mice were prepared by crossing. The Shh induction by Doxycline (Dox) starts 3 days and 90 days of IR for 7 days. SMG samples were collected.
4. Hh activation is induced in female mice by Smoothened Agonist (SAG).
5. SMG samples were also cultured to examine the effect of transient Hh activation following IR on salisphere formation.
6. Saliva flow rate were measured and SMG samples of mice were subjected to qRT-PCR, X-gal staining, immunofluorescence staining to analyze the mRNA expression of Hh target gene and other related signal activities.

Results: The lack of expression of LacZ Hh reporter in Ptch1-LacZ mice after IR indicated that Hh pathway is not activated by radiation damage. Transient activation of Hh induced by Dox significantly ameliorated the negative effect of IR on maintenance and differentiation of salivary stem/progenitor cell in male mice by up regulating related signal activities including that of Wnt, Bmi1, Chrm1/HB-EGF, Notch, Laminin α1 and Integrin α2. The increase in salisphere forming cells after Shh induction also indicated an expansion of salivary stem/progenitor cell. SAG induced Hh activation in female has similar effects on SMG.

Conclusion: Transient Activation of Hh pathway can preserve IR damaged salivary stem cells. Further research on transient activation of Hh pathway and its downstream molecular cues will improve the functional restoration of salivary glands.

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Protein HbpA and Deformin Activity in Erythrocytes

Gemel A. Joseph and Laura Hendrix, Ph.D.
Department of Microbial and Molecular Pathogenesis
Texas A & M Health Science Center College of Medicine, College Station, TX

Introduction and Background: Erythrocytes (red blood cells) transport oxygen throughout the circulatory system. Hemoglobin in the red blood cells binds to the oxygen as the cells deliver the oxygen to body tissues. Mature red blood cells are biconcave and lack a nucleus and most organelles which are features ideal for transporting oxygen molecules. *Bartonella bacilliformis*, a species of the pathogenic genus *Bartonella*, enters human red blood cells and this parasitic relationship leads to Carrion's disease, which is characterized by severe acute anemia. Although *Bartonella bacilliformis* is contained within the specific geographic area of Peru, Ecuador, and Colombia and can be monitored with antibiotics and insecticides, it remains an organism of great interest because its parasitic capabilities and the disease states in which it induces makes it unique among human pathogens. Characteristics and features of Bartonella bacilliformis include a gram-negative bacterial cell wall containing LPS (lipopolysaccharide), a small size of up to 1-2 mm in diameter and a relatively slow growth rate at a temperature of 28 ˚C and pH of 7.8. The protein deformin, which can be found in culture supernatants of *B. bacilliformis*, produces indentations on red blood cell membranes that allow the bacterium to enter the cell. The *Bartonella* protein HbpA (hemin-binding protein A) contains a repeated region of approximately 1,400 Daltons in *B. bacilliformis* strain KC583 that is missing in strain KC584. Strain KC584 is unable to produce deformin in culture supernatants. Researchers discovered that the region required for producing deformations has a molecular weight of 1,400 Daltons, but they have been unable to sequence the region.

Specific Aims: In this project, several methods will be performed to determine the role HbpA plays in the deformin activity of *Bartonella bacilliformis*.

Hypothesis: *Bartonella bacilliformis* KC583 hemin-binding protein A (HbpA) is required for the appearance of indentations on human red blood cell membranes known as deformations and thought to be required for entrance of the bacterium into erythrocytes.

Materials and Methods:

1. Perform deformin assays with strains KC584 & KC583
2. Ammonium sulfate precipitation of proteins in strains KC584 & KC583
3. Perform protein assays of culture supernatants and cell pellets of strains KC584 & KC583
4. Indirect Fluorescent Antibody Stain of *Bartonella bacilliformis* in red blood cells
5. Purify antibodies and sera (αcom1, αHbpA, αBb, and normal rabbit serum) using protein A agarose
6. Inhibit deformin activity using antibody and sera (αcom1, αHbpA, αBb, and normal rabbit serum)
7. Analysis of IgG Fractions by Electrophoresis

Results: Additions of the αcom1, αHbpA, αBb antibodies and normal rabbit serum have no significant effect on deformin activity of *Bartonella bacilliformis*. These results do not indicate a relationship between these proteins and the deformin activity of *Bartonella bacilliformis*.

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Flammability of Surgical Materials in Varying Oxygen Concentrations

Alex Kimbrough, Sarah Luna, William C. Culp, Jr., M.D.
Division of Cardiothoracic Anesthesiology, Scott & White Memorial Hospital, Temple, Texas
Texas A&M Health Science Center

Introduction: For a fire to occur there must be three things present: an oxygen source, an ignition source, and a fuel source. With the elements of the fire triad present during most operations, it is estimated that over 600 operating room fires occur each year. Of the reported operating room fires, 81% involve surgical drapes, yet minimal data exist about the flammability of surgical materials in varying oxygen concentrations.

Purpose: The purpose of this study is to assess the flammability characteristics of surgical materials under varying oxygen concentrations. Hopefully, this study will raise awareness amongst surgical staff about potential fire risk and mitigate the threat of patient injury or death.

Methods: Five fuel sources were analyzed: a blue OR towel, utility drape, surgical gown, surgical drape, and laparotomy sponge. Test samples of each material were burned in a fashion similar to that established by the Consumer Product Safety Commission. The test sample was placed within a 46 cm by 46 cm by 46 cm test chamber constructed with transparent walls. Inside the test chamber, a 5 cm by 15 cm sample was placed on a burn rack and held at 45 degrees with a match flame source located at the base of the test sample. Three test samples of all the materials were used in each concentration of oxygen. The oxygen concentrations were set at 21%, 50%, and 100% and measured by a multi-gas analyzer. Time to sample ignition and time to complete burn were measured using video analysis. Two-way ANOVA was used to analyze the interaction between the oxygen concentration and test material with burn time as the dependent variable.

Results: The average ignition time in 21% oxygen was 1.1 seconds, in 50% oxygen 0.5 seconds, and in 100% oxygen 0.2 seconds. The average burn time in 21% oxygen was 20.7 seconds, in 50% oxygen 4.2 seconds, and in 100% oxygen 1.6 seconds.

Conclusions: Both ignition and burn times decreased as oxygen concentration increased. Although the utility drape and surgical gown did not support combustion in room air, they became highly flammable in increased oxygen concentrations. Flash fires occurred at high oxygen concentrations with materials composed primarily of cotton. Understanding the flammability characteristics of surgical materials in oxygen-enriched environments and proper product selection may help reduce perioperative fire risk.

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The effect of acute oral amino acid intake on whole-body fat oxidation

Christopher A. Latz, Nicholas Hurren, Elisabet Børsheim
UTMB Heath, Department of Surgery, Metabolism Unit
Texas A & M University Health Science Center College of Medicine,
College Station

Introduction: Coronary artery disease is the number one cause of death in the United States in both males and females. A significant, independent risk factor for coronary artery disease is an elevated triacylglycerol concentration in the blood – hypertriglyceridemia (Assmann et al. 1998; Austin et al. 1998). Hypertriglyceridemia commonly increases with age, making it a significant health risk among the elderly population (Miller et al. 2011).

Triacylglycerols are hydrophobic in nature and therefore must be packaged within larger macromolecules, known as lipoproteins, in order to circulate within the blood. In the fasting state, the majority of circulating TAG is carried within very-low-density lipoprotein (VLDL) particles, which are synthesized by, and secreted from, the liver. Once within the circulation, TAGs within lipoproteins can be hydrolyzed by lipoprotein lipase, an enzyme present within the capillary endothelia of tissues such as skeletal muscle, adipose tissue and the heart. Such hydrolysis releases free fatty acids (FFAs) from the glycerol backbone of the triacylglycerol molecule. The FFAs are then taken up into the tissue via fatty acid transporters and can either be oxidized, or re-esterified and stored intracellularly.

It has previously been shown by our group that chronic supplementation of a normal diet with essential amino acids can lower plasma TAG concentrations; Fig 1 (Børsheim et al. 2009). We have also shown that acute amino acid intake stimulates VLDL secretion from the liver (Børsheim et al., unpublished data).

Specific Aim: To determine if acute amino acid intake will increase the rate of whole-body fat oxidation and overall energy expenditure.

Methods: Data for this study were collected within the context of a 10-hour constant infusion trial. During the experiment, the first five hours represented the basal period, in which the subject had no caloric intake. The final five hours represented the amino acid intake period.

During the amino acid intake period, subjects ingested small boluses of an essential amino acids + arginine mixture (3.26% histidine, 8.57% isoleucine, 35.88% leucine, 17.08% lysine x HCl, 3.59% methionine, 4.65% phenylalanine, 9.57% threonine, 7.44% valine, 9.97% arginine) given in the form of a 25 mL drink, from 5-10 hours, every 10 minutes, adding up to a total of 30 g of amino acid intake.

Indirect calorimetry was performed at 2.5 hours (during the basal period) and again at 7.5 hours (during the amino acid period), for approximately 30 minutes, to determine VCO2 ($L \bullet min^{-1}$) and VO2 ($L \bullet min^{-1}$). VCO2 and VO2 were then used to calculate whole-body fat oxidation, carbohydrate oxidation, and energy expenditure.

Results: Whole-body fat oxidation, resting energy expenditure, and the percentage of resting energy expenditure due to fat oxidation, were all increased during the amino acid intake period compared with the basal period.

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Role of miRNAs, miR-1 and let-7f in Post-Stroke Pathogenesis in Middle-Aged Female Rats

S. Lavasani, A. Selvamani, F. Sohrabji
Neuroscience and Experimental Therapeutics
Texas A & M University Health Science Center College of Medicine, Bryan Campus

Introduction: In the United States, stroke is a leading cause of major disabilities, with older women at a higher risk for stroke, more severe post stroke cell damage and cognitive decline. Age is a predominant factor in post stroke recovery due to accompanying estrogen decline and a spike in hormonal therapy used by the older female population. Previous work from the lab has indicated that young females sustain a smaller infarct as compared to mature adult females, and that estrogen treatment to older females further increases cortical infarct volume (Selvamani and Sohrabji, 2010a). Post-stroke infusions of IGF-1 (insulin-like growth factor-1) reverse estrogen-mediated toxicity (Selvamani and Sohrabji, 2010b).

MicroRNAs (miRNA) are a class of therapeutic molecules with potential therapeutic use for inflammatory diseases such as cardiovascular disease, cancer and stroke. They function as translational repressors by either inhibiting translation and/or degradation of mRNA. There are several miRNA that have been associated with stroke including, let-7f, and miR-1. Both age and increased estrogen therapy decrease IGF-1 availability in the older female populations, and administering IGF-1 to older female rats post stroke improves infarct volume. Thus, increasing IGF-1 is important to protect the brain from cell loss post-stroke. Bioinformatics approaches indicate that miRNAs, such as miR1 and let7F, target the IGF-1 signaling pathway to repress translation. Thus, antagonizing these miRNAs with anti-miR1 and anti-Let7f therapy might lead to an up-regulation of IGF-1 signaling. In fact, post-stroke anti-mir1 therapy minimizes infarct volume in the cortex while anti-Let7f therapy minimizes infarct volume in the cortex and striatum in young females (Selvamani et al., 2012).

Hypothesis: Since young females benefit from IGF-1 therapy and there is evidence for let-7f and miR-1 miRNA's therapeutic role in post-stroke pathogenesis in young female rats, we hypothesized that these miRNA's might have therapeutic effects in the middle-aged population as well.

Methods: All animals were purchased from Harlan Laboratories (IN). Females were purchased as retired breeders (9-11months old). All animals were maintained in a constant 12-h dark: 12-h light cycle in AAALAC accredited vivarium facilities. Food and water was available ad libitum. The animals were subjected to stereotaxic surgery to occlude the left middle cerebral artery (MCAo). MCA occlusion was induced by microinjecting 3 ul of Endothelin-1. Four hours post-stroke, animals were administered intracerebroventricular (ICV) injections of either scrambled miR (control), anti-Let 7f, or anti-miR1. At termination, the brain was rapidly removed and processed for TTC (Triphenyl Tetrazolium Chloride) staining to assess infarct volume and biochemical analysis. All animals were sacrificed on day 5 post-MCAo. Motor impairment following MCAo was assessed using the vibrissae evoked forelimb placement task and the sticky tape test.

Results: The vibrissae evoked forelimb placement task indicated that all animals had impaired sensory motor performance post stroke. Infarct volume data indicated that in intact middle-aged females, anti-Let7f treatment resulted in a significantly larger infarct in the cortex and striatum than the control group post stroke, while anti-miR1 treatment had no effect on infarct volume compared to the control group. Consistent with the infarct data, the "sticky tape" test showed significant impairment on the right side only (contralateral to the ischemic hemisphere) with anti-Let7f treatment. In conclusion, unlike younger adults, anti-Let7f treatment appears to be neurotoxic in middle aged female rats, implying a therapeutic role of Let7f in this population post stroke.

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Epigenetic Modifications at Histone H3 and Reversal of Drug Resistance in B-Cell Lymphoma

Devesha Lester[1], Behrooz Mousavi[2], S.A. Maxwell[2]
[1]*Prairie View A&M University,* [2]*Molecular & Cellular Medicine, College of Medicine – College Station*

Introduction and Background: Resistance to the CHOP chemotherapy drug regimen is an ongoing problem which needs to be resolved in order to develop a successful cure for Diffused Large B-cell Lymphoma (DLBCL), an aggressive subtype of non-Hodgkin's lymphoma. For over two decades the standard anthracycline-based chemotherapy mixture CHOP (consisting of cyclophosphamide, doxorubicin, vincristine, and prednisone), has been the most effective treatment for DLBCL. Regrettably, approximately half of DLBCL patients develop a chemoresistant disease with a high mortality rate. Thus, characterization of the molecular basis of CHOP resistance is urgently needed to develop a rational strategy to overcome drug resistance. Several CHOP-resistant DLBCL cell lines were generated by repeated treatments with CHOP as models for studying the mechanism of multi-drug resistance. We have discovered that agents that induce reactive oxygen species (ROS) act to sensitize CHOP-resistant cells. Since epigenetic processes such as Histone H3 methylation have been implicated in drug-resistance in several other cancers, we explored whether changes in histone methylation, possibly mediated by ROS, were associated with CHOP resistance in DLBCL. According to the theory of the 'histone code', transcription of genes is regulated by the chemical modification of histones such as methylation on their amino terminal ends. The study of cancer epigenetics hypothesizes that certain genes are activated or deactivated when such modifications occur. Methylation of lysines at the amino terminus of Histone H3, are believed to play roles in the emergence of drug resistance in lung, prostate, and breast cancer. We suspected that a similar situation occurs in drug resistance observed in DLBCL. We observed changes in lysine-9 methylation at Histone H3 upon sensitization of CHOP-resistant cells mediated by a novel ROS-inducing agent (ROS-IA).

Hypothesis: We hypothesize that H3 dimethylation at lysine-9 of histone plays a role in reversal of drug resistance seen in DLBCL. We also predict that reversal of drug-resistance in lymphoma cells induced by a ROS-IA will be associated with decreases in the dimethylation of Histone H3 lysine residue-9 (H3-K9).

Methods:

- We generated CHOP resistant DLBCL cell lines as a model for CHOP resistance. The CHOP resistant cells were generated from originally CHOP sensitive cell lines by repeated cycles of "on-off" exposures to increasing concentrations of CHOP, which is similar to the clinical regimen.
- Western blot analysis was conducted on chemosensitive 2631 DLBCL cells, resistant G3 cell derivatives, and G3 cells treated with the ROS-IA. Antibody Histone H3 (D1H2) Rabbit mAb was used to identify the total histone in each cell sample. Afterwards a dimethyl lysine-9 specific antibody was used to compare levels of H3 lysine-9 dimethylation in drug resistant cells treated with ROS-IA (G3+ROS-IA).

Results: A significant down regulation was observed in the lysine-9 dimethylation at Histone H3 in CHOP-resistant G3 cells sensitized with an ROS-IA.

Conclusion:

- Epigenetic modifications at lysine-9 Histone H3 are implicated in reversal of CHOP resistance. Additional experiments are now planned to pursue the role of H3 methylation in CHOP resistance in DLBCL.

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Regulation of Diastolic Function in Cardiac Myocytes by Mechanical Stress-Related Kinases

Toby J. Mathew, Damir Nizamutdinov, Fnu Gerilechaogetu, David E. Dostal
Division of Molecular Cardiology,
Central Texas Veterans Health Care Center, Temple, TX, USA
Texas A & M University Health Science Center College of Medicine, Temple, TX, USA

Introduction: Heart disease is the leading cause of death for both men and women in the United States. When stimulated by various neuroendocrine factors or an increase in mechanical stress, the myocardium has a compensatory hypertrophic response to preserve cardiac output. Long-term cardiac hypertrophy is a predictor of more serious complications such as heart failure, and can evolve into a decompensated state with contractile dysfunction. A large number of cardiovascular pathologies are related to a decrease in heart pump function. A disorder in intracellular calcium homeostasis is one of the hallmarks of structural heart disease. The Mitogen Activated Protein Kinase (MAPK) signaling pathways have been implicated in directing cellular responses to a wide array of stressors, including those that lead to cardiac hypertrophy. In this study, we measured the activation state of several MAP kinases (extracellular signal regulated kinases (ERK), p38, c-Jun N-terminal kinases (JNK)), as well as, focal adhesion molecules (FAK), protein kinase B (Akt) and their effects on cytoplasmic calcium regulation at different beating frequencies using isolated neonatal rat ventricular myocytes (NRVM).

Hypothesis: To investigate the role of FAK, Akt and MAP kinases (ERK, p38 and JNK) in regulation of intracellular Ca^{2+} mobilization in paced NRVM.

Methods: NRVM were isolated from ventricles of neonatal rats by enzyme digestion of minced tissue, separated from non-myocytes by centrifugation using a discontinuous Percoll gradient and plated on gelatin-coated cell-culture dishes (Corning). After 48 hr of incubation, cells were starved with serum free medium for 8-9 hr and subjected to Ca^{2+} measurements. A microscope-based fluorometer (Ion Optix System) was used to measure intracellular Ca^{2+} levels in response to pharmacological inhibitor treatments. NRVMs plated on dedicated cover slips were pretreated with various kinase inhibitors for 30 min and loaded with 2 μM Fura-2 AM for 15 min. Intracellular levels of Ca^{2+} in NRVM were measured at various pacing frequencies (0.5 – 3.0 Hz) using the Ion Optix System and data recording and analysis were performed using Ion Wizard 6.2 software. Experimental results were compared to the corresponding control (vehicle) treatment.

Results: Treatment with AKT inhibitor showed a 40% decrease in intracellular calcium mobilization in comparison of different pacing rates, specifically 30 beats per minute (BPM) and 180 BPM. FAK inhibitor showed a 40% decrease. p38 inhibitor showed a 25.1% decrease. JNK inhibitor showed a 81.3% decrease. However, ERK inhibitor showed a 3% increase in calcium mobilization.

Conclusion: We observed a significant decrease in the ratio of cytoplasmic bound/free Ca^{2+} levels with Akt, FAK, p38, and JNK inhibitors. Diastolic changes accounted for the majority of the percent change, while systolic values stayed relatively consistent with inhibitor treatments. The predominant effect on diastolic calcium with increased pacing frequencies indicate that these signaling molecules play an important role in regulation of the mechanisms responsible for pumping calcium ions from the cytosol into the sarcoplasmic reticulum, other organelles (e.g. mitochondria, nucleus) and/or across the plasma membrane. Subsequent studies are required to determine the specific mechanisms regulated by each of the signaling molecules examined in this study. During cardiac hypertrophy, often the levels of MAPK, Akt, and FAK pathways are increased. The increase in diastolic calcium is consistent with diastolic heart failure, which is poorly understood pathology that affects a large number of patients. These results suggest that JNK, Akt and FAK may serve as therapeutic targets for the treatment of acute diastolic dysfunction.

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MicroRNA, ethanol, and the implications in directing neurodevelopmental stem-cell EMT associated behaviors

Phillips Prung Nagsuk
Dept. of Neuroscience and Experimental Therapeutics
Texas A & M University Health Science Center College of Medicine,
College Station

Introduction: Epithelial – Mesenchymal Transition (EMT) is a behavioral process of transitioning cellular morphology from epithelioid origin to that of mesenchymal phenotype. This process is well known in its roles of cancer metastasis, embryonic gastrulation, and neurulation. MicroRNA (miR) are relatively short sequences of nucleic acids (often between 20 and 25 ribonucleotides in length) that perform the role of transcriptional and post-transcriptional regulators. In post-transcriptional regulation, they operate by binding to the 3'UTR of targeted mRNA transcripts, and often have the effect of mRNA silencing. Research has evidenced strong miR directed controls over the mechanistics of the progression of cancer growth and metastasis, of which EMT plays a most intimate role. Neurulation during embryogenesis is one of the most critical moments in Central Nervous System (CNS) development. During the first and second trimesters, neural epithelial stem cells of the neural tube undergo EMT as they migrate across basement membrane and ingress into the developing brain matrix where they shall differentiate into neurons of the gray matter. Congenital diseases such as Fetal Alcohol Spectrum Disorder (FASD) often present with decreased viable neural tissue, potentially through the loss of normal EMT migration of neuronal progenitors. Although it is understood that EMT is the mechanism that facilitates the migration of neural epithelium, it has not been shown whether miR operates in the same manner in this process as in cancer metastasis. Understanding of the mechanisms of specific miR in CNS embryological development is a potential gateway to the understanding of neurological disease as well as to potential therapeutics through manipulation of progenitor stem cell morphologies via directed miR signaling pathways.

Hypothesis: Downregulation of miR-9 and 21 leads to decreased EMT of neural epithelium in an analogous behavior to cancer metastasis. Ethanol consumption by the mother during the 1st and 2nd trimesters may play a role in miR-9 and 21 downregulation, thus influencing the progression of pathologic CNS development. Since upregulation of miR-9 destabilizes E-cadherin, thus liberating beta-catenin, which consequently co-localizes within the nucleus where it acts as a transcription factor for VEGF translation leading to angiogenesis, miR-9 downregulation may also play a role in reduced angiogenesis during neurodevelopment.

Methods: I cultured neurospheres from homogenized brain tissue surgically removed from a mouse fetus. These neurospheres served as the source of neural stem cells for experimentation. These cells were then passaged into several hundred million neural stem cells, which were then treated into control and ethanol groups. Transfection was then performed with control inhibitor as well as miR-9 and 21 inhibitors, and cells were subjected to differentiation in minimum differentiation media with laminin coating (yielding adherent neuronal precursors) or no differentiation in mitogenic growth medium yielding non-adherent stem cells. These cells were consequently worked up into doubled stained immunofluorescence slides, with antibodies for beta-catenin, Vimentin, E-cadherin, MAP2, neurofilament, and nestin. Quantitative Real Time PCR was also performed on all samples to obtain miR-9 and 21 levels, as well as and mRNA levels of beta-catenin, E-cadherin and N-cadherin, and Western Blotting was performed to quantify protein levels. I designed the qrtPCR primers through analysis of gene mappings through the UCSC Genome Bioinformatics tools.

Results:

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Role of biogenic amines and tumor associated macrophages (TAMs) in the cholangiocarcinoma tumor microenvironment

Nisha Patel, Dinorah Leyva-Illades, Mayghan Findley, Li Huang, Gabriel Frampton, Hae Pae Yong, Sharon DeMorrow
Department of Internal Medicine, Texas A&M Health Science Center College of Medicine, Temple, TX
Scott & White Digestive Disease Research Center, Temple, TX
Central Texas Veterans Healthcare System, Temple, TX

Introduction: Cholangiocarcinoma (CCA) arises from the neoplastic transformation of cholangiocytes, the epithelial cells that line the intra and extrahepatic bile ducts. Chronic inflammation is an important risk factor in the development of CCA, inflammatory diseases such as primary sclerosing cholangitis and hepatitis C can predispose patients to CCA. Patients with cholangiocarcinoma have a poor prognosis due to the late onset of symptoms and resistance to chemotherapy and radiation therapy. The tumor microenvironment consists of neoplastic cells and a biologically complex stroma comprising stromal cells and extracellular matrix. Stromal cells, particularly inflammatory cells, vascular endothelial cells, and fibroblasts have been shown to support tumor growth. Furthermore, neoplastic cells can secrete factors that recruit and activate stromal cells in a paracrine fashion. Most tumor-associated macrophages (TAMs) have an M2-like phenotype, thus having reduced anti-tumor activities, and increased production of angiogenic and proliferative mediators. How TAMs are involved in cholangiocarcinoma development and progression remains unclear. We have shown that CCA cells secrete increased amounts of the biogenic amines serotonin and dopamine leading to growth promoting effects, however, additional consequences of this dysregulation remain to be studied.

Hypothesis: Our central hypothesis is that TAMs present in CCA will have an M2 phenotype due to factors secreted by the CCA cells. Our long-term goal is to identify the signaling mechanisms that promote the M2 phenotype, thus allowing future development of strategies to inhibit the M2 phenotype switch and restore these macrophages to an anti-tumor M1 phenotype.

Methods: Immunohistochemical staining (IHC) of CD68 and IL-10 was performed on human benign and cholangiocarcinoma tumor tissue. THP-1 monocytes were differentiated into macrophages with 50 ng/ml of PMA for 48 hrs. Cells were pretreated for 24h with conditioned media from the CCA cell line Mz-Cha-1, serotonin (10^{-7}M) or dopamine (10^{-7}M). To determine the effects of conditioned media, serotonin and dopamine on the activation of inflammatory pathways, macrophages were then treated with IFN-γ (15ng/ml) for 30min, 4h or 24h. MHCII expression was measured by flow cytometry using a FITC labeled anti-HLA-DP-DQ-DR antibody and CIITA expression was measured by qRT-PCR. Stat1 activation was measured by western blotting and confocal microscopy.

Results and Conclusions: Macrophages were found in both benign and cholangiocarcinoma tumors as evidenced by CD68 expression, while IL-10 immunoreactivity (as a marker of the M2 macrophage phenotype) was only found in cholangiocarcinoma tissue. The effects of serotonin and dopamine on macrophage activation were determined, IFN-γ increased cell surface MHCII expression whereas cells pretreated with Mz-Cha-1 conditioned media, serotonin, or dopamine had dampened MHCII expression compared to IFN-γ treatment. CIITA expression (the major regulator of MHCII expression) was upregulated after IFN-g treatment; its expression was reduced in the presence of serotonin after 24h. Together, these data suggest that serotonin and dopamine secreted by cholangiocarcinoma cells may play a role in the phenotypic switch from M1 to M2 of TAMs in the cholangiocarcinoma tumor microenvironment.

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Activation of P38 MAPK Results in Cardiac Insulin Resistance and Heart Failure

*Demetria Perry, Yajuan Qi, Qinlei Zhu, Zihui Xu, Shaodong Guo
**Baylor University; Cardiovascular Research Institute, Division of Molecular Cardiology, Texas A&M University Health Science Center, Central Texas Veterans Health Care Center Temple, Texas, USA*

Introduction and Background: Diabetic cardiomyopathy is widely present in diabetic patients, and the major cause of death is cardiac failure. However, the molecular mechanism that links diabetes to cardiomyopathy is incompletely understood. Whether insulin itself is a determinant factor leading to cardiac insulin resistance and dysfunction is uncertain. Our early study indicated that the mouse model- L-DKO mice, deficient in hepatic insulin receptor substrate 1, 2 (IRS1, IRS2), displayed type 2 diabetic features: high levels of blood insulin and glucose. Here we found the L-DKO mice reduced systolic and diastolic cardiac function and diminished both IRS1 and IRS2 protein levels in the heart, leading to inactivation of Akt and activation of P38MPAKinase. Here we further provided cell-based evidence, demonstrating that chronic insulin stimulation decreased both IRS1 and IRS2 protein levels via activation of P38MAPKinase, which also is sufficient for reducing the IRS1 and IRS2 protein levels and causes myocardial insulin resistance.

Specific Aims: The aim of this research is to investigate whether cardiac insulin resistance occurs in the heart of diabetes mellitus. Initially, we will detect expression of IRS1 and IRS2 protein and activation of Akt and MAPKinase in the in the heart of a diabetic mouse model- L-DKO mice, deficient in hepatic IRS1 and IRS2. Secondly, we will examine the potential mechanism of diabetes-induced cardiac insulin resistance in neonatal rat cardiac myocytes by assessing the activation of MAPK that may underlie the mechanism of the insulin resistance induced by hyperinsulinemia

Materials and Methods: (1)L-DKO mice: L-DKO mice were generated using the Cre-loxP genetic approach resulting in deficient in hepatic IRS1 and IRS2 and the cardiac function was examined through echocardiography. (2) Primary cultures of neonatal cardiomyocytes were prepared from the ventricles of 1-day-old neonatal Wistar rats by enzymatic dissociation. Then insulin signaling molecules and kinase activation were assessed in vitro. (3)Western-blot: Heart tissue or cellular proteins were prepared, resolved by SDS-PAGE and transferred to nitrocellulose membrane for immune-blotting analysis using specific antibodies. (4) Cardiomyocytes were infected with adenovirus expressing active form or inactive form of P38MAPKinase. Subsequently, the cells were cultured in serum-free Dulbecco's modified Eagle's medium/F12 media for an additional 24 h before treatment or cell lysates and protein preparation and analysis.

Results: The L-DKO mice increased both insulin and glucose concentration, reducing systolic and diastolic cardiac function and diminishing both IRS1 and IRS2 protein levels in the heart, in consistent with that insulin-induced AKT/Foxo1 signaling cascade was down-regulated, while P38MAPK was enhanced. Further in vitro cell-culture experiment indicated that the protein level of IRS1 and IRS2 in cardiomyocytes significantly decreased upon chronic insulin stimulation and insulin-induced activation of AKT/Foxo1 signaling cascade also largely prevented. Clearly, inhibiting P38 largely rescued the decrease of IRS1 and IRS2, whereas, expression of P38MAPKinase using adenovirus infection led to the degradation of IRS1 and IRS2 proteins.

Conclusion: Chronic hyperinsulinemia can result in insulin resistance, which contributes to the heart failure in diabetic condition, and the major underlying mechanism is involved in the activation of P38MAPK.

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

Anjali Rai, Irina Nizamutdinova, Rakeshwar Guleria, Jonny Kendall, Sen Zhu, Kenneth Baker, Jing Pan
Department of **Medicine**
Texas A & M University Health Science Center College of Medicine,
Temple

Introduction: Diabetic cardiomyopathy is one of the leading causes of increased morbidity and mortality in patients with diabetes. It is characterized by cardiomyocyte loss and myocardial fibrosis, leading to decreased elasticity and impaired contractile function. Cardiac fibroblasts are the predominant secretary cells of collagen in the heart, prolonged activation of cardiac fibroblasts, defined by increased proliferation, differentiation and subsequent increased extracellular matrix, which largely consists of collagens, leads to cardiac fibrosis, a condition characterized by excess collagen deposition and a stiff myocardium, thus impairing the systolic and diastolic functions of the heart. All-*trans* retinoic acid (ATRA), a derivative of vitamin A, has been shown to inhibit the development of cardiac remodeling (hypertrophy and fibrosis) induced by various pathological stimuli, suggesting that ATRA may have inhibitory effects on high glucose-induced fibroblast proliferation and fibrotic changes.

Hypothesis: We propose that ATRA alleviates high glucose-induced increase in cell growth and collagen secretion in cultured cardiac fibroblasts, through regulation of transcription factor NF-κB, which has been shown to be involved in regulation of the development of cardiac remodeling.

Methods: Cultured neonatal cardiac fibroblasts were treated with or without ATRA, and then exposed to high glucose (25 mM) for 24-48 h. JSH-23, a NF-κB specific inhibitor, was used to determine the role of NF-κB in ATRA-mediated effects. Gene expression of TGF-β and collagen type1 I and type III was determined by Real-time RT-PCR. The activation of NF-κB was determined by Western blotting.

Results: ATRA inhibited high glucose-induced cardiac fibroblast proliferation, differentiation and collagen synthesis. High glucose stimulated gene expression of TGF-β and collagen type I and III was also significantly inhibited by ATRA. NF-κB signaling was activated by high glucose, which was inhibited by ATRA. Inhibition of NF-κB signaling by JSH-23 attenuated high glucose-induced gene expression of collage type I and III. These results suggest that ATRA inhibits high glucose induced cardiac fibrosis through regulation of NF-κB signaling.

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The Identification of Lifestyle Factors in CHF, AMI, and Pneumonia Patients at Baylor Health Care System

Manoj P. Reddy, Jenny Reed, Mae M. Centeno, Cliff Fullerton, MD
Institute of Chronic Disease and Care Redesign
Baylor Health Care System, Dallas, TX

Introduction: CHF, AMI, and PN have been identified as diseases that predominantly plague the elderly population. 20% of acute myocardial infarction patients (AMI), 25% of congestive heart failure patients (CHF), and 18% of pneumonia (PN) patients >65 years and older were readmitted within 30 days after discharge between 2007-10. Increased hospital readmission rates and mismanaged care have contributed to the soaring costs. In recent years, an emphasis has been placed on reducing readmission rates of chronic diseases in an effort to control rising costs. The management of CHF, AMI, and PN involves the use of pharmacological interventions in combination with lifestyle modifications. Behavioral factors have been shown to contribute to early readmission rates in patients. Recent studies have shown the benefits of lifestyle modifications in the reduction of readmission rates, improvement in the quality of life and the decrease in overall medical costs. Identification of advantageous modifications may help in the formation of institutional specific management guidelines. The purpose of this study was to determine the specific factors that increase or decrease the likelihood of a patient with acute myocardial infarction, congestive heart failure, and pneumonia being readmitted within 30 days after discharge.

Hypothesis: We hypothesized that lifestyle factors will correlate specifically with patients who were either readmitted or non-readmitted with AMI, CHF, or PN at Baylor Health Care System hospitals.

Methods: We conducted a retrospective survey of AMI, CHF, and PN patients that were admitted to BHCS facilities between September 2011 and April 2012. Data for patients that were readmitted for AMI, CHF, and PN were taken from in-hospital evaluations. Information from admitted patients were obtained via phone interview approximately six months post-admission. We performed a statistical comparison between non-readmitted (NR) and readmitted (R) patients responses to questions involving their lifestyle behavior after admission.

Results: As expected, non-readmitted patients demonstrated markedly improved lifestyle modifications in comparison with the readmitted group. Significant differences between the groups as they pertain to lifestyle considerations were apparent in pillbox usage, follow-up appointment compliance, adherence to a special diet (i.e. heart healthy), and BHCS discharge guidelines.

Limitations to the study include the retrospective design, relatively small sample size, and the reliability of patient responses. The results of the study correlate with similar studies guidelines for CHF, AMI, and PN management. Institutional recommendations should focus on patient education and repetition in an effort to increase compliance with lifestyle modifications and guidelines.

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Body Mass Index in Relation to Total Hip Arthroplasty Complications

John Reilly, MPH, Matthew Jordan, MD, Daniel Jupiter, PhD, Kindyle Brennan, PhD, PT, Kirby Hitt, MD, Christopher Chaput, MD
Department of Orthopedic Surgery at Scott and White Hospital
Texas A&M Health Science Center
Temple, Texas

Introduction: As the prevalence of obesity in America increases, the number of obese patients who undergo THA is expected to increase as well. While it is commonly thought that obese patients have a higher rate of surgical complications following THA, studies on the association of BMI and THA report conflicting results.

Methods: A retrospective review of 1,504 patients treated at a single institution over 9 (2003-2011) years was performed. Data collected included patient age, gender, BMI, occurrence of revision surgery and types of complications (superficial wound infection, wound complication, deep infection, deep venous thrombosis/pulmonary embolism, pneumonia, cerebrovascular event, myocardial infarction, urinary tract infection, death, transfusion, other medical complication, and other surgical complications).
Logistic regression modeling using covariates age, gender and WHO classified BMI, was applied to determine independent risk factors for complications (surgical, medical, or any).

Results: There were a total of 1,447 patients that met the study inclusion criteria, 830 (56.19%) women and 647 (43.81%) men; 57 were lost during the 3- or 12-month follow up or their BMI could not be located. The mean age was 66.12 (12.82) years. The average BMI was 29.20 (5.86) (Table 1, see below).

For medical complications multivariate logistic regression analysis using the WHO classification of obesity demonstrated an association between occurrence of complication and underweight status, significant obesity (WHO Class III) and for each year of increased age, with odds ratios of 4.29 (95% CI 1.36-13.5), 2.32 (95% CI 1.05-5.17), and 1.04 (95% CI 1.02-1.06), respectively.

Conclusions: The underweight population showed the strongest association with medical complications; there was also an association with medical complication for patients classified as category III obese. Age showed a minor association with medical complications. Understanding patient characteristics that increase the risks of having a complication after THA is important in order to appropriately counsel patients, and possibly intervene pre-operatively to decrease modifiable risk factors related to increased or decreased BMI.

Table 1. Number and percentage of patients in each weight category (WHO = World Health Organization)

WHO Underweight (<18.5)	WHO Normal (≥18.5 – <25)	WHO Overweight (≥25 – <30)	WHO Obese I (≥30 – <35)	WHO Obese II (≥35 – <40)	WHO Obese III (≥40)
19 (1.29%)	321 (21.76%)	555 (37.63%)	352 (23.86%)	146 (9.90%)	82 (5.56%)

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Rapid actions of estrogens to modulate synaptic activity in the rodent brain

Casey Roth, Joel D. Turtle, Joanne C. Damborsky, William H. Griffith

Introduction: Traditional actions of estrogens are thought to regulate normal physiology through ligand-activated transcription factors mediating long-term genomic effects over the course of days, months and years. Rapid, non-genomic signaling effects of estrogen occur over the time course of minutes and are postulated to contribute to neuronal excitability, neuroprotection, homeostasis, synaptic plasticity and cognition (Woolley, Rev Pharmacol Toxicol, 47:657-680, 2007). The understanding of the cellular mechanisms responsible for these rapid effects of estrogens is in its infancy. Detailed knowledge of estrogen receptor function is critical because declining estrogen levels during menopause result in the increased incidence of stroke, cognitive impairment and inflammatory responses. It is unknown if rapid non-genomic actions of estrogens play a role in the clinical management of these diseases. The purpose of the present investigation is to begin to delineate the estrogen receptors' mechanism(s) that are responsible for modulating synaptic transmission in young male and female subjects.

Hypothesis: Estrogen agonists work at the G-protein-coupled estrogen receptor (GPER) affecting synaptic transmission

Methods: Female and male rats aged between 30 and 70 days were anesthetized with isoflurane and decapitated. The hippocampi were removed from both hemispheres, and a tissue chopper was used to cut 500μm transverse sections from the middle third of each hippocampus. Slices were held in oxygenated ACSF and hippocampal slices were held in a recording chamber with a constant flow of ASCF. Slices were stimulated via a bipolar stimulating electrode placed in CA1 stratum radiatum. Extracellular responses were recorded using a glass electrode filled with 1M NaCl solution. I/O curves were recorded at 5 different voltage inputs determined on a per slice basis to establish a baseline, then 17 β-E 100 nM was applied for ten minutes and a second I/O curve at the same voltage ouputs was recorded. A follow-up study with the GPER agonist G-1 used the same method but G-1 100 nM was used in place of 17β-E. Responses were recorded and analyzed using pCLAMP 9 software.

Results and Conclusions: Female slices (64%) were more responsive to 17 β-E compared to male slices (46%) suggesting that estrogen may act differently at the G-protein-coupled estrogen receptor in females and males. A follow-up study was conducted with the same methods using G-1 (100nM), an agonist of the G-protein-coupled estrogen receptor (GPER), in female subjects. G-1 increased the slope of fEPSP's significantly in female hippocampus slices. These findings suggest that estrogen agonists act through a GPER to enhance synaptic transmission in the hippocampus. We cannot rule out any contribution of the classical ERα/β receptors and future studies will have to focus on the interactions between all the different estrogen receptors.

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Factors Influencing Outcomes of Lumbar Fusion

Antoine C. Scott, MS II
Texas A&M Health Science Center
Bryan, Texas

Introduction and Background: Persistent chronic lower back pain (LBP) is a major problem throughout industrialized countries. In the US, it's the second most common chief complaint for those seeking medical care. LBP contributes to approximately fifteen percent of all sick-leave from work and is the principle cause of disability for persons under forty-five years of age. Persistent LBP is difficult to treat due in large to its etiology being even harder to define. No specific diagnosis can be made in over 85% of the reported cases although it has such a high prevalence.

Discogenic pain, LBP due to degenerative disc disease (DDD), attributes a considerable malady in 5% of the population annually. Several forms of treatment are offered with varied degrees of success. Spinal lumbar fusion, considered the more controversial treatment for discogenic pain, has yielded ambivalent results over the past twenty years.

Disc pathology has multiple diagnostic subsets and presentation criteria. Correct etiological identification plays a major role in successful surgical treatment. A majority of discogenic pain clinically presents *without the classical symptoms* of lumbar spinal stenosis. With that consideration, initial assessment needs to be based on a thorough medical patient history, imaging studies, physical exam findings, and functional survey of the LBP. MRI radiographic studies with T2-weighted imaging is the recommended method for evaluating the patient for degenerative changes however; they are not the sole determinant for the use and success of spinal lumbar fusion.

Hypothesis: Are chronic low back pain patients best treated with or without posterior lumbar spinal fusion?

Methods: A database will be constructed using the stratifications and further subgroups established based on the surgical technique, approach (posterolateral or circumferential), unilateral or bilateral fusion, single or multi-level fusion, and intrabody device installed. The inclusion of each factor aids in securing the most appropriate etiology, which, ensures the optimum surgical treatment, and allows for stronger correlate outcomes for respective patient subgroups. Given its extreme prevalence, reliable measures of success for lumbar spinal fusion with instrumentation will impact population health and the domestic workforce.

Conduct combined clinical meta-analysis and retrospective cohort study on surgical procedure used for treating discogenic back pain due to degenerative disc disease. Using a patient population of 361, medical records will be securely accessed and patients will be stratified into surgical indication subgroups based on multiple parameters. Selection for this study requires minimum of 6 months of atraumatic chronic LBP (previous history of chief complaint) and stratification is based on conservative treatments attempted, previous imaging studies, vocation, gender, age, and body-mass-index (BMI). Outcomes were assessed eight weeks post-procedure with inclusion of radiographic findings, complications, duration of recovery, rehabilitation regimen, and subsequent patient complaint.

Results: ------

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Generation of a Lentiviral Transposon for Screening of Proliferative Cardiomyocytes

Edward Seto, Yixin Jin, and Xu Peng
Department of Systems Biology and Translational Medicine
Texas A&M University Health Science Center College of Medicine - Temple, Texas

Introduction:

Determining ways to cause a fully differentiated cardiomyocyte to re-enter the cell cycle as a means to generate more cardiomyocytes could provide the basis for a novel therapeutic approach for the treatment of cardiac dysfunction. Insertional mutagenesis has become a powerful tool for identifying crucial genes involved in controlling cell proliferation. Transposons are small sequences of DNA that can be "cut and pasted" into random sites in a genome, and can be utilized as a tool in insertional mutagenesis to activate or disrupt the function of a gene depending on the insertion site of a transposon. In addition, an easy and efficient genetic material delivery system is necessary for a high-throughput screening analysis. To this end, a lentivirus will be created that encodes a transposon, serving as a genetic mutagenesis tool.

Methods:

Construction of the Transposon Element into a Lentiviral Vector: To clone the transposon element into the lentiviral vector, plasmid 7TC was first digested with SalI, filled in with T4 DNA polymerase to create two blunt ends, and then digested with ClaI, resulting in one blunt end and one sticky end. Plasmid pT2/Onc, containing the transposon element, was cloned using PCR methods, wherein PCR primers were designed that flanked the transposon element to be amplified. PCR purification was performed afterwards, and the transposon element was subsequently digested with ClaI, producing a blunt end and sticky end. Lastly, the lentiviral backbone and transposon element were ligated together.

Construction of the Transposase Sleeping Beauty into a Lentiviral Vector. To clone the *Sleeping Beauty* transposase into the lentiviral vector, plasmid 7TC was digested with ClaI, filled in with T4 DNA polymerase to create two blunt ends, and then digested with SalI to create one sticky end. Then, plasmid pCMV/SB10, which contains the *Sleeping Beauty* 10 transposase, was digested with BsaAI and SalI, creating a blunt end and sticky end, respectively. Lastly, the lentiviral backbone and *Sleeping Beauty* transposase were ligated together.

Results:

1) Confirmation of plasmids from manufacturer by using PCR and restriction enzyme digestion.
2) Amplification of Transposon Element fragment from pT2/Onc plasmid by PCR.
3) Preparation of Transposase from pCMV/SB10 plasmid by restriction enzyme digestion.
4) Subcloning of Transposon Element and Transposase into lentiviral vectors.

Confirmation of cloned Lentiviral-Transposon vector by using PCR and restriction enzyme digestion

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Role of Akt in Regulating Calcium Handling Through Signaling Interactions during Acute Hypoxia

Furqan Shah, Scott & White Memorial Hospital, Temple, TX; Linley E Watson, Scott & White Memorial Hosp, Temple, TX; Donald M Foster, Central Texas Veterans Health Care System, Temple, TX; David E Dostal, TAMHSC CVRI, Temple, TX; Honey B Golden, TAMHSC CVRI, Temple, TX

Introduction: We previously identified the MEK7-JNK1/2-Akt-phospholamban (PLB) signaling axis as a direct target in anthrax lethal toxin (LT)-induced cardiac dysfunction and an important regulator of intracellular calcium (Ca^{2+}_i) handling. Although LT-induced hypoxia and tissue necrosis are also common symptoms of anthrax toxicity, the effects of acute hypoxia on the MEK7-JNK-Akt signaling axis are unknown.

Hypothesis: We hypothesized that acute hypoxia induces a cardioprotective mechanism that couples MEK7 and Akt signaling to facilitate the phosphorylation of PLB-T^{17} and mitochondrial hexokinase II. Specifically, we hypothesized that MEK7, an upstream regulator of JNK, would improve JNK survival signaling through activation of Akt, and that JNK may serve as a functional regulator of Ca^{2+}_i handling through complex formation with Akt to phosphorylate (and thus inactivate) PLB-T^{17}.

Methods: A time-course study of acute hypoxia was performed using isolated neonatal rat ventricular myocytes (NRVM) at 0 min, 1min, 5 min, 15 min, 30 min, 1 h, 2 h and 4 h 5% O_2/CO_2. Western blotting was performed with cell lysates to determine phosphorylation of mTOR, MEK7, JNK1/2, Akt-T^{308}/S^{473} and PLB. The formation of JIP1 (scaffold protein for JNK), PLB and Akt complexes with JNK1/2, MEK7 and hexokinase II were determined by co-immunoprecipitation experiments at 0 min, 1 min, 15 min and 2 h hypoxia..

Results: We demonstrated that acute hypoxia phosphorylates (activates) MEK7, JNK, Akt and inactivates PLB-T^{17} after 15 minutes of incubation in a hypoxic (5% O2/CO2) environment, while activation of mTORC2 was induced at earlier time-points in isolated cardiac myocytes. Although phosphorylation (inactivation) of PLB-T^{17} subsided before 1 h of hypoxia, PLB-T^{17} phosphorylation increased after 2 h. Immunoprecipitation experiments using anti-JIP antibody revealed complex formation with phosphorylated MEK7, JNK and Akt-T^{308} at 15 min hypoxia, although these signaling interactions were lost at 2 h hypoxia treatment. JIP1 also formed complexes with phosphorylated JNK1/2 as early as 1 min. Interactions between JIP1 and phosphorylated Akt-S^{473} were not as apparent as with Akt-T^{308}. Immunoprecipitation using anti-Akt (pan) antibody revealed complex interactions with JIP, hexokinase II and JNK1/2 ($\alpha1\beta1$ isoforms) throughout the time-course. Furthermore, PLB formed complexes with Akt at 1 min and 15 min, although these interactions subsided at 2 h hypoxia. Interestingly, phosphorylated Akt-S^{473} immunoprecipitated with larger, high molecular weight complexes of PLB-T^{17}, instead of monomeric PLB.

Conclusions: We have characterized a novel signaling pathway in myocytes by which activation of JNK1 by MEK7 positively regulating Akt targeting to PLB and subsequent phosphorylation of PLB-T^{17}. Thus, integration of the JNK signaling module with Akt regulation represents an important stress-activated signalosome that may confer protection to sustain cardiac contractility and maintain normal levels of Ca^{2+}_i during different aspects of cellular stress.

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Determining the LC_{50} of the Glycogen-Synthetase-Kinase-(GSK)-3β inhibitor bromoindirubin-3'-monoxime (BIO) on Multiple Myeloma cell lines *in vitro*

Irtiza N. Sheikh, Ulf Krause, Bret Clough and Carl A. Gregory
Institute for Regenerative Medicine, Texas A & M University Health Science Center College of Medicine, (Temple Campus)

Introduction: Multiple myeloma (MM) is a malignancy of plasma cells arising from the bone marrow and is known to cause skeletal symptoms in patients such as osteolytic bone lesions and pathological bone fractures (myeloma bone disease, MBD). These cells have shown the ability to impair the osteogenic differentiation of mesenchymal stem/osteoprogenitor cells (MSCs) through secreted inhibitors of the canonical Wnt-signaling pathway with glycogen-synthetase-kinase (GSK)-3β as the key enzyme.
The current treatment for multiple myeloma involves the use of chemotherapeutic drugs such as thalidomide which reduce the viability of the MM cells but do nothing to stimulate osteogenic differentiation, or in the case of dexamethasone, even further increase osteoporosis. Bisphosphonates, the standard agents used to delay bone resorption, can have severe adverse effects after long-term use. There is a clear need for novel agents that not only kill the MM cells but reverse the damage to bones caused by the malignancy.
In previous research in a murine model of MBD, the GSK-3β-inhibitor bromoindirubin-3'-monoxime (BIO) has been found to stimulate MSCs to differentiate into osteoblasts and aid in self-healing of the bones. Surprisingly, BIO treatment also directly increased tumor necrosis and a pro-apoptotic effect on MM cell lines was demonstrated *in vitro*.

Specific Aims: The aim of this project is to determine the concentration of BIO which will reduce the viability of various MM cell lines to less than 50% (LC_{50}).

Methods: To achieve this aim, IL-6-dependent and -independent MM cell lines harboring different known mutations in apoptotic pathways were incubated with increasing concentrations of BIO. In a second set of experiments, the MM cell lines were co-cultured in direct contact with human MSCs to mimic some of the tumor-stroma interactions that are occurring *in vivo* and to test for the influence and predicted protective effect of the microenvironment. After 72h, cell numbers and viability were assessed by trypan blue staining and cell cycle analysis was performed by flow cytometry. All experiments were run in triplicates. Differences in viability and cell cycle were analyzed using ANOVA after arcsine transformation of proportions. LC_{50} values of BIO were calculated using probit analysis. Statistical significance was defined at a P value $< .05$.

Results: The data show that different MM cell lines show varying resistance to BIO (3-92% viability), so the LC_{50} could be determined for some but not all lines tested. In no case was the viability of MM cells increased over controls. The results also show that BIO treatment can reduce the overall cell number of the MM cells by slowing down or arresting the cell cycle. Furthermore, results indicate that co-culture with MSCs can protect the malignant cells from apoptosis.

Conclusion: The results of this experiment support the use of BIO for targeting certain MM cell lines that are susceptible to the inhibition of GSK-3β. In follow-up studies, these viability/cell cycle data will be correlated with changes in microRNA/mRNA/protein expression to provide more information on the mechanism of action of BIO. Use of more specific GSK-3β/cdk inhibitors will further elucidate the involved pathways. Overall, BIO and other
GSK-3β inhibitors might serve as a valuable adjunct therapy in combination with established treatment protocols through their enhancement of bone regeneration and therefore, improving patients' quality of life.

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Testing the effects of an artificial scaffold to promote blood vessel formation

E.J. Summers, S. Tsai, K.D. Northern, C. Abbey, J. Patterson, S.E. Bondos, K.J. Bayless
Department of Molecular and Cellular Medicine
Texas A & M Health Science Center, College of Medicine - College Station

Introduction: Cytokines and growth factors are proteins that regulate certain cellular functions such as growth, proliferation, differentiation and apoptosis to control key processes like angiogenesis, the formation of blood vessels from pre-existing vasculature. Consequently, these proteins can potentially be used to manipulate cell growth and behavior in 3D scaffolds used for tissue engineering. However, the harsh conditions necessary to form these materials can damage certain cytokines through denaturation or diffusion. The *Drosophila melanogaster* protein Ultrabithorax (Ubx) has the ability to self-assemble into materials, such as fibers or sheets, when placed in mild buffers and exposed to the air-water interface. This gentle approach to assembly allows proteins that are fused genetically to Ubx to preserve their original structure and function. We have preliminary evidence that Ubx is compatible with living eukaryotic cells and has mechanical properties similar to extracellular matrix proteins. This, coupled with its ability to assemble into these structures when fused to other proteins, enables Ubx to be an ideal tissue engineering scaffold. Different proteins such as enhanced green fluorescent protein (EGFP), mCherry, myoglobin and luciferase have already been combined successfully with Ubx. Consequently, angiogenesis-promoting proteins such as vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF-2), stromal cell-derived factor-1 (SDF-1a/CXCI12) and bovine serum albumin (BSA, which binds the angiogenic lipid S1P) will be effectively fused in the same way. Cytokines are usually found as soluble molecules and protein receptors are internalized by cells causing signaling to terminate. With different proteins and angiogenic factors covalently attached to Ubx fibers, it is predicted that protein receptor internalization will be prevented, allowing a stronger, more concentrated and longer lasting signaling cascade to promote cell proliferation and attachment to Ubx.

Hypothesis: We propose that adding human umbilical vein endothelial cells (HUVECs) to a sheet of VEGF-Ubx would cause sustained expression of the downstream second messenger pERK compared to adding soluble VEGF in solution and also would see more cell proliferation than HUVECs added to plain Ubx or mCherry-Ubx. It is also hypothesized that signaling responses would be longer and stronger, as fused VEGF may prevent the internalization of VEGF receptors and the termination of the signal that occurs with addition of soluble VEGF.

Methods: Testing the effects of soluble verses immobilized VEGF was accomplished by either adding recombinant VEGF in solution to HUVEC monolayers or allowing HUVEC to attach to immobilized VEGF. In the Bondos lab, the Ubx gene was fused to the gene encoding mCherry fluorescent protein, and then to both EGFP and VEGF. The cytokine genes were obtained from cDNA libraries of Clontech. The *E. coli* Rosetta bacterial strain expressed the proteins under the T7 promoter and those bacteria expressing the fused protein were then lysed and, using phosphate buffered saline, were diluted 100 fold in a Teflon-coated tray. Sheets and films of both mCherry-Ubx and EGFP-VEGF-Ubx protein were pulled using inoculation loops and 20,000 HUVECs were added to each. At time= 1 hr, 2 hrs and 3 hrs, a loop from each fusion protein was boiled in 150µl of hot sample buffer. For soluble VEGF studies, 2ml of buffer containing 40mg/ml of VEGF were added to HUVEC monolayers for 0, 5, 10 and 15 min. Lysates were collected. Western blots were used to test for the levels of ERK (antibody p44/42 MAPK Erk1/2, Rabbit mAb) and phospho-ERK (antibody phospho-p44/42 MAPK Erk1/2, Rabbit, (Cell Signaling Technology)) in all samples.

Results: pERK expression levels in HUVECs were highest at 15min after the addition of soluble VEGF. HUVEC exposed to VEGF-Ubx fibers displayed higher pERK expression at the 2hr time point compared to mCherry-Ubx. These initial studies suggest VEGF immobilized to Ubx may exhibit unique signaling properties to enhance angiogenic responses. Experiments are still being conducted to confirm these findings.

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Susceptibility of C57Bl/10, C57B10.D2 and C57B10.Q Mice to Epilepsy Following Theiler's Virus Infection

A. Toribio[a], M. Bijalwan[b], C.R. Young[b], C.J. Welsh[a&b]
[a]College of Medicine, Texas A&M Health Science Center, Bryan, Texas
[b]Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, Texas

Introduction: Epilepsy is a chronic neurological condition affecting millions of individuals worldwide with potential causes ranging from genetic and congenital abnormalities to trauma and infection. Previously, Theiler's murine encephalomyelitis virus when injected into susceptible SJL mice causes Theiler's virus-induced demyelination (TVID), a model of human multiple sclerosis. Susceptibility to TVID has been mapped to the H-2D region of the major histocompatibility complex (MHC). C57Bl/6 mice, when injected with Theiler's virus do not show symptoms of multiple sclerosis, up to nine months post-infection. However, it has recently been shown that Theiler's virus induces epilepsy in 50-60% of C57Bl/6 mice, which peaks at 5 days post infection. Interestingly, SJL mice do not develop epilepsy, at any time post infection. Our study was to examine the susceptibility of C57Bl/10 congenic strains of mice following Theiler's virus infection. The overall aim of this project is to attempt to map susceptibility genes to epilepsy within the mouse MHC.

Methods: 4-6 week old C57Bl/10, C57B10.D2 and C57B10.Q mice were purchased from The Jackson Laboratory while the C57Bl/6 mice were bred in house, 10 mice per strain (five male and five female). Mice were anesthetized with isoflurane and injected intracerebrally with Theiler's (BeAn strain) virus. Each mouse was weighed and given a clinical score using the Racine seizure scale. At day 10 and 13 post-infection mice were sacrificed by intraperitoneal injection of terminal euthanasia, exsanguinated and perfused in-vivo using phosphate buffered saline. Brains were either cryopreserved or processed for immunohistochemistry. Sagittal brain sections were stained for astrocytes (with antibody to glial fibrillary acidic protein), microglia (antibody to actin-binding protein IBA), nuclear staining (Hoescht) and neurons (Cresyl violet).

Results: 90% of C57Bl/10 mice had clinical signs with a maximum clinical score of 5, 100% of B10.D2 mice had clinical signs with a maximum clinical score of 6 and 100% of B10.Q mice had clinical signs with a maximum clinical score of 6. We developed an amended Racine score system since we observed additional clinical symptoms not included in the original Racine scoring scale such as hyper-salivation, arched back and hind-limb paresis. Astrocyte and microglial immunofluorescence shows a marked increase of activated cells in the hippocampus that extends to the substantia nigra. Nuclear and neuronal staining shows degeneration of pyramidal cells in the hippocampus while no astrocytosis or microgliosis was evident in the cerebellum.

Discussion: Extensive proliferation of astrocytes and microglia coupled with neuronal cell death in the hippocampus may set the stage for the development of epilepsy. Recent work has demonstrated that reactive astrocytes and microglia release proinflammatory molecules (i.e. cytokines, chemokines) that may interact with neurons increasing neuronal excitability. Increasing the excitability of neurons in the hippocampus may lead to seizure formation. Astrocytes play a role in the sequestering of potassium from the extracellular space around neurons. Astrocytosis may increase potassium uptake that may reduce neuron threshold potential leading to hyperexcitability of neurons and seizures. Neuronal loss in the hippocampus correlates with the seizures observed but may also play a role in memory deficits in affected mice.

Conclusion: C57Bl/10, B10.D2 and B10.Q mice all develop epilepsy following injection with Theiler's virus. Proliferation of astrocytes and microglia is evident in the hippocampus, extending to the substantia nigra. There is no apparent astrocytosis or microgliosis in the cerebellum. This study has laid the foundation for examining the role of the MHC in the control of epilepsy in a murine model. We have developed a more refined infectious agent model of murine epilepsy that could be used for examining intervention therapies for the treatment of epilepsy.

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Contribution of Interleukin 6 (IL-6) *Trans* Signaling to Pulmonary Fibrosis

Xuan Tran[1], TT Le[2], H Karmouty-Quintana[2], E Melicoff[3], M Pedroza[4], L Garcia-Morales[5], S La Francesca[5], H Seethamraju[5], and MR Blackburn[2]

1. Texas A&M Health Science Center, College of Medicine, Bryan, TX; 2. Department of Biochemistry and Molecular Biology, University of Texas Health Science Center, Houston, TX; 3. Department of Pediatrics, Baylor College of Medicine, Houston, TX; 4. Department of Medicine – Rheumatology, Baylor College of Medicine, Houston, TX; 5. The Methodist Hospital Research Institute, Houston, TX

2.

Introduction: Idiopathic Pulmonary Fibrosis or IPF is a lethal lung disease that causes progressive scaring and fibrosis of the lung. IPF, unlike other chronic lung diseases like COPD and emphysema, has no known cause. IPF is different than other interstitial lung diseases in that there is no effective treatment available nor is there a way to stop the progression of the disease. This is largely because, as the name of the disease indicates, little is known about its pathogenesis. Since little is known about the etiology of IPF specifically, we first asked ourselves: What are the causes & mechanisms that regulate pulmonary fibrosis? We then apply these concepts to further understand IPF. In trying to answer the previous question of what mechanisms could be involved in regulating pulmonary fibrosis, we take into consideration the current view that pulmonary fibrosis develops as a result of abnormal repair processes in response to lung injury. The focus of our lab is predominately on understanding the interplay of the factors normally generated during tissue injury and then further investigating what happens when there is an up-regulation or down-regulation of such factors. We are particularly interested in studying the purine signaling nucleoside adenosine and subsequent activation of the cytokine interleukin 6 (IL-6) signal transduction in particular. Previous studies from our lab showed that IL-6 contributes to pulmonary fibrosis and its removal results in attenuation of fibrosis. We further demonstrated that levels of soluble IL-6 receptor alpha (IL-6Rα), indicative of IL-6 trans signaling, are elevated in the lungs of IPF patients suggesting that IL-6 trans signaling plays a role in the pathogenesis of IPF.

Hypothesis: Will blocking IL-6 trans signaling result in attenuation of pulmonary fibrosis in the adenosine-deaminase (ADA)-deficient mouse model?

Methods: Using the adenosine-deaminase (ADA)-deficient mouse model of pulmonary fibrosis, either phosphate buffered saline (vehicle) or soluble GP130 (intervention) was given via intraperitoneal injections once a day from Day 30 to Day 42. Soluble GP130 is a reagent used to block IL-6 *trans* signaling in vivo. The mice were sacrificed at the end of the experiment and assessed for changes to pulmonary phenotype. Sample collections included: plasma, bronchoalveolar lavage (BAL) fluid and whole lungs. Total cell count and cell differential were performed on BAL fluid. Supernanant from BAL fluid was analyzed with ELISA and SIRCOL assays. Whole lungs were fixed in formalin and embedded in paraffin for immunohistochemistry staining. Frozen lungs were used to prepare RNA and protein for PCR and western blotting analyses.

Results: In comparison to ADA-deficient mice given saline (who developed pulmonary fibrosis), ADA-deficient mice treated with soluble GP130 had significant reductions in total cell counts, collagen deposition, and alpha smooth muscle actin (α-SMA) expression, which translated to less fibrosis.

Conclusions: These findings suggest that IL-6 *trans* signaling plays an essential role in the development of pulmonary fibrosis, and neutralization of *trans* signaling is enough to attenuate disease.

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Cardiovascular Inflammation following TBI

Stacey Velasquez, Suzanne Zeitouni, Lee Shapiro
Scott & White Hospital, VA Central Texas Health Care System, Texas A & M University Health Science Center College of Medicine-Temple

Introduction: Traumatic brain injury (TBI) is a relatively common condition that can arise following everyday activities, as well as from battlefield related injuries to soldiers. TBI can be caused by a direct blunt trauma causing contusion to the brain, or from tissue damage as the result of sudden changes in acceleration/deceleration. Such injuries can also occur from blast or percussive forces, where extreme air movement results in neurotrauma. The majority of research into TBI has focused on examining central nervous system (CNS) deficits. However, it is possible that peripheral organ dysfunction is also involved in TBI-induced pathology. For example, brief periods of hypoxia/ischemia are known to occur following TBI. As a result, cardiovascular abnormalities are often observed as part of the TBI syndrome (1). Previous studies demonstrate that cardiovascular dysfunction is associated with up regulation of pro-inflammatory molecules. Considering that the blood brain barrier is compromised following TBI, it is possible that inflammatory proteins released from the heart could enter the brain and have detrimental effects. However, no studies have conclusively demonstrated cardiovascular inflammation following TBI. Therefore, this study was taken to address this possibility.

Hypothesis: TBI results in pro-inflammatory cytokine release in the heart.

Methods: The purpose of this study was to investigate cellular and inflammatory signaling molecules released in response to TBI. For this study, the well described fluid percussion injury (FPI) model (2) was used to assess up regulation of inflammatory molecules in the heart. Tissue samples from the heart were collected, flash frozen, and stored at -80 degrees Celsius for protein extraction. The proteins were solubilized using an extraction solution consisting of PBS containing 1 mM MgCl2, 1% (w/v) SDS (Sigma), 0.1% Triton X-100 (Fisher Lifesciences) and 10 fold protease and phosphotase inhibitor cocktail (Roche Diagnostics, Nutley, NJ). Tissue was homogenized using a cell lysis buffer so that analysis could be carried at western blot. Protein levels were determined using coomassie blue R250 stained gels. TBI was performed using a specially designed pendulum to deliver FPI through a fluid-filled chamber attached to the skull of adult mice (23-25 gms). Tissue was collected at 2, 6, and 24 hours post injury.
Western blot analysis was carried using standard techniques. Briefly, Samples were reduced by heating for 5 min at 95ºC and were run on an SDS-PAGE Tris Glycine gel at 150 volts and then transferred to a PVDF membrane. The membrane was incubated in primary antibody overnight and this was followed by incubation with an HRP-conjugated secondary antibody for two hours. The blot was visualized using chemiluminescence. Phosphorylated levels of the following proteins were examined: Akt, p38, Jnk, Erk. The phosphorylation of these proteins is affected by cytokine secretion, and they are involved in inflammatory, cell death or cell survival pathways.

Results: Evidence for inflammation is observed in the heart at 2, 6, and 24 h after TBI. pAkt is more robust at the 2 h time point, whereas pErk elevation was most robust at the 24 h time point. Jnk and p38 analysis is ongoing. Future work will include looking at total protein levels and examining the ratio of phosphorylation to total. Once cardiovascular inflammation is established, future studies will examine their putative role in CNS dysfunction.

References: (1) Brett E. Larson, David W. Stockwell, Stefan Boas, et al. Cardiac Reactive Oxygen Species after Traumatic Brain Injury. Journal of Surgical Research 173, e73-e81 (2012)
(2) Sanjib Mukherjee, Khurshed Katki, Gabriel Arisi, et al. Early TBI-induced cytokine alterations are similarly detected by two distinct methods of multiplex assay. Frontiers in Molecular Neuroscience (2011)

TAMHSC SUMMER RESEARCH PROGRAM

August 8, 2012: 9:00 AM - 2:00 PM
Health Professions Education Building
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Characterization of *Coxiella burnetti's* Toxic Substrates

Brett Walker, Mary M. Weber, Chen Chen, Zhao-Qing Luo, James Samuel
Department of Microbial and Molecular Pathogenesis
Texas A & M University Health Science Center College of Medicine, Bryan

Introduction: *Coxiella burnetii* is a Gram-negative obligate intracellular pathogen that causes acute and chronic Q fever in humans. This organism is primarily associated with disease in livestock, but is readily transferred between hosts by the inhalation of aerosolized bacterial particles. Because the LD50 is only one *Coxiella burnetii* organism, it is considered to be one of the most infectious known organisms. In most cases host defenses limit infection to a flu-like illness that dissipates without antibiotics, but in rare incidents, patients can develop serious complications or possibly chronic Q fever.

Coxiella burnetii, like *Legionella pneumophila*, encodes a specialized Dot/Icm secretion appraratus that is essential for intracellular replication and for the formation of an acidic parasitophorous vacuole derived from the host lysosomal network. This system delivers bacterial effector proteins into the cell's cytoplasm. Recent advances in the field, including a mariner-*Himar1* transposon and the development of an acidic culture media for axenic growth, have allowed for basic manipulate of this pathogen and have allowed for the identification of over 90 *C. burnetii* T4SS substrates. Furthermore, recent data indicates that like in *L. pneumophila*, a functional Dot/Icm secretion is essential as secretion mutants fail to replicate intracellular and do not form a spacious parasitophorous vacuole. While numerous studies have sought to identify *C. burnetii* T4SS substrates, little is currently known about the role of these substrates in *C. burnetii* pathogenesis. In the current study we used several large-scale screens to characterize these substrates.

Hypothesis: The working hypothesis is that heterologous expression of *C. burnetii* T4SS substrates in yeast will identify several substrates that impair normal host cell processes and the function of these substrates in *C. burnetii* pathogenesis can be determined using a yeast suppressor screen.

Methods: In order to identify substrates capable of interfering with normal host cell processes, each of our 90 T4SS substrates were cloned into pYesNTA under a galactose-inducible promoter. Heterologous expression in yeast identified four substrates (CBU0041, CBU1524, CBU0885, and CBU2052) that moderately impaired yeast growth. Ectopic expression in Hela cells revealed that these substrates traffic to distinct subcellular compartments. In order to identify pathways targeted by these substrates we overexpressed host proteins to identify host proteins capable of rescuing the growth defect. Using this approach we isolated over 200 colonies that displayed normal growth.

Results: We confirmed the toxicity of CBU1524, CBU2052, CBU0885, and CBU0041; as well as, rescued approximately 100 yeast colonies from each CBU1524, CBU2052, and CBU0885.

Conclusions: Characterizing the toxic substrates of *Coxiella burnetii* and their corresponding host suppressors will allow us to identify pathways targeted by host. We can then determine how the pathogen modulates it by using pull-downs to identify binding partners. Understanding and characterizing these substrates will further our understanding of host pathogen interactions.

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August 8, 2012: 9:00 AM - 2:00 PM
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Regulation of IL-5 receptor trafficking by serine residues in the common beta chain

Ashley Way, Jonathan T. Lei and Margie Martinez-Moczygemba
Department of Microbial and Molecular Pathogenesis and Clinical Science
and Translational Research Institute
Texas A & M University Health Science Center College of Medicine,
Houston Campus

Introduction: Eosinophils are multifunctional leukocytes implicated in the pathogenesis of many inflammatory diseases including allergic asthma and hypereosinophilic syndrome. Eosinophil physiology is dependent on interleukin-5 (IL-5) and the IL-5 receptor (IL-5R), which is composed of two chains: the IL-5Rα and the common beta chain (βc). IL-5 is the principle cytokine for eosinophil differentiation, activation, and prolonged cell survival. Key to these biological functions is βc tyrosine phosphorylation which regulates signal transduction. However, the role of βc serine phosphorylation in IL-5R function has not been examined. Previous bioinformatics analysis of the βc cytoplasmic domain revealed the presence of three serine residues located in functionally relevant binding domains, two of which are ubiquitin ligase binding sites. In this study, we asked whether or not the presence of these three serine residues played a role in IL-5 receptor function.

Hypothesis: The presence of three potentially phosphorylated βc serine residues play a role in the intracellular trafficking and cell surface expression of the IL-5 receptor.

Methods: Three βc mutants, βc Fbw7 S543A, βc β-TrCP S683G, and βc S585G, were stably-transduced into HEK293 cell lines expressing IL-5Rα. All cell lines were assayed for receptor function by flow cytometry, microscopy, and biochemical techniques. The trafficking patterns of each βc mutant were monitored by labeling cell surface IL-5 receptors with Cy3 IL-5 and the lysosomal marker, LAMP-1.

Results: Our data demonstrate the requirement for the presence of two βc serine residues, S543 and S585, for accurate IL-5 receptor trafficking and efficient cell surface expression. We predict that these βc serine residues are physiologically phosphorylated and mass spectrometry analyses will be performed to test this hypothesis. Moreover, we further speculate that phosphorylation of these serine residues serve as docking sites for the binding of regulatory adaptor proteins to βc which guide activated receptors through the endocytic pathway and deliver them to the lysosomes.

TAMHSC SUMMER RESEARCH PROGRAM

August 8, 2012: 9:00 AM - 2:00 PM
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Role of obstacles to primary care and emergency department utilization for Medicaid beneficiaries

AJ Widmer[1], MPH; R Basu[2], PhD; A Hochhalter[1,2], PhD
[1]Department of Internal Medicine; [2]Center for Applied Health Research
Texas A & M University Health Science Center College of Medicine – Temple

Introduction: Emergency Departments (EDs) serve as an entry portal to America's health care system for many individuals due to convenience and availability regardless of ability to pay. Medicaid beneficiaries utilize the ED for conditions that are sensitive to ambulatory care at a higher rate than individuals with other insurance types or no insurance. By definition, the optimal settings in which to care for these ambulatory care sensitive conditions (ACSCs) are primary care or other office-based settings. A critical need exists for information on why Medicaid beneficiaries tend to utilize the ED for conditions that may be more appropriately addressed in primary care offices. The objective of this retrospective study is to test the association between barriers to obtaining and maintaining primary care services and utilization of the ED and other healthcare services by Medicaid beneficiaries.

Hypothesis: Self-reported barriers to continued primary care services are associated with the rate of ED utilization for Medicaid beneficiaries.

Methods: Data were extracted from the 2009 Medical Expenditure Panel Survey (MEPS), a nationally representative sample of the civilian non-institutionalized population in the United States. The MEPS is conducted annually by the Agency for Healthcare Research and Quality (AHRQ) and the National Center for Health Statistics (NCHS) to provide national estimates of health care use, expenditures, payment sources, and insurance coverage. The publicly available 2009 MEPS full year consolidated data file contains 36,855 individuals who participated in the MEPS Household Component of the 2009 survey. For this study, variables were selected from the self-reported Household Component and the provider-submitted Medical Provider Component (MPC). Our study sample included 1578 respondents who had full year (12-month) Medicaid coverage during the year 2009. The variables of interest included insurance coverage type, usual source of care provider characteristics, ambulatory care sensitive conditions, place of medical care visits, and patient characteristics. This poster presents preliminary analyses of individual characteristics and office visits associated with ED use. The emergency department visits were considered as a categorical variable having 3 categories as no ED visits, ED visits between 1 and 2, and 3 or more ED visits.

Results: On average, 21% of respondents had one or two emergency department visits and 4% had three or more ED visits. Respondents had about 6 physician outpatient visits in the study year. The chi-square-test was performed to assess the differences in ED utilization for beneficiaries with and without several chronic conditions (each considered separately). Beneficiaries with hypertension, coronary heart disease, and asthma (considered individually) had higher rates of ED utilization than beneficiaries without each condition (in all cases, $p<0.001$). Number of office based physician visits was significantly associated with more ED visits ($\lambda^2=92.2$, $p<0.001$).

Conclusion: Presence of some chronic illnesses and office visits were associated with higher ED utilization among Medicaid beneficiaries. The next step in this study is to test multivariate models to identify groups of variables associated with ED use. By identifying subgroups at highest risk of ED utilization, interventions may be better targeted to groups at highest risk of ED utilization.

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August 8, 2012: 9:00 AM - 2:00 PM
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The Effects of Indole on Susceptibility of *Salmonella typhimurium* to Host Antibacterial Compounds

Laura Zinke, TAMHSC SRP participant; Candice Devora, Madhu Katapali, Nandita Kohli, Arul Jayaraman, Robert Alaniz, PI
Microbial and Molecular Pathogenesis
Texas A & M University Health Science Center College of Medicine,
College Station

Introduction: *Salmonella typhimurium,* a pathogenic strain of bacteria, infects the human digestive tract through ingestion of contaminated foods. *Salmonella* uses Salmonella pathogenicity island 1 (SPI-1) coded type III secretion system (T3SS), a needle-like structure, to inject invasion proteins into the host cell, causing engulfment the invading *Salmonella* by epithelial cells. *Salmonella* can subsequently spread to other parts of the body and cause salmonellosis. During this journey through the GI tract, *Salmonella* interacts with both the host and the host microbiota, and with different compounds produced by them. The compounds this study looked at were indole, sodium deoxycholate, and hydrogen peroxide.

Indole is a tryptophan metabolite produced as extra-cellular signaling molecule by gut-colonizing *E. coli* and has been shown to affect *Salmonella*. Specifically, indole reduces the expression of TTSS-1 genes involved in the initial infection of the gut cells, including those that affect motility and flagella formation. It also down-regulates genes in the SPI-1, including those in T3SS. However it has not been shown to affect the growth and survival of *Salmonella* once inside the invaded cell.

Sodium deoxycholate (DOC) is a known component of bile in the human intestinal tract, that acts within the digestive tract as an antibacterial through membrane disruption, DNA damage, and protein denaturation. A minimum inhibitory concentration (MIC) of DOC for *Salmonella* has been shown to be 7%.

Hydrogen peroxide is a reactive oxygen species (ROS) which is found in high concentrations in lysosomes in the host cells. After *Salmonella* bacterium is engulfed, the phagosome fuses with a lysosome, exposing the bacterium to the H_2O_2 and its bacteriacidal effects.

Hypothesis: Will growth of *Salmonella typhimurium* in the presence of indole affect its response to compounds used as host defenses against bacterial invasion.

Objectives: Determine the differences in reaction to DOC and H_2O_2 between *Salmonella* grown in the presence of indole and grown without it using a fluorescence and absorbance.

Visualize and confirm these results using zone of inhibition disc assays.

Determine differences in cellulose and fimbriae formation related to indole treatment.

Methods: In a minimum inhibitory concentration series of experiments wildtype *Salmonella typhimurium* was cultured in overnight LB broth both in the presence of 1 mM indole and without indole. Cultured *Salmonella* was treated with sodium deoxycholate or hydrogen peroxide and Alamar blue, and absorbance/fluorescence was read every 30 minutes for 4 hours using a plate reader. In related experiment *Salmonella* cultured overnight with and without indole were diluted to 10^6 cells/ml and grown into lawns. Discs with sodium deoxycholate, hydrogen peroxide, or HCl added to measure the differences in zones of inhibition, and antibiotics were used as positive controls for growth inhibition. The clearings were measured after 8 hours of incubation at 37°C and again at 18 hrs of incubation to determine the inhibitory effect of the compounds on indole-treated and non-indole-treated *Salmonella* and the differences between the two.

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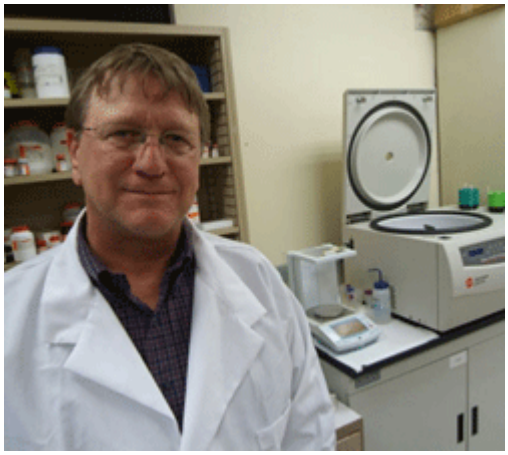
Pydi Akella	Dr. Samba Reddy
Cecilia Benz	Dr. Emily Wilson
Jessica Heibel	Dr. Farida Sohrabji
Charles Howard	Dr. Emily Wilson
Shadi Lavasani	Dr. Farida Sohrabji
Phillips Nagsuk	Dr. Rajesh Miranda
Casey Roth	Dr. William Griffith
Antoine Scott	Dr. Jonathan Friedman
Antonio Toribio	Dr. Jane Welsh
Brett Walker	Dr. James Samuel
Undergraduate Students	
Arielle Baker	Dr. Farida Sohrabji
Gilbert Encarnacion	Dr. Jeffrey Cirillo
Gabrielle Henslee	Dr. Mari Muthuchamy
Gemel Joseph	Dr. Laura Hendrix
Devesha Lester	Dr. Steve Maxwell
Eleanor Summers	Dr. Sarah Bondos and Dr. Kayla Bayless
Laura Zinke	Dr. Robert Alaniz

Temple	
Medical Students	
Jordan Buess	Dr. Thomas Kuehl
Peter Chen	Dr. Art Frankel
Shannon Essler	Dr. David Morgan
Hai Hoang	Dr. Cynthia Meininger
Nisha Patel	Dr. Sharon DeMorrow
John Reilly	Dr. Chris Chaput
Edward Seto	Dr. Xu Peng
Undergraduate Students	
Lai Jiang	Dr. Fei Liu
Bradly Kimbrough	Dr. William Culp
Demetria Perry	Dr. Shaodong Guo
Anjali Rai	Dr. Kenneth Baker
Irtiza Sheikh	Dr. Carl Gregory
Stacey Velasquez	Dr. Lee Shapiro

Houston	
Medical Students	
Zach Henderson	Dr. Mingyao Liu
Sheryl Jacob	Dr. Magnus Hook
Undergraduate Students	
Ashley Way	Dr. Margie Moczygemba

2012 College of Medicine
Summer Research Program Seminar Series

Date	Time	Topic	Presenter
6/4	12:00 PM	Record Keeping	Dr. Van Wilson
6/5	12:00 PM	Bridging the Translational Research Gap Through Exercise and Nutrition Research	Dr. Richard Kreider
6/8	9:00 AM	Translating Science into Clinical Medicine	Dr. David Huston
6/12	12:00 PM	Engineering Healthcare and Bringing Innovative Concepts to Market	Dr. John Criscione
6/15	9:00 AM	Orthopedic Oncology and Pediatric Orthopedics; Fertile Ground for Translational Medicine	Dr. Suzanne Yandow
6/19	12:00 PM	Human Experimentation	Dr. John Quarles
6/22	9:00 AM	Scientific Misconduct	Dr. Vernon Tesh
6/26	12:00 PM	Clinical Research for Physicians in Academic Medicine	Dr. John Friedman
6/29	9:00 AM	Development of Therapies for Medullary Thyroid Carcinoma	Dr. Robert F. Gagel
7/3	12:00 PM	MD/PhD Program	Dr. Julian Leibowitz, Anita Mantri, and Evan Cherry
7/6	9:00 AM	Use of Animals in Biomedical Research: Why, Which Animal and How Many?	Dr. James Elliott
7/10	12:00 PM	Clinical Science and Translational Research Grand Rounds	Dr. Carl Gregory
7/13	9:00 AM	Science and the Microbe	Dr. Samuel Shelburne III
7/17	12:00 PM	Scientific Method	Dr. David McMurray
7/20	9:00 AM	Medical Research… Why Me?	Dr. William Culp
7/24	12:00 PM	Biotechnology/Ethics	Dr. James Samuel
7/27	9:00 AM	Translating Basic Airway Epithelial Biology to Asthma, Pneumonia, and Lung Cancer	Dr. Burton F. Dickey
7/31	12:00 PM	Student Oral Presentations	
8/3	9:00 AM	Student Oral Presentations	
8/8	9:00 AM-2:00 PM	Poster Presentations and Reception	



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

Department of Systems Biology & Translational Medicine
Texas A&M Health Science Center
College of Medicine
Rm. 310B Reynolds Medical Building
College Station, TX, 77843-1114
Email: WEZimmer@medicine.tamhsc.edu
Phone: 979-845-2896