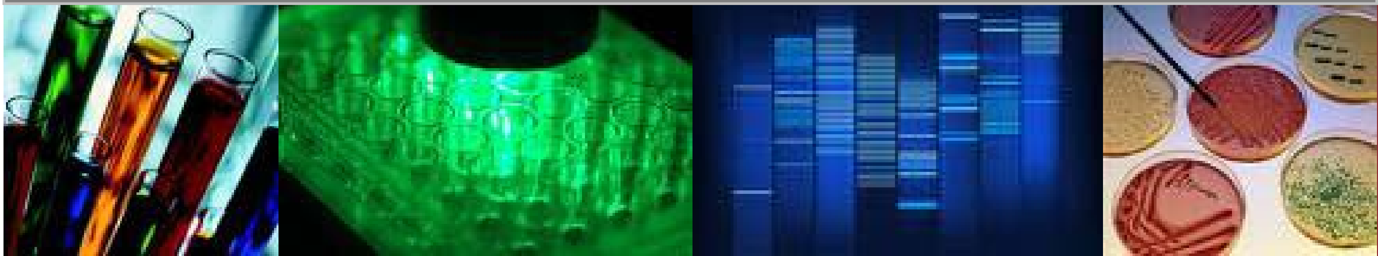
Poster Session

and Reception

2009

Summer Research Program



July 29, 2009

9 a.m. – 2 p.m.

Reynolds Medical Building

Texas A&M
Health Science Center
College of Medicine

Program

July 29, 2009

9 a.m.-2 p.m.	Poster Presentations, lobby of the Reynolds Medical Building
11:30-12:30 p.m.	Lunch 150 Reynolds Medical Building
12:30-1:00 p.m.	Speech by Dr. Christopher Colenda, Dean College of Medicine Lobby Reynolds Medical Building
1:00-1:45 p.m.	Presentation of Certificates by Dr. Warren Zimmer, Director of the Summer Research Program
1:45-2 p.m.	Presentation of Dean's Recognition Awards
2 p.m.	Adjourn

Speaker's Bio:

Christopher C. Colenda, M.D., M.P.H.

The Jean and Thomas McMullin Dean of Medicine
Health Science Center Vice President for Clinical Affairs

Christopher C. Colenda, M.D., M.P.H. became the Jean and Thomas McMullin Dean of the Texas A&M Health Science Center College of Medicine in January 2003. As dean, Dr. Colenda is the chief administrative and academic officer of the college. He is responsible for the organization, operation, development and evaluation of instruction and research programs, as well as leading the faculty and administration for all academic programs of the college.

A geriatric psychiatrist by training he is currently is a member of the Liaison Committee for Medical Education, the National Board of Medical Examiners, the Accreditation Council for Graduate Medical Education, and the American Board of Psychiatry and Neurology. He was recently selected to serve on the Psychological Health External Advisory Subcommittee for the Defense Health Board, Department of Defense, and elected to the Administrative Board of the Council of Deans for the Association of American Medical Colleges.

Dr. Colenda's awards are numerous. He was selected as a delegate to the White House Conference on Aging in Washington, D.C. in 2005. Some of his past recognitions include APA's Jack Weinberg Award in Geriatric Psychiatry, the Alumni Star for the School of Medicine of the Medical College of Virginia, Outstanding Faculty Award from the College of Human Medicine at Michigan State University and a Special Commendation from the Council of Aging of the American Psychiatric Association. He is a Member of Alpha Omega Alpha and Sigma Xi Honorary Societies. Dr. Colenda was elected to the American College of Psychiatrists in 1998, and has been listed among the Best Doctors in America since 1994.

Dr. Colenda came to the College of Medicine from Michigan State University, where he served as Professor and Chairman of the Department of Psychiatry and Associate Dean for Programs and Projects. Prior to his service at MSU, Dr. Colenda was a faculty member and administrator at Wake Forest University School of Medicine and at the Medical College of Virginia of Virginia Commonwealth University.

Dr. Colenda received a B.A. in chemistry from Wittenberg University in 1974, followed by his M.D. degree in 1977 from the Medical College of Virginia. He completed his training in psychiatry at the University of Virginia Hospitals and at Emory University where he served as Chief Resident and Fellow. Dr. Colenda also received a Masters of Public Health in Health Services Administration from Johns Hopkins University in 1982.

Acknowledgements

A major goal of the Texas A&M Health Science Center College of Medicine Summer Research Program is to provide students a foundation to the "how and why" of conducting biomedical research. The program serves two separate student populations. Undergraduate students from all over the country join the program to gain research experience and assess their desire to pursue graduate studies, perhaps in the combined MD/PhD program. Additionally, upon completion of their first year, our medical students have the opportunity to participate with the faculty in a research project. This is the first year we have had students on three separate campuses: College Station, Temple and Houston (see pages 40, 41). With the expansion to multiple sites we have opened many more opportunities for the students, allowing them to gain unique perspectives and a better understanding of basic research and academic medicine.

All the program participants have attended research-related talks in addition to working very hard in the labs during the program. On display at today's poster session/reception is the products of their hard work, and I invite you to come view the posters, ask questions and be prepared to lean.

The program would not be successful without the dedication of faculty, both as research advisors and for providing stimulating and informative lectures. Please see the content of these talks on page 42. In addition, it is difficult, but not impossible, to provide content simultaneously to three locations. The work of Drs. Murray (College Station), Mitchell (Temple) and Huston (Houston) as site coordinators keeping things running efficiently is greatly appreciated.

We obtained funding from a number of sources and would like to thank Dr. Christopher Colenda, College Dean; Dr. Don Wesson, Vice Dean of the Temple Campus; and Dr. David Carlson, Vice President for Research and Graduate Studies for major contributions to our budget. In addition, we could not have had a successful year without the generous support of Dr. Roy Smythe, Chairman of Surgery; Dr. Alejandro Arroliga, Chairman of Medicine, Dr. Harris Granger, Chairman of Systems Biology and Translational Medicine, Dr. Val Runge, Radiology; and Dr. William Culp, Cardiothoracic Anesthesiology. Finally, I would like to thank Dr. Van Wilson and his staff in College Station, **Josephine Hernandez and Mary Ann Wolff;** Dr. Huston's staff in Houston, **Anna Wirt;** and the Dean's staff in Temple**, Loria Lynce and Cari Cummings** for making certain that the entire program got off the ground and running effortlessly.

Dr. Warren Zimmer

Director, Summer Research Program

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Accurate Prediction of PTCA outcomes

Uchenna O. Aduba, Dr Catherine McNeal, Dr Ronald Macfarlane

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Texas A & M Health Science Center College of Medicine

Coronary artery disease (CAD), the number one killer of both men and women in America today afflicting over 13 million people currently[1]; begins when plaque (composed of cholesterol, fatty acids, cells and lipids) deposits along arterial walls. Plaque build up is dependent on an individual's lipid chemistry, metabolism, endothelial health and risk factors (hypercholesterolemia, hypertension, diabetes mellitus) eventually causing stenosis (narrowing) of the arterial walls with clinical manifestations of angina pectoris and possibly heart attacks due to lack of perfusion.

Treatment options of stents, surgical intervention and lipid lowering interventions are problematic because physicians have no diagnostic marker ensuring with high probability, the best course of action. Current studies indicate possible diagnostic markers based density, surface charge, and constitution of apolipoproteins of an individual's lipoprotein chemistry but are limited to only total cholesterol, HDL-c (high density lipoprotein), LDL-c (low density lipoprotein) and triglyceride.[2] Over 50% of CAD patients have normal lipid profiles based on such vectors. Additionally, such factors do not allow one to predict short and long term outcomes of Percutaneous Transluminal Coronary Angioplasty (PTCA) or Coronary Artery Bypass Graft (CABG).

PTCA involves opening of coronary arteries with a balloon, with arteries kept open by a stent (tube). There has been recent return to bare metal stents (BMS) due to increased risk of thrombosis with drug eluting stents. Even with BMS, restenosis is rather frequent with rates of 30% to 40% with 6 months.[3] Current studies have shown a strong correlation between C-reactive protein levels and lipoprotein-a in predicting in-stent restenosis.[4] Additionally, there may be a correlation between serum lipid levels and restenosis.

Analysis of lipid particle subspecies and associated apolipoproteins provides a gateway to an array of over 100 potiential diagnostic markers ensuring accurate outcome predictions. We seek to identify characteristics of lipoprotein particles that promote in-stent restenosis.

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Androgen responsiveness of Nkx3.1 gene is due to binding and transactivation of androgen receptor

Douglas K. Armour, Chinatsu Kojima, and Warren E. Zimmer

Department of Systems Biology and Translational Medicine

Texas A & M Health Science Center College of Medicine

Nkx3.1 is a conserved homeobox gene that plays a critical role in the development, differentiation and maintenance of prostate epithelial cells in a variety of animal models, but the precise molecular mechanism is not well understood. There is not a clear understanding of the molecular means of transcriptional control of Nkx3.1. Nkx3.1 is expressed in an androgen dependent manner in normal prostate epithelial cells. We show that the 2.7 kilobase in front of mouse Nkx3.1 gene enhances transcription in prostate epithelia cells, but this segment is not androgen responsive. We have shown that there are many potential androgen responsive elements (AREs) found within the adjacent sequences of Nkx3.1 and within the inton of the gene. We seek to identify the particular groupings or pairs that are androgen responsive. In the course of our investigation we have shown that there are two ARE half-sites found within the single intron of Nkx3.1 which were demonstrated to confer androgen-dependent transcriptional activation. Each element referred to as ARE A and ARE B contain the base pair core sequence, TGTTCT, that is identical to previously described androgen receptor half-site sequence. The canonical androgen receptor (AR) sites are typically palindromic sequences of the two ARE half-sites placed end to end with no spacer sequence. There have been androgen responsive genes that contain the half-sites separated by short sequences of basepairs. In Nkx3.1 the two half-sites are non-canonical and separated by 491 basepairs; a spacer sequence of this length has not been described previously. The conservation of these sequences across many species suggests that the appropriate transcriptional control especially at this site is vital for normal prostate maturation and differentiation. We have shown that in androgen responsive prostate cancer cells (LNCaP) when transfected with luciferase reporter gene under control of the normal intron of Nkx3.1, there is considerable luciferase activity. Finally we have shown that the activity seen is a direct result of androgen receptor and not caused by secondarily activated pathways. We sought to investigate this by utilizing cotransfection of androgen receptor and the same luciferase reporter into cells that do not endogenously express androgen receptor. These two pieces of data taken together show the androgen responsiveness of Nkx3.1 is due to the direct binding and transactivation of androgen receptor.

Novel Biocompatible Matrices for In Vivo Delivery of Mesenchymal Stem Cells for Bone Repair

Darryl T. Blalock, Ulf Krause, Suzanne Zeitouni and Carl A. Gregory

Institute for Regenerative Medicine, Texas A & M Health Science Center College of Medicine

Introduction and Background: Mesenchymal stem cells (MSC's) are pluripotent cells with the ability to differentiate into many cell types, including osteoblasts. These cells are usually extracted from adult bone marrow and easily expanded in culture using established methods. The differentiation of MSC's into osteoblasts has been shown to rely upon Wnt signaling and this information is important for their use in repairing bone. Through manipulation of this pathway, MSC's can be induced to differentiate into osteoblasts but a reliable solid matrix for their delivery is still necessary. In the case of critical size defects there is a need to regrow bone to reestablish stability and support in the area of injury. Current treatment involves the use of metallic implants, but this treatment sometimes carries with it serious side effects due to the foreign nature of the implant. It is the overall goal of this research to determine methods for the production of a biocompatible osteoinductive matrix that maintains viability and functionality when administered to the patient.

Specific Aims: This first aim of this research is to produce cultures of MSC's and test the effectiveness of different methods for extraction of the resultant extracellular matrix (e.g. trypsin digest). Following this the next aim is to co-culture MSC's with the extracted matrices and measure cell viability as well as ability of cells to differentiate into osteoblasts. Furthermore we will attempt to co-culture MSC-matrix composites with bone samples *ex vivo* and examine the ability of the construct to integrate with bone tissue. Finally we will examine the osteogenic characteristics of the Wnt signaling modulator 7-azaindolyl-pyrazinyl-maleimide (7AIPM) for possible use being cross-linked to the matrix for delivery to injury sites.

Materials and Methods: MSC's were cultured as monolayers and induced to differentiate by addition of beta-glycerophosphate, ascorbic acid, and dexamethasone. The osteogenic matrix was extracted from monolayers and separated from cell components by using a series of enzyme treatments and detergent/solvent extractions. Half of the samples were treated with trypsin while the other half were untreated. MSC's expressing green-fluorescent-protein (GFP) were co-cultured with the matrix and cell viability (by GFP fluorescence intensity) and differentiation (using enzyme assays, ELISA, and microscopy) were measured. MSC's were cultured as monolayers and induced to differentiate by addition of previously described osteogenic media as well as varying concentrations of 7AIPM. ELISA's for Osteoprotegerin (OPG) and Dkk-1 were performed to determine osteoinductive characteristics of 7AIPM.

Results: The data suggests that MSC's were successfully co-cultured with the extracted matrices. Furthermore, results indicate that these cells were induced to become osteogenic. Finally, 7AIPM was found to have increased osteogenic characteristics.

Analysis of Motility Characteristics of Substrates of the Twin-Arginine Translocation System in *Salmonella enterica* serotype Typhimurium

Danial Bokhari

Mentors: Megan Reynolds and Dr. Helene Andrews-Polymenis

Microbial and Molecular Pathogenesis

Texas A & M Health Science Center College of Medicine

The twin arginine translocation (TAT) system exports folded proteins containing a twin arginine signal sequence from the cytoplasm to the periplasm. The TAT system has been shown to be significant for virulence and motility in many pathogenic bacteria including *L. pneumophila* and *E. coli* O157:H7, among others. Components of the TAT system are also present in *Salmonella enterica* serotype Typhimurium, but not previously shown to have a phenotype. This *Salmonella* serotype is significant to humans as it can cause salmonellosis, a form of gastroenteritis. The TAT system is encoded by four genes: *tatA*, *tatB*, *tatC* and *tatE*. The folded proteins transported by the TAT system are transported to the periplasm or the extracellular space and include proteins such as metabolic proteins and redox enzymes bound to cofactors. Mutants deleted for *tatC* were shown by our lab to have a defect in swimming motility, but not swarming motility, and this defect correlates with the ability to express flagellins (FliC and FljB) on the surface of the bacteria. Approximately 43 substrates have been predicted to contain a twin arginine signal sequence in *Salmonella* (Dilks et al, 2003), but none have been shown to be directly related to flagellar export. In order to determine what TAT substrates were responsible for the defect in swimming and flagellar export, each of the potential TAT substrates was deleted using a homologous recombination method described by Datsenko and Wanner (2000). Six out of 43 mutants showed a statistically significant defect in the ability to swim when compared to the wild type: ΔSTM1383 (*ttrA*), ΔSTM1622 (*mdoD*), ΔSTM2099 (*wcaM*), ΔSTM2991 (*amiC*), ΔSTM3172 (*sufI*), ΔSTM4557 (*holD*). These mutants were also tested for ability to swarm and surface flagellin expression. Identifying genes with an effect on *Salmonella* swimming ability can be useful as specific targeting of gene products may reduce *Salmonella* motility, which may in turn reduce *Salmonella* virulence. This could lead to development of vaccines targeting TAT substrates or the TAT system itself to treat diseases such as salmonellosis.

17-alpha Estradiol, Stroke & the Reproductively Senescent Female Rat

Calender JP, Selvamani A, Sohrabji F

NExT

Texas A & M Health Science Center College of Medicine

Roughly 3.9 million women suffered from strokes in 2005, and more women than men are affected by stroke each year. Changes in circulating and CNS levels of ovarian steroids could contribute to this, and previous studies have shown 17b-estradiol to be neuroprotective in young females. Much less is known about the role of the naturally occurring 17-alpha stereoisomer. Normally 17-alpha estradiol is found at very low circulating levels but is elevated in the brains of adult female, gonadectomized female, and adrenalectomized female mice. Since the role of 17b-estradiol in neurovascular disease such as stroke is controversial in postmenopausal women and reproductive senescent animals, the aim of the present experiment was to investigate the role of 17-alpha estradiol has in an experimental stroke model in reproductively senescent female rats. Twenty-six female rats (~11m old) were determined to be reproductively senescent by vaginal smears, ovariectomized and replaced with a pellet containing 17b-estradiol or a sham pellet (control). Three weeks post-ovariectomy the rats received transient intracerebral middle-cerebral artery occlusion (MCAo) using ET-1 and given an injection 4 hours post-occlusion of either 17a-estradiol in corn oil or corn oil alone (control). Behavioral testing was done pre-MCAo and 24, 72, and 96 hours post MCAo. All surviving rats were terminated 96 hours post MCAo and brains harvested. Severity of stroke was measured by TTC staining of brain tissue and behavioral testing.

Human Basophil Response to Thymic Stromal Lymphopoietin

Caram, B.M., Vohra, R., Tavana, G., Moore, J.P., Huston, D.P.

Microbial and Molecular Pathogenesis

Texas A & M Health Science Center College of Medicine Houston Campus

Background: Thymic stromal lymphopoietin (TSLP) is a hematopoietic cytokine principally expressed by epithelial cells in the lungs, skin, and gut, and has been implicated as the master switch of allergic inflammation. TSLP signals via a heterodimeric receptor that is comprised of the cytokine-specific TLSPRα and the IL-7Rα. TSLP predominately targets immature myeloid dendritic cells (mDC) and induces a Th2 immune response characterized by production of cytokines that promote IgE production and eosinophil development. Recent murine studies suggest that TSLP can also activate basophils to induce this same response. However, differences in TSLP biology between mice and humans are known to exist. While mDC are responsive to TSLP in mice and humans, in contrast to humans, murine T and B cells are also responsive to TSLP. There are no reports on the biology of TSLP on human basophils.

Hypothesis: This study tests the hypothesis that human basophils express the TSLPRα and IL-7Rα, and can respond to TSLP.

Methods: Basophils will be isolated from leukopaks using gradient density separation and negative selection with immunomagnetic beads coated with mAb specific for cell surface molecules on non-basophils. The purity of the basophils will be confirmed by light microscopy of Wright Giemsa stained cells and by flow cytometry using anti-CD123 and anti-IgE. Basophils will be cultured with and without Cra-1 (anti-FcεR1α), to investigate potential changes in TSLPRα and IL-7Rα expression. TLSPRα and IL-7Rα message will be measured by real-time PCR. Cell surface expression of TLSPRα and IL-7Rα will be assessed by flow cytometry. Basophil responses to TSLP will be determined by phosphorylation of STAT5 using Western analysis.

Results: Isolation of basophils demonstrated density separation predominantly in the peripheral blood mononuclear layer rather than the granulocyte layer. Immuomagnetic bead isolation yielded approximately 88% purity by light microscopy and flow cytometry. Preliminary PCR analysis of purified basophils demonstrated expression of message for both TSLPRα and IL-7Rα. Ongoing experiments will investigate cell surface expression of these receptors, changes in receptor expression with cell activation, and functional response to TSLP.

A 3-Dimensional Invasion Model to Study Drug-Resistant Metastasis

Evan Cherry, Dr. Kayla Bayless, Dr. Steve Maxwell

Molecular and Cellular Medicine

Texas A & M Health Science Center College of Medicine

Diffuse Large B Cell Lymphoma (DLBCL) is a common form of cancer, accounting for 30% of all lymphoma diagnoses. Patient survival rates of DLBCL with the standard chemotherapeutic cocktail consisting of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP therapy) are roughly 30-40%. Approximately 50% of patients develop chemoresistance to CHOP therapy and succumb to metastasis of vital organs. To penetrate tissues and organs, lymphoma cells must invade the extracellular matrix surrounding blood vessels. We investigated the ability of CHOP-resistant lymphoma to invade three-dimensional collagen matrices mimicking the extracellular matrix. We evaluated the potential for cellular dyes to quantify invasion responses and tested an automated system for quantifying invasion responses. Further, because the cytoskeleton is critical for cell locomotion and morphology, we investigated the role of key cytoskeletal proteins vimentin, actin, and tubulin in lymphoma invasion. Our data indicate that microtubule stabilization, but not depolymerization, inhibits CHOP-resistant lymphoma invasion. Additionally, depolymerization of actin and vimentin completely blocked invasion responses. Altogether, this work develops a quantifiable model to study lymphoma invasion, mimic metastasis, understand cytoskeletal function, and gain further insight into molecular signals required for cellular invasion.

MR imaging of pain generation from degenerative lumbar disc disease

Stephen A. Coffman, Jonathan A. Friedman

Departments of Surgery, Neuroscience and Experimental Therapeutics

Texas A & M Health Science Center College of Medicine

Magnetic resonance imaging is used to visualize the lumbar spine in patients with suspected degenerative lumbar disc disease. However, the correlation between lumbar disc structural abnormalities and intractable lower back pain is currently unclear. Past studies have demonstrated that many patients with lumbar disc abnormalities experience no pain. Other studies also found that there were no significant lumbar disc structural abnormalities in patients whose physical history exams might have indicated lumbar disc disease. Due to these inconsistencies it has been difficult for clinicians to accurately distinguish the origin of lower back pain in many patients. The diagnoses could drastically alter the course of treatment, thus it is important to develop a method to determine whether lower back pain is attributable to a specific lumbar disc or to a different pathology.

To address this issue we have proposed a novel method that utilizes magnetic resonance imaging to visualize the generation of neuropathic pain in symptomatic lumbar discs. Symptomatic lumbar discs are known to increase production of inflammatory cytokines that are involved in the progression of neuropathic pain. Through literature reviews we have identified TNF-α, IL-1β, and IL-6 as primary cytokines in this process. Currently we are investigating methods to modify one or all of these molecular structures by utilizing monoclonal antibodies to attach an MR contrast agent such as gadolinium-DPTA. Once that is accomplished the modified cytokine could then be introduced into the patient intravenously local to the site of back pain and theoretically would highlight the disc responsible for the origin of pain. Animal testing will be conducted using small rodent models with induced lumbar disc structural damage. Strategies to identify the presence of pain in the animal models and MR techniques to visualize the lumbar disc and contrast agent will be discussed. Human testing is a goal for the future but will require more extensive work and safety testing depending on the MR contrast agent used in the modification process.

Ovarian Aging and Hypoxia: Effects of Astrocytes on Neural Progenitor Cells

Rebekah Condit, Danielle Lewis, and Farida Sohrabji

Department of Neuroscience and Experimental Therapeutics

Texas A & M Health Science Center College of Medicine

Stroke is a major health problem in the United States effecting both men and women. Post-menopausal women are more likely to have a stroke and women in general are more likely to die from stroke. In order to understand the increased risk and severity for stroke in menopausal females, our lab uses an animal model that replicates significant characteristics of pre and post menopausal women. Specifically, premenopausal women are modeled by multiparous mature adult female rats (MA) that have normal but lengthened estrus cycles, while post menopausal women are modeled by older acyclic females (RS) that are in constant diestrus (a permanent low estrogen state). Our previous work indicates that astrocytes, which are an important brain support cell, from mature and senescent females differ in their ability to promote neuronal differentiation of neural progenitor cells (NPCs). Furthermore, in an in vitro model of stroke (hypoxia), hypoxic astrocytes from mature adults facilitated neuronal differentiation while hypoxic astrocytes from senescent females did not. In the present study we determined two related issues: 1. would astrocytes from senescent animals affect cell fate decisions in NPCs, using astrocyte and NPC co-cultures and 2. to determine the mechanism underlying ovarian age-related differences in the ability of astrocytes to promote neuronal differentiation. For the latter studies, we examined the expression of Tenascin-C, an important component of the ECM that is involved in neuronal differentiation and outgrowth, and its receptor β1-integrin, as well as insulin-like growth factor-1 an important pro-survival/growth factor. Our data indicate that Tenascin is reduced in reproductive senescent females as well as age matched males, indicating that this ECM is regulated by chronological age. However, β-1 integrin expression is reduced in reproductive senescent females but not age-matched males, indicating that this receptor is regulated by ovarian aging and not chronologic aging. IGF-1 secretion is also decreased in normoxic and hypoxic astrocytes from senescent females as compared to mature adult females. To determine the effect of hypoxia on neuronal fate decisions, MA or RS, astrocytes were placed in hypoxic conditions for 6 hours and co-cultured with GFP+ NPCs 24h later. Immunohistochemistry is being used to identify neuronal cells (NeuN labeled cells) from the population of GFP+ NPCs. The results from this experiment are still being examined.

mRuby: An Imaging Tool

Luis Dlouhy, Jeffery Cirillo, Suat Cirillo, Ying Kong

Microbial and Molecular Pathogenesis

Texas A & M Health Science Center College of Medicine

Tuberculosis currently infects nearly one-third of the world's population or over 1 billion people and there are millions of new infections each year. Our goal is to develop new approaches to visualize these important bacteria in the laboratory as well as during animal infections. This technology would facilitate all areas of tuberculosis research because this organism normally takes months to grow in the laboratory, so an immediate readout for bacterial numbers is extremely important. We have chosen to utilize a new fluorescence protein named mRuby because of its bright fluorescence and wavelength that could allow visualization directly in animals. mRuby is a red fluorescent protein showing excitation and emission at 558nm and 605 nm. The protein is able to resist denaturation at high and low pH levels. Because of these qualities, it has the ability to be used as a tool for *in vivo* imaging of infections. 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 mRuby gene cloned through a combination of the polymerase chain reaction and appropriate restriction enzyme digestion and ligation. Four different plasmid combinations, with different antibiotic resistance and transcriptional promoters, were used. With the appropriate vector plasmids constructed, the plasmid can be transformed into mycobacteria to evaluate the presence of mRuby through the use of a fluorescent microscopy and plate dilutions. If the presence of mRuby is detected, the plasmid can be transformed into BCG strain of Tuberculosis to infect mice and imaged with the aid of the mRuby to determine bacterial loads in different tissues. We have successfully cloned mRuby and are characterizing its fluorescence in different bacterial strains. These constructs should have broad utility in the study of tuberculosis pathogenesis as well as development of novel therapeutics and vaccines.

Himar1-based Transposon Mutagenesis in *Coxiella burnetii* Phase II 439

Ganapathy, A., K. Mertens, and J.E. Samuel

Department of Microbial and Molecular Pathogenesis

Texas A & M Health Science Center College of Medicine

Coxiella burnetii is an obligate intercellular bacterium and the causative agent of acute and chronic Q Fever in humans. *C. burnetii* resides and replicates within the host cell in an acidic parasitophorous vacuole, which has characteristics of a terminal phagolysosome. This constraint has limited the ability to genetically manipulate this bacterium until the recent development of a *Himar1*-based transposon mutagenesis system. To understand the pathogenic process of *C. burnetii*, a mutant library is being created using *Himar1*-based mutagenesis.

Himar1-based transposition is characterized by random insertions and independence from host encoded factors. The *Himar1*-delivery plasmid, pKM225, carries genes for chloramphenicol acetyltransferase and the fluorescent protein mCherry on the transposable element. After electroporation, the plasmid encoded transposase mediates insertion of the transposable element via the 'cut and paste' mechanism. Expression of the above selection markers and transposase is driven by *Coxiella*-specific promoters for optimal expression. An integrated origin of replication allows rescue cloning of chromosomal fragments containing the transposable element for insertion site determination.

L929 mouse fibroblast cells were infected with one of 4 separate transformations of *C. burnetii* Phase II 439 and grown in the presence of chloramphenicol (Cm). These 4 cultures were monitored for vacuoles containing mCherry expressing *C. burnetii* using fluorescence microscopy. Individual host cells infected with Cm resistant and mCherry expressing bacteria were isolated by **F**luorescence **A**ctivated **C**ell **S**orting (FACS) with ≈10,000 positive events per transformation and diluted for isolation and expansion of individual insertion clones. After expansion of 384 seed cultures, 115 samples were positive for mCherry expression and selected for DNA isolation. Bacterial DNA was isolated from 39 of these tissue culture cells and analyzed by Southern Blotting with a mCherry-specific probe. 8 cultures were confirmed to contain clonal populations as indicated by the presence of a single hybridization band. These clones are currently being subjected to rescue cloning for characterization of the *Himar1* insertion site. Once the insertion site has been identified, the specific mutations and their corresponding phenotypic changes may provide insights in the survival mechanism or virulence factors of *C. burnetii*.

Detection and Prevention of Lipid Oxidative Damage in Alzheimer's Disease

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Neuroscience and Experimental Therapeutics

Texas A & M Health Science Center College of Medicine

Alzheimer's disease (AD) is increasing in the aging population. A possible cause of AD is lipid oxidative damage, which precedes pathological formation of amyloid plaques. Two features of AD, amyloid β protein (Aβ) misfolding and increased oxidative damage, may be linked in a pathological feedback mechanism. For example, Aβ's direct oxidation of lipids may drive plaque formation. Lipid oxidation also increases ceramide and sphingosine levels, lipids which may be biomarkers of AD. Finally, it is crucial to prevent this pathological feedback mechanism of oxidative damage, especially with drugs already approved by the FDA for other uses. Of interest is the drug hydralazine, which has antioxidant function.

Hypotheses:

1) Does lipid oxidative damage occur at amyloid plaques?

2) Can we detect Alzheimer's disease by measuring lipid biomarkers in CSF?

3) Can we identify drugs that prevent oxidative damage and Aβ misfolding?

We used two independent techniques to demonstrate lipid oxidative damage in amyloid plaques in the brains from the presenilin/amyloid protein precursor mouse model of Alzheimer's disease. Biocytin tagging of and immunostaining for HNE showed extensive lipid oxidation co-localization with amyloid plaques. Using liquid chromatography-mass spectrometry, we detected the potential lipid biomarkers sphinomyelin, ceramides, and sphingosines in pig cerebrospinal fluid (CSF). Future studies will detect these biomarkers in CSF from Alzheimer's disease patients. Finally, we chemically disrupted this oxidative process; hydralazine inhibited HNE-Aβ misfolding and unfolded HNE-Aβ.

In conclusion, our findings provide evidence of a pathological feed-forward mechanism, linking lipid oxidative damage and amyloid plaques, and they suggest that hydrazides may potentially prevent or treat AD.

Survival of the Mayo conservative femoral stem compared to a conventional system

A consecutive, single surgeon series

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Short stem hip prostheses have been designed for younger patients in attempt to preserve bone stock for future revision. One such design, the Mayo Conservative Hip (Zimmer International, Warsaw, IN) utilizes a tight proximal interference fit to achieve stability. To our knowledge, only two studies have been published regarding midterm performance of this prosthesis. In this study, we compare outcomes in patients who received Mayo conservative stems to those who received cemented and uncemented VerSys (Zimmer International, Warsaw, IN) stems, with the VerSys system representing a more conventional device. At a single institution between 1997 and 2003, a single senior surgeon placed 56 VerSys press fit stems in 55 patients, 59 VerSys cemented stems in 55 patients, and 139 Mayo stems in 125 patients, all consecutively. Demographic variables, Harris hip scores, perioperative complications, and outcomes including revision were recorded. Kaplan-Meier survivorship curves were constructed with revision due to aseptic loosening as an endpoint. Excluding deaths, survivorship data at 2 years or beyond were available for 41 (80.4%) VerSys press fit, 36 (70.6%) VerSys cemented, and 95 (70.4%) Mayo stems, with mean follow-up of 67 months, 46 months and 43 months, respectively. Five-year survivorship without mechanical loosening was 82.3% for the Mayo and 100% for the VerSys stems. The excellent survivorship reported elsewhere for the Mayo prosthesis was not realized, and survival of the Mayo stem was significantly worse than that of the conventional stems.

Identification of new markers and targets for Glioblastomas progenitor cells.

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Glioblastomas are the most common and aggressive form of brain tumors in adults. Glioblastomas have a high rate of occurrence because slow growing progenitor cells are resistant to standard therapy procedures. We sought to characterize the expression of protein markers of stem cells and mi-RNA within samples of Glioblastoma, using immunohistochemistry and in situ hybridization respectively. Characterization of the expression of these markers would allow for the possible identification of tumor progenitor cells at earlier branch points of lineage creation. If present, these markers will provide therapeutic targets for new treatment modalities. Nestin was not observed to be selective for tumor stem cells as it appeared to be expressed heavily in tumor cells. It was observed that ABCG2 and Musashi-1 were selectively expressed in tumor cells and may best serve as a marker for tumor stem cells. Further work will be done to determine if the tumor cells that expressed these markers are in fact tumor stem cells. mi-RNA 335 and mi-RNA137 will be studied to determine its expression levels in tumor cells.

Novel toxin therapy specifically targeting sub-populations of glioma cells.

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The treatment of patients with glioblastoma multiforme is, unfortunately, rarely curative. This tumor's ability to survive and spread in spite of surgical resection, radiation, and chemotherapy explains the terrible prognosis faced by patients, families, and caregivers. Over the last decade, data have been gathered supporting a tumor progenitor cell hypothesis – that cells most resistant to current therapies are similar to stem cells in their ability repopulate a tumor. Therefore, targeting these tumor progenitor cells by engineered toxin offers the potential to improve outcomes.

In this laboratory, we hypothesize that the use of targeted toxin therapy, alone and in combination with interfering genetic constructs, will enhance the efficacy of current glioblastoma therapy.

In this laboratory, we are pursuing novel therapeutic modalities for the treatment of glioblastoma. We engineer the pore-forming toxin to both targeting and delivery of small molecules against glioblastoma. We modify the toxin to target glioblastoma progenitor cells in two ways. First, by adding a linker protein to the toxin, we can use various tagged antibodies to direct the toxin to a multitude of specific receptors. Streptavidin-toxin chimera is used for the screen and selects the specific tumor targeting mates such as biotinylated monoclonal antibody. Second, we take advantage of the pore formed by the toxin on the cell membrane, to target the unique genetic makeup of glioblastoma progenitors. Thus, interfering genetic material (directed against the tumor cell's regulatory machinery) can pass through the pore, into the cell, and specifically halt its growth.

This summer, we made the initial steps towards treatment of glioblastoma with targeted staphylococcal alpha-hemolysin.

MicroRNA Regulation in Bone Tissue is Affected by Alcohol Consumption

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MicroRNAs are small, single stranded, 20-30 nucleotides, non-coding RNAs that play important roles in the regulation of gene expression at the posttranscription level, usually through repression of mRNA translation or gene silencing.

In both brain and liver tissues, alcohol's detrimental effects on cell function and development appears to be through a disturbance of microRNA regulation of these events. To better understand the effects of alcohol on bone, RNA from alcohol and pair-fed animals was extracted and microarray analyses of mRNA against the entire rat genome and microRNA arrays were conducted. Smad4, an important step in the TGF-B and BMP signaling pathway, was found to be down regulated with alcohol consumption. Smad4 has been reported to be a target for miR-21 and miR-301a which we found increased.

In this study, we use human multipotent stromal cells (hMSC) which are driven towards osteogenesis and incubated with ethanol. After 10 days exposure to osteogenic stimulus with ethanol, hMSCs were counted using a gDNA quantification assay. Ethanol had a dose-dependent detrimental effect on cell recovery. Levels of the early osteogenic markers, alkaline phosphatase (ALP) and osteoprotegerin (OPG) were measured by colorimetric assay of the monolayer or ELISA of the media respectively. When OPG values were normalized to the remaining cells, the hMSCs had produced greater amounts of OPG on a per cell basis. Similar results were obtained when monolayers were assayed for ALP activity by p-nitrophenol phosphate exposure.

These data demonstrate that in tissue culture, ethanol inhibits the proliferation of hMSCs or kills a substantial subpopulation. Due to reduced cell-numbers, overall activity for both markers in the in the entire culture is reduced. These data support the observation that ethanol is detrimental to bone repair *in vivo*. Unexpectedly, the surviving hMSCs have increased ALP activity and OPG output. These data suggest that for a subpopulation of hMSCs, ethanol can upregulate early osteogenic markers, or select for a more osteogenic subpopulation in the culture. Unfortunately, the remaining osteoblasts, irrespective of their increased marker expression, are not sufficient to counteract the detrimental effects of ethanol exposure on bone repair observed in *in vivo* experiments. Thus, the effects of ethanol on bone repair are likely due to an overall reduction in cell viability or a much earlier stage in the healing processes unrelated to osteoblasts.

TARGETED ACTIVATION OF RETINOID RECEPTOR MEDIATED SIGNALING PROTECTS FROM FREE FATTY ACID-INDUCED APOPTOSIS IN CARDIOMYOCYTES

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Dyslipidemia is an increasingly important pathogenic factor in type II diabetes and in overall cardiovascular disease risk. The cytotoxicity of free fatty acids has been implicated in the pathophysiology of cardiovascular disease, though effects on cardiac myocytes are incompletely understood. Using neonatal cultured cardiomyocytes, we determined the role of retinoid receptors in palmitic acid (PA) induced cytotoxic stress. PA increased intracellular lipid droplets, along with cell apoptosis, as demonstrated by an increased number of TUNEL positive and caspase-3 activated cells, and high glucose accelerated PA-induced cellular injury. Using a selective agonist for RARα (retinoic acid receptor, Am580) and RXRα (retinoid x receptor, SR11345), we demonstrated that the increased intracellular lipid accumulation and apoptosis was inhibited by both Am580 and SR11345. Silencing RARα and RXRα expression by siRNA transfection resulted in increased cell apoptosis, indicating that both RARα and RXRα are required in maintaining cell survival. The renin-angiotensin system (RAS) has an important role in diabetes induced cardiac remodeling. To determine whether the RAS was involved in the protective effects mediated by activation of RAR/RXR, we analyzed the expression of RAS components. PA increased gene expression of angiotensinogen and renin, and the synthesis of angiotensin II, which were inhibited by Am580; but, not by SR11345, indicating that RARα is the main receptor subtype contributing to the inhibitory effect on PA-mediated expression of RAS components. In conclusion, targeted activation of RARα and RXRα protects cardiomyocytes from free fatty acid induced cellular injury, and the RARα mediated inhibitory effect on expression of RAS components has an important role in free fatty acid-induced cytotoxic stress. These data suggest that targeted activation of RAR/RXR mediated signaling may have therapeutic potential in the prevention and treatment of type II diabetes induced cardiac complications.

Effects of Early Embryonic Alcohol Exposure in Zebrafish on Specific miRNA Profiles

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Neuroscience and Experimental Therapeutics

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Despite research showing the harmful effects of drinking alcohol during pregnancy on the developing fetus, many pregnant females continue to use alcohol throughout the entire period of pregnancy. Alcohol consumption during pregnancy can lead to Fetal Alcohol Spectrum Disorder, with fetal alcohol syndrome being the most severe form of this disorder showing a collection of birth defects which include facial dysmorphology and neurological dysfunctions. Recently, it has been suggested that alcohol-mediated changes in microRNA profiles may account for the deficits associated with fetal alcohol exposure. MicoRNAs (miRNA) are non-coding RNAs approximately 21-23 nucleotides in length that are able to regulate gene expression at the post-transcriptional level by binding to target mRNA and either causing transcript degradation or halting translation. In this study, a zebrafish embryo model was used to examine an early alcohol exposure regimen during gastrulation on specific 2 miRNAs: miR-430a and let-7h. In early development of the zebrafish, miR-430 has been shown to regulate morphogenesis, clearance of maternal mRNAs, and brain rescue in MZ*dicer* mutants; and let-7 is known to be involved in cell migration in the nervous system development.

The zebrafish embryos were exposed to 0, 0.32, or 2% (w/v) alcohol (ethanol) beginning at 3.5 hours post-fertilization (hpf) and the treatment lasted for 4 hours during the gastrulation stage of the zebrafish embryo development. These developing embryos were evaluated for morphological alterations at 48 hpf and were sacrificed for miRNA analysis. MiRNA was isolated using the mirVana Isolation Kit (Ambion) according to the manufacturer's protocol. Real time RT-PCR was used to verify the quantitative changes in miRNA expression with SYBR green based RT-PCR using MyiQ™ single-color RT-PCR detection system (BioRad). The RT-PCR data was normalized to U6 snRNA. The results showed that zebrafish embryos exposed to 2% alcohol exhibited significant morphological alterations, such as cardiac edema and a curved vertebral column. However, such defects were not observed in embryos exposed to 0.32% alcohol. The miRNA expression data are currently being analyzed and will be discussed during the poster presentation session.

Oxidative damage in Alzheimer's disease model and effects of hydralazine in rat stroke model

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Intro: Ischemic stroke and Alzheimer's disease (AD) share features of increased oxidative damage. Oxidative damage occurred around amyloid plaques in AD models and it is important to identify cells involved in this process. Also, drugs that reduce the detrimental effects of this oxidative damage, and such studies can rapidly be performed in the rat stroke model than in an AD mouse models. The drug hydralazine, is of interest for several reasons: it is an FDA approved anti-hypertensive which increases vasodilation and it has antioxidant function.

Hypotheses

1. Identification of the source of lipid oxidative damage around amyloid plaque.

2. Hydralazine will reduce infarct size and oxidative damage in the rat stroke model

Stroke model: Middle cerebral artery occlusion (MCAo) was induced via endothelin-1 injection. Hydralazine was administered via tail vein to stroke induced and sham treated rats 4hrs post stroke. Motor control behavior was evaluated via forelimb placement test before and after MCAo. The infarct volume was measured using 2,3,5-triphenyltetrazolium chloride (TTC) staining. Infarct volume and histological evaluation of oxidative stress were evaluated 5 days post stroke.

Alzheimer's disease model: Oxidative damage, neuronal structure, microglia and astrocyte activation around amyloid plaques were examined histologically.

Fracture Risk in Women 60 Years of Age and Older with Diabetes Mellitus

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Objective: Diabetes Mellitus (DM) is a metabolic disorder characterized by an inability to maintain normal blood glucose levels. A large proportion of the literature reports an increased risk of insufficiency fracture in the diabetic population. However, a number of studies concluded no difference in insufficiency fracture rate in diabetic and non-diabetic populations. Since most related studies highlight the risk of insufficiency fractures in women with and without diabetes, the objective of this study was to investigate if there is an association between DM and insufficiency fracture risk in women ≥60 years of age.

Research Design and Methods: This study was a retrospective search of the Scott & White Health Plan Data Base from 1999 to 2008 (n= 35,724). Diagnostic codes for diabetes, fracture, and renin-angiotensin inhibitor (RAI) use were used to refine the search. Patients included in the study were placed into 11 categories based on the diagnosis of diabetes, fracture, and use of RAI. Data related to RAI use was found to be unreliable and therefore our focus shifted to characterizing the relationship between diabetes and fracture rate only. Random sampling of 10 female and 10 male charts from each year was performed to assess how useful diagnostic codes were at excluding patients with fractures unrelated to bone insufficiency. Due to high sampling error in the male population (10%), our final sample involved only women ≥60 years of age.

Results: We tested the assumption of independence between DM and fracture risk and found it to be robust. Mean incidence of fracture in the diabetic population (1999-2008) was 2.44% (95% CI 1.47%-3.81%). Mean incidence of fracture in the non-diabetic population (1999-2008) was 2.21% (95% CI 2.00%-2.44%). The prevalence of DM for the patient population was 9.45% (95% CI 9.14%-9.75%).

Conclusions: Contrary to a large portion of the literature, our data indicates that the risk of insufficiency fracture in women ≥60 years of age is no higher than the corresponding risk in non-diabetic women ≥60 years of age.

Modification of Tel and its potential importance on the alteration of the Bcl-XL transcription level

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Bcl-XL, a member of Bcl-2 family, is an anti-apoptotic protein that interrupts programmed cell death and is an important regulator for mediating cellular survival and death. Bcl-XL is strongly up-regulated in diseases such as mesothelioma, lung adenocarcinoma, esophageal cancer, and colon cancer.

The over-expression of Tel, a member of Ets family of transcription factors, has been found to repress Bcl-XL via interactions with mSin3A, N-CoR and SMRT co-repressors. Typically, Tel is negatively regulated by phosphorylation and sumoylation, and such regulations remove Tel from the promoter and inhibit its transcriptional repression effect on Bcl-XL. CRM1 mediated nuclear export system exports Tel to the cytoplasm, and, consequently, Tel is phosphorylated by specific MAPK and removed of its transcriptional repressive properties by decreased DNA-binding ability. In addition, E2 SUMO-conjugating enzyme UBC9 interacts with the Point Domain of Tel, with K99 providing the predominant SUMO-1modification site.

The aim of this study was to create combinations of mutants targeting two phosphorylation sites (S213 and S257) and two sumoylation sites (K11R and K99R) to counter the modifications that hinder its repressive activity on Bcl-XL. Initially, four single mutants were created and have demonstrated slight decrease in Bcl-XL expression in mesothelioma cell lines via Western-blot. Additional six double mutants (S213 x S257, S213 x K11 R, S213 x K99 R, S257 x K11 R, S257 x K 99R, K11 R x K 99R) have been created using mutagenesis system. After another treatment with mesothelioma cell lines, K11 R x K 99R double mutant has been determined to be the most effective mutant.

Conversion of human dermal fibroblasts into induced pluripotent stem cells with TAT-STEMIN

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Introduction Stem cell based therapy has been proposed as a promising new means for repairing damaged tissues. Induced pluripotent stem (iPS) cells have most of the characteristics of embryonic stem cells without the obstacles such as low availability and ethical considerations. IPS cells can be reprogrammed from human somatic cells such as human dermal fibroblasts (HDFs) by introduction of essential stem cell marker genes Oct4, Sox2, cMyc, and Klf4. Dr. Schwartz's lab recently discovered that treatment of HDFs with lentiviral Stemin, a mutant form of serum response factor (SRF), induced stem cell–like activity. Hence, we hypothesized that Stemin is an upstream regulator of many stem cell marker genes, including Oct4, Nanog, and Sox2, that reprogram HDFs into stem-like cells. We will characterize the iPS cells resulted from Stemin treatment for stem cell behavior and ability to differentiate into cardiac myocytes.

Results and Methodology We have evaluated the efficacy of using nonviral TAT-fused Stemin to convert HDFs into iPS cells. The expression vectors for TAT-protein fusions contain protein coding sequences inserted in frame after the plasmid pTAT-HA region that consists of the six-histidine region, TAT signal sequence, and the HA tag. The six-histidine region enables Stemin to bind to metal affinity beads. TAT signal sequence helps Stemin bind to cell membrane and transport through the cell membrane. The HA tag, consisting of hemagglutinin sequence, helps detect foreign proteins inside a cell. Stemin was overexpressed in E. coli BL21(DE3)pLysS cells at 37ºC with IPTG induction. Cells were lysed, centrifuged, and the supernatant was applied to a column. The proteins were eluted into fractions during Talon metal affinity binding chromatography at certain concentrations of imidazole. Protein-containing fractions were desalted using PD-10 column using PBS/20% glycerol. Purified Stemin protein was then used for cell culture experiments. HDFs were treated with Stemin for 6 hours for immunostaining analysis to verify cell localization. Another set of HDFs were treated with Stemin for 4 days for iPS conversion, which may be detected by cell morphology and immunofluorescence. STEMIN-induced iPS cells can then be used to form embryoid bodies and expected to initiate differentiation based on forced aggregation mechanism.

Significance The ability of the iPS cells to successfully differentiate into cardiac myocytes will help treat heart failure. Cardiomyocytes do not normally regenerate after birth, and without the ability for renewal, loss of functional cardiomyocytes eventually lead to deterioration of pump function, resulting in heart failure. Regenerative medicine may benefit from customized stem cell treatment based on patient's own skin cells. Hence, we propose that using TAT-STEMIN will set in motion the autoregulatory stem-cell factor loop to reprogram patient's HDFs to more closely resemble actual stem cells that may also have the capacity to fully differentiate into cardiac progenitors for cardiac repair.

Anesthetic Implications of Extremely Hypertensive Patients Undergoing Implantation of the Rheos Anti-Hypertensive Device

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Mild to moderate hypertension—140 to 179 mm Hg systolic and 90-109 mm Hg diastolic—is not a major perioperative risk factor in anesthesia and surgery. However, the risks associated with extreme hypertension—pressures higher than 180 mm Hg systolic and 110 mm Hg diastolic—have not been studied. The objective of this study is to examine the degree of perioperative risk of anesthesia and surgery among this extremely hypertensive population. The patients participating this study have been unable to manage their blood pressure and are receiving a Rheos baroreceptor stimulation device to help manage their hypertension. We will perform a retrospective chart review of 20 patients undergoing surgical implantation of this device to determine the prevalence of adverse perioperative events, including acute renal failure, acute myocardial infarction, congestive heart failure, blood pressure lability, stroke, and death. The retrospective chart review will involve evaluation of preoperative notes, intraoperative anesthetic records, and postoperative follow-up notes of each patient up to 45 days after the procedure. While we predict that there will be significant blood pressure lability—especially hypotensive episodes—during the surgical procedure, we believe that these drops in blood pressure will be corrected with the use of vasoconstrictors, reducing the likelihood of perioperative complications. We expect that elective surgery can be performed safely in this patient population with proper intraoperative management.

Role of Stress Activated Protein Kinases in Mediating Angiotensinogen Expression in Pressure Overloaded Myocardium

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Mitogen-activated protein (MAP) kinases have been implicated in hemodynamic load induced heart failure. Both angiotensin II (Ang II) and mechanical stretch activate MAP kinases in cardiac myocytes. Angiotensinogen (Ao) is the precursor to angiotensin II and therefore a vital component of the mechanical signaling cascade seen in hemodynamic load induced heart failure. Pilot studies conducted by our laboratory suggest that left venticular Ao gene expression in aortic banded rats and mice is initially depressed at 1 day, compared to control (sham operated). However, at later times (>3 days) Ao mRNA levels appears to be elevated in left venticular pressure-overloaded rats and mice. As with cell culture studies, Ao mRNA expression corresponded to reciprocal changes in JNK and p38. Hemodynamic load initially resulted in activation of JNK and p38 (1 day) in left ventricles of rats. However, afterwards JNK activation declined, whereas p38 remained activated in both the rat (3 and 7 days) and mice (14 days). These data suggest that JNK and p38 may have similar roles in regulating Ao gene expression in the hemodynamically overloaded myocardium in rats and mice, as described using isolated cardiac cells.

The purpose of this study was to conduct studies on additional rats and to use echocardiography to correlate systolic and diastolic functional changes of the myocardium with activation of the cardiac renin-angiotensin system and activity of the MAP kinases. Baseline echocardiography was performed obtain baseline data prior to randomization of rats into sham or abdominal aortic constriction surgery groups. Echocardiography was performed again on days 1, 3, or 7 after surgery, after which rats were euthanized and left ventricular tissues and blood obtained was obtained for biochemical analysis of Ao, p38, and JNK using reverse-transcriptase polymerase chain reaction, immunohistochemistry and/or Western blot analysis.

The Effects of Statin Therapy on Vascular Function in Children with Hypercholesterolemia

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Young children with high cholesterol are at developing cardiovascular disease in adulthood. However, not all children (or adults) with hypercholesterolemia risk for will develop premature cardiovascular disease (CVD). The overarching goal of our proposed study is to determine if surrogate markers of vascular health can be used to identify children who have the greatest risk for developing premature CVD. The purpose of this research project is to a) determine if the lipoprotein subclass profile measured by Density Gradient Ultracentrifugation (DGU) is better correlated with markers of vascular health compared to standard lipid levels and the correlation with demographic information such as body mass index (BMI); b) evaluate differences in the lipoprotein subclass profiles between hypercholesterolemic and normocholesterolemic youth , and c) determining how statin therapy impacts the lipoprotein subclass profile. We utilized serum from hypercholesterolemic youth who participated in a randomized, double-blinded, placebo controlled trial. Youth ages 10-20 years of age were randomized to simvastatin or placebo. Subjects in the active treatment arm started with a 20 mg dose of simvastatin which was increased to 40 mg after 24 weeks followed by a 3 month wash-out. Blood draws were taken at 0, 24, 48 and 60 weeks. A group of health children also served as a comparator group. Surrogate vascular markers that were measured include high sensitivity C-reactive protein (hsC-RP) and various ultrasound measurements of the peripheral arteries. This study will focus on correlations with hsC-RP. DGU was used to characterize the lipoprotein subclasses. Serum was combined with an EDTA gradient and NBD-C_6 ceramide to stain the lipoprotein particles and then spun at 120,000 rpm at 5° C for a total of 6 hours. Ultracentrifuge tubes were then imaged and analyzed using ORIGIN software to create a profile based on intensity levels and tube coordinates. Using ANOVA analysis, we evaluated changes in the lipoprotein subclasses and their correlation with standard lipid levels, hsC-RP and clinical variable (BMI, gender, etc.). Data analysis is being completed at this time. Because evidence suggests that the lipoproteins, especially high density lipoproteins (HDL) are affected by inflammation, we expect that the HDL subclass distribution will be correlated with surrogate vascular markers, most notably hs-CRP.

Identification of Phosphoproteins Involved in CHOP Resistance of Large Diffuse B Cell Lymphoma

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Every year 20,000 to 30,000 new cases of diffuse large B-Cell lymphoma (DLBCL) occur. The standard treatment for DLBCL is the chemotherapeutic agent CHOP which consists of cyclophosphamide, doxorubicin, vincristine, and prednisone. In approximately 40% of DLBCL cases, the cancer becomes CHOP resistant, which often leads to the eventual death of the patient. We isolated CHOP-resistant DLBCL cells from CHOP-sensitive cultures as an *in vitro* model to elucidate the pathways that mediate CHOP-resistance. CHOP-resistant cells were found to overexpress the Akt protein kinase relative to CHOP-sensitive cells. Since the Akt protein kinase promotes cell survival and plays a role in drug resistance in other types of cancers, we hypothesized that alterations in the phosphorylation state of proteins plays a role in CHOP-resistance in DLBCL. We conducted a phosphoproteomics analysis of CHOP-resistant and –sensitive DLBCL cells to identify phosphoprotein targets that were associated with the drug-resistant phenotype. Phosphoproteins in whole-cell detergent extracts of DLBCL cells were isolated using phosphoprotein-binding columns (Pierce Chemical). A phosphoprotein stain (Molecular Probes) confirmed the isolation and enrichment of phosphoproteins from whole-cell lysates. The enriched phosphoprotein eluates from the phosphoprotein-binding columns were subjected to two-dimensional differential in-gel electrophoresis (2D-DIGE) analysis. Markedly increased amounts of phosphorylated proteins were purified from CHOP-resistant cells than CHOP-sensitive cells. The results of the 2D DIGE are still in progress, but preliminary data has identified several candidate phosphoproteins that are unique to the CHOP-resistant form of DLBCL. Through use of mass spectrometry, we hope to identify differentially-expressed phosphoproteins. The sequence information will not only indentify phosphoprotein targets but may also reveal which kinases are involved in the phosphorylation events. We anticipate that this work will allow for the better understanding of the CHOP resistance pathway, which could lead to the development of a new chemotherapeutic agent that could greatly increase the survival rate of patients affected by DLBCL.

Teratogenic Effect Of Ethanol In Presence Of Retinoic Acid

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Alcohol is a strong teratogen that is highly consumed by human population. Heavy alcohol exposure during fetal development can lead to a constellation of brain, crani-facial, cardiovascular and limb defects that are collectively termed the "Fetal Alcohol Syndrome". Our laboratory is focused on understanding what developmental processes render the developing fetus vulnerable to ethanol. The second-trimester-equivalent period of human fetal development is one such period of vulnerability, because during this period fetal stem cells proliferate and mature to give rise to most of the neurons of the adult brain. Our experiments used neural stem and progenitor cells, derived from the Gestational Day 12.5 mouse dorsal telencephalic vesicle, and maintained in defined medium as neurospheres, to model the second trimester of human fetal development. Our hypothesis was that ethanol has teratogenic effects on neural stem and progenitor populations, resulting in persistent defects in neural maturation. To address this hypothesis, we cultured neural progenitors in the presence of ethanol (320mg/dl) or control medium for 5 days to approximate the in vivo period of neurogenesis. After 5 days, ethanol exposure was terminated and neurospheres were transferred to mitogen-depleted medium, and cultured on laminin-coated plates supplemented with retinoic acid to promote neural differentiation. In the presence of laminin and retinoic acid, neural progenitors disperse out of their parent neurospheres, and assume first a bipolar morphology characteristic of migrating neuroblasts, and subsequently a multipolar morphology characteristic of differentiating neurons and glial cells. Photomicrographs were obtained at time points from 24 to 72 hours during the differentiation period and the growth and branching of neuritic processes quantified using the ImageJ software package with the NeuronJ plug-in module. Next, immunostaining was performed on them to quantify neural maturation, and flow cytometry was conducted to quantify cell cycle. Preliminary analyses indicates that neural progenitor cells, pre-treated with ethanol exhibited longer processes and more complex branching compared to control progenitors. Our initial findings suggest that ethanol promotes premature maturation of neural progenitors.

COMPARISON OF MAGNETIC RESONANCE IMAGING TECHNIQUES AS TOOLS IN TRACKING ACUTE ISCHEMIC STROKE VOLUME IN ADULT FEMALE RATS

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Electrical Engineering and Neurology

Texas A & M Health Science Center College of Medicine

In practice, computed tomography (CT) and magnetic resonance imaging (MRI) are used as diagnostic tools for acute hemorrhagic and acute ischemic stroke, respectively. Unlike CT, which exposes the subject to harmful ionizing radiation, MRI is considered low risk for most subjects and uses non-ionizing magnetic gradients to create an image of acute stroke. There are many subtypes of MRI and this study examined the uses of diffusion weighted (DW) and fluid-attenuated inversion recovery (FLAIR) MRI in tracking acute ischemic stroke infarct volumes in adult female Sprague-Dawley rats over a period after middle cerebral artery occlusion (MCAo). The researchers achieved MCAo by injecting endothelin-1, a potent vasoconstrictor, into tissue surrounding the MCA, causing gradual vessel occlusion and reperfusion over a period of 12-16 hours. MR images were taken at intervals following MCAo on a magnet with specified values for sequence parameters, including echo time (TE), repetition time (TR), slice thickness, and b value. At sacrifice, DW and FLAIR MR images of ischemic stroke infarcts may be compared to histological sections to assess the image accuracy in measuring infarct volume. Trends in lesion growth may be identified by tracking the volume of the lesion surrounding the MCA through the infarct images at different post-stroke intervals.

Regulation of Mouse Aortic Smooth Muscle Cells

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Department of Systems Biology and Translational Medicine

Texas A & M Health Science Center College of Medicine

The reciprocity between the extracellular environment (ECM) and intracellular environment is vital to the function multicellular organisms. Cells are able to communicate with the extracellular environment through integrin, a membrane receptor protein. Integrin plays a vital role in the conveying of the status of the ECM to the native intracellular environment, allowing the cell to respond to changes in the environment. Focal adhesion contacts reinforce and stabilize integrin connections, and aid in the formation of intracellular signaling complexes in which cytoskeletal proteins play a key role. We seek to identify the role that α-smooth actin isoform participates in the formation of focal adhesions and aids in the transduction of information from these focal points. Smooth muscle cells are an active and dynamic cell family and have many functional roles in organisms. They can contract to modulate the systemic blood pressure as well as differentiate into a fibroblast like cell that aids in wound healing. Focal adhesion contacts modulate smooth muscle cells these two functional phenotypes. We used mouse aortic smooth muscle cells - wildtype (MOAOSMC – WT) and mouse aortic smooth muscle – smooth muscle α-actin knockout cells (MOAOSMC – SMAKO) in order to ascertain the importance of α-actin in focal adhesion contacts. We utilized two different ECMs, collagen IV and fibronectin which correspond to normal state and an injured state. We examined changes in proliferative potential of cells as well as the changes in the location of specific proteins, both structurally and regulatory, of smooth muscle cells. The cells did retain some functional capacity of the focal adhesion and integrin signaling complex, and the functional significance of α-actin in integrin signaling complexes is as yet unclear. We hope to further investigate signal transduction pathways and gene expression involved in upstream and downstream regulation of smooth muscle cells and the role that α-actin plays in these complexes.

Expression of Thymic Stromal Lymphopoietin by Human Basophils

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Microbial and Molecular Pathogenesis

Texas A & M Health Science Center College of Medicine Houston Campus

Background: Thymic Stromal Lymphopoietin (TSLP) is a hematopoietic cytokine primarily expressed by epithelial cells. Its primary target is immature myeloid dendritic cells. TSLP has also been dubbed the master switch for induction of the Th2 immune response characteristic of asthma and allergic inflammation. Recent murine studies have suggested that TSLP can be expressed by activated basophils.

Hypothesis: Since the biology of TSLP in mice and humans has many differences, we investigated the hypothesis that human basophils have the potential to express TSLP message and protein following activation by crosslinking their FcεRIα.

Methods: Isolation of purified human basophils from leukopaks is achieved by density gradient separation followed by negative selection using immunomagnetic beads coated with mAb specific for cell surface molecules for non-basophils. Basophil purity is established by Wright Giemsa staining of cytospun cells and by flow cytometry by dual detection of cell surface CD123 and IgE. Activation of the basophils is done by cross-linking cell surface FcεR1α with Cra1mAb. Basophil expression of TSLP mRNA is measured by real-time PCR at time points before and after activation. Expression of TSLP protein will be measured by ELISA of basophil supernatant.

Results: Basophils were demonstrated to be predominantly located in the peripheral blood mononuclear cell layer instead of the granulocyte fraction. Basophil isolation yielded 88% purity by light microscopy and flow cytometry. Preliminary PCR analysis of resting basophils demonstrated no detectable message for TSLP.

Conclusion: Ongoing studies will clarify whether activated human basophils can produce TSLP, and thereby provide insight into their potential to promote the development of Th2 immune responses in humans.

Evaluation of Gadobutrol vs Gadodiamide, Two MRI Gadolinium Chelate Agents, in Rat Brain Glioma Model at 1.5 T and 3 T

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In this study we compare two MRI gadolinium chelate agents, gadobutrol and gadodiamide, in rat brain glioma model at 1.5 T and 3 T field strengths. The experiment consists of three cohorts of rats. In the first group of 9 rats, we injected gadobutrol and gadodiamide at 0.1mmol/kg in each rat at 24 hours apart and measured the contrast effect on a 3 T MR scanner. For the second group of 7 rats, we injected the same contrast agents as the first group but scanned at 1.5 T. The third group of rats we compared 0.1mmol/kg of gadobutrol scanned at 1.5 T versus 0.05mmol/kg of gadobutrol scanned at 3 T. The results of group 1 and group 2 show that gadobutrol improves signal to noise ratio and contrast enhancement of rat tumors by about 20% at both 3 T as well as 1.5 T over gadodiamide. The third group shows that half amount of gadobutrol dosage scanned at 3 T achieves similar signal enhancement as full gadobutrol dosage at 1.5 T. These results are significant because gadodiamide has been known to cause a rare but fatal disease called nephrogenic systemic fibrosis (NSF) in patients with renal insufficiency. This is due to the instability linear structure of the chelate complex which leads to the release of toxic gadolinium ions in the body. Gadobutrol has a macrocyclic structure and binds to the gadolinium ion tightly. Gadobutrol is not yet FDA approved in the US but has been approved to use widely throughout Europe and gadobutrol has not been found to cause any NSF. Switching to gadobutrol and at lower concentration 0.05mmol/kg with scanning at 3 T instead of 1.5 T, will minimize any possible toxic effect of the gadolinium contrast agent and eliminates NSF in patients with renal problem.

A Phase I Study of DT2219ARL for the Treatment of B-Lineage Hematologic Malignancies

Rickesha L. Wilson

Hematology/Oncology and Cancer Research Institute at Scott & White

Texas A & M Health Science Center College of Medicine

Leukemia is a common type of cancer characterized by uncontrolled blood cell growth in the bone marrow or blood. There are approximately 245,225 people in the United States living with leukemia and an estimated 44, 790 will be diagnosed in 2009. Acute leukemia represents 30% of childhood malignancies with 80% of these cases being B-lineage acute lymphoblastic leukemia. Though the 5-year survival rates have improved over the decades, leukemia causes more death than any other cancer. Immature malignant cells (blasts) become resistant to chemotherapy and are often the cause for treatment failure. In the last few decades, immunotoxin therapy has been developed to more effectively and selectively target hematologic malignancies.

DT2219ARL is a bispecific ligand-directed toxin (BLT) that targets highly expressed CD 19 and CD 22 receptors on malignant tumor cells. It consists of catalytic DT390 and two single chain antibody variable fragments that target CD22/19, genetically mutated for improved anti-leukemic efficacy. The bispecificity of DT2219ARL has shown greater activity in vitro than its monospecific counterparts, and the BLT has shown to significantly prevent the onset of the highly aggressive B cell malignancies in SCID mice.

The Cancer Research Institute at Scott & White has added the DT2219ARL protocol to its Phase I clinical trials program. Qualifying patients with relapsed ALL/CLL are given immunotoxin therapy as a 4 hr IV infusion QOD x 4. Doses are escalated with each patient (.5, 1.25, 2.5 ug/kg/dose for #1-3) and subsequent patients cohorts receive higher doses until MTD has been determined. Our objectives are to determine the safety and tolerability of DT2219ARL as well as observe the response rates, response duration, PK profile, and immunogenicity in patients. The CRI has treated two patients and counting on the DT2219ARL protocol. The first two patients (ages 64 and 71, white males with CLL) have been free of grade 3 toxicities without adverse events, but no remissions have been seen based on bone marrow and blood studies. These results could be due to the low doses of drug given (.5 and 1.25 ug/kg/dose) and the aggressive nature of relapsed B-cell malignancies.

Conversion of Human Dermal Fibroblasts into Cardiac Myocytes

Musi Zhang, Dr. Robert Schwartz
Texas A & M IBT Center for Molecular Development and Disease
Texas A & M Health Science Center College of Medicine

Heart failure is a major public health concern worldwide, and coronary artery disease in particular is the leading cause of death in the United States. Management of ventricular remodeling and chronic ischemic cardiomyopathy after myocardial infarction remains a challenge. Stem cell therapy is a promising new approach to restoring cardiac function and preventing ventricular remodeling after acute myocardial infarction. However, the therapeutic effects of stem cells in heart disease have been limited thus far, and it is not clear whether adult stem cells can differentiate into cardiac muscle cells and fibers. Identifying specific somatic cell–derived cardiogenic cells that can survive and proliferate in the harsh, proapoptotic microenvironment of the damaged heart is a key goal that would increase the efficacy of stem cell therapy. To that end, the recent discovery that induced pluripotent stem (iPS) cells can be generated by reprogramming adult somatic cells has provided a promising new direction for optimization of stem cell therapy. Recent studies have demonstrated that ectopic expression of four transcription factors: c-Myc, Sox2, Klf4, and Oct4, could reprogram murine somatic cells to induced pluripotent stem cells (iPSCs), and iPSCs could be subsequently generated using similar genetic manipulation. In order to examine the reprogramming of primary human fibroblasts into iPS cells and the subsequent converting to cardiac myocytes using TAT – c-Myc, Sox2, Klf4, and Oct4, codons of human *Pou5f1/ cMyc* (NP_002458), *Sox2* (NP_003097), *Klf4* (NP_004226), and *Oct4* (NP_002692), will be first optimized for high level protein expression in *E. coli*; the protein transduction protocol will be further examined with Nkx2.5 red tomato-puromycin selectable system. The induced iPS cells will be used to form embryoid bodies in noncoated plastic dishes. Cells will be harvested by trypsinization and transferred to bacterial culture dishes in ES medium without LIF. It is expected that, like ES cells grown in tissue culture dishes, the embryoid bodies from the infected clones will attach to the bottom of the dish and initiate differentiation. Finally, a forced aggregation system will be used for iPS cells to determine which signaling systems would induce cardiac mesoderm progenitors. Cell based therapy has been proposed as a promising new means for repairing damaged heart muscle after myocardial infarction. Recent observations have shown that the combined addition of c-Myc, Sox2, Klf4, and Oct4 can reprogram mouse and human primary embryonic fibroblasts into stem cells and set in motion the autoregulatory stem-cell factor loop to reprogram HDFs to more closely resemble actual stem cells that may also have the capacity to fully differentiate into cardiac progenitors for cardiac repair. Hopefully, the use of protein transduction of these stem cell marker proteins will allow us to take reprogram cardiac myocytes into the clinic.

Detecting Missed Ligamentous Injuries of the Occipito-Cervical Complex (OCC)

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Texas A & M Health Science Center College of Medicine

BACKGROUND CONTEXT: Recent case series demonstrate that Occipito-cervical complex (OCC) injuries are potentially survivable. Delay in diagnosis can lead to increased morbidity and mortality.

PURPOSE: The primary aim of this study is to detect OCC injuries initially missed at a Level 1 trauma center.

STUDY DESIGN/SETTING: Retrospective radiographic and clinical review of patients in a comprehensive trauma database.

METHODS: Normative maximum values (NMV's) that included 97.5% of the population were defined with a sample of 251 consecutive normal CT scans for the Basion-Dens Interval (BDI), Atlanto-Occipital Interval (AOI) and Lateral Mass Interval (LMI) of C1-2. Then, 844 cervical CT scans from consecutive polytrauma patients were reviewed for evidence of OCC injury. Measurements above the NMV's were considered suspicious for injury. A BDI>12mm or a BDI>10mm with a confirmatory MRI was considered definite evidence of an OCC injury, as was an LMI$\geq$4mm with confirmatory MRI. The electronic medical record was reviewed to determine if an injury was detected on the final neuroradiologist's report or during follow up.

RESULTS: 5 patients had evidence of atlanto-occipital dissociation (AOD), and 2 had atlanto-axial dissociation (AAD). Of these, 3 cases of AOD and 2 cases of AAD were missed on the final report by the neuroradiologist. The undiagnosed patients were subsequently diagnosed by orthopaedic surgeons consulted for axial spine or other musculoskeletal trauma. No patients that were diagnosed with AAD or AOD in the electronic medical record were missed using the criteria of BDI > 10mm and LMI $\geq$ 4mm to define OCC injuries.

CONCLUSIONS: OCC injuries can be missed even with standardized MDCT with MPR. High quality normative data used to determine a reliable, Picture Archiving and Communication System (PACS) based measurement of the OCC anatomy can detect ligamentous injuries initially missed in polytrauma patients.

Summer Research Program Participants (M1s) 2009

College Station -10
Temple – 13
Houston - 3

M1 Student	Mentor	Department/Affiliation
Uchenna Aduba	C McNeal	Internal Medicine/Temple
Doug Armour	W Zimmer	SBTM/CS
Darryl Blalock	D Prockop	Regen Med/Temple
Danial Bokhari	H Andrews-Polymenis	Micro Mol Path/CS
Jeffery Calender	F Sohrabji	NExT/CS
Bianca Caram	D Huston	Medicine/Micro Mol Path/ Houston
Stephen Coffman	J Friedman	Next/ Inst Brain & Spine/CS
Rebekah Condit	F Sohrabji	NExT/CS
Joseph Hellman	E Childs	Surgery/Temple
Jonathan Jan	I Murray	NExT/CS
Drew Kelly	C Chaput	Orthro/Temple
Andrew Kovoor	G Toussaint	NExT/ Inst Brain & Spine/CS
Michael Laney	J Friedman	NExT/inst Brain & Spine/CS
Hao Jun Li	H W Sampson	SBTM/Temple
Tony Lu	K Baker	Medicine/CVRI/ Temple
Ben Morrissey	W-J Chen	NExT/CS
Ulfat Nisa	I Murray	NExT/CS
Samantha Otokunrin	H W Sampson	SBTM/Temple
Kuan Park	R Smythe	Surgery/Temple
Hiral Patel	R Schwartz/D Huston	Medicine/Micro Mol Path/ Houston
Vivek Patel	W Culp	Medicine/Anesthesiology/Temple
Marcelo Ribeiro	D Dostal	Medicine/CVRI/ Temple
Leslie Schornack	C McNeal	Internal Medicine/Temple
Rahat Vohra	D Huston	Medicine/Micro Mol Path/Houston
Lan Vu	V Runge	Radiology/Temple
Rickesha Wilson	A Frankel	Internal Medicine/Temple

Summer Research Program Participants (Undergrads) - 2009

Student	School	Mentor	Department
Evan Cherry	TAMU	Bayless	Mol Cell Med
Luis Dloughy	TAMU	Cirillo	Micro Mol Path
Ashlyn Spring	TAMU	Maxwell	Mol Cell Med
Anand Ganapathy	TAMU	Samuel	Micro Mol Path
Mohammed Taha	Purdue	Miranda	NExT
Victoria Vo	UT Austin	E. Wilson	SBTM
Musi Zhang	UT Arlington	Huston	Medicine/Houston
Jillian Vitter	Georgetown	Friedman	NExT/Inst Brain & Spine

2009 Summer Research Program

Sites: College Station RMB: Lecture Hall II
TPL: MEC Lecture Hall I
Houston IBT: Rm 1105

Updated: 5/13/09

DATE	TOPIC	PRESENTER
May 19; noon	Graduate program	Dr. Emily Wilson and grad students (Will originate in Temple)
May 22; 9:00 am	Record Keeping	Dr. Van Wilson
May 26; noon	Animal Research	Dr. Andrews-Polymenis
May 29; 9:00 am	Scientific Method	Dr. David McMurray
June 2; noon	Medical Research …Why Me?	Dr. Culp
June 5; 9:00 am	Biotechnology/ethics	Dr. James Samuel
June 9; noon	MD/PhD Program Molecular Pathogenesis	Dr. Leibowitz and MD/PhD students Dr. James Samuel
June 12; 9:00 am	Scientific Misconduct	Dr. Vernon Tesh
June 16; noon	Neuroscience	Dr. Rajesh Miranda
June 19; 9:00 am	Commercialization in Medicine	Bruce Leander, Retired President, Amhion, Inc.
June 23; noon	Cardiovascular Research	Dr. David Dostal
June 26; 9:00 am	Human Experimentation	Dr. John Quarles
June 30; noon	Translational Research	Dr. David Huston
July 3	**HOLIDAY**	
July 7; noon	Cell and Mol Biol Biochem and Structural Biol	Dr. Kayla Bayless Dr. Sarah Bondos
July 10; 9:00 am	Cancer Research	Dr. Roy Smythe
July 14; noon	Student Oral Presentations	
July 17; 9:00 am	Student Oral Presentations	
July 21; noon	Student Oral Presentations	
July 24; 9:00 am	Student Oral Presentations	
July 29; 9:00 am – 2:00 pm	**ALL – POSTERS PRESENTATIONS AND RECEPTION** **Reynolds Medical Building**	

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

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