UTHealth
The University of Texas
Health Science Center at Houston

McGovern
Medical School

2016 SUMMER RESEARCH PROGRAM
STUDENT ABSTRACTS

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Preface

The University of Texas Medical School at Houston (UTMSH) Summer Research Program provides intensive, hands-on laboratory research training for MS-1 medical students and undergraduate college students under the direct supervision of experienced faculty researchers and educators. These faculty members' enthusiasm for scientific discovery and commitment to teaching is vital for a successful training program. It is these dedicated scientists who organize the research projects to be conducted by the students.

The trainee's role in the laboratory is to participate to the fullest extent of her/his ability in the research project being performed. This involves carrying out the technical aspects of experimental analysis, interpreting data and summarizing results. The results are presented as an abstract and are written in the trainees' own words that convey an impressive degree of understanding of the complex projects in which they were involved.

To date, more than 1,900 medical, college, and international medical students have gained research experience through the UTMSH Summer Research Program. Past trainees have advanced to pursue research careers in the biomedical sciences, as well as gain an appreciation of the relationship between basic and clinical research and clinical practice.

UTMSH student research training is supported by a grant from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and/or by financial support from the Dean and the departments and faculty of the medical school and School of Dentistry.

Biomedical science education remains a vital and integral part of our nation's interests. The UTMSH Summer Research Program, and the dedication of our faculty and administration exemplify the institution's commitment to training and educating the future leaders in our biomedical scientific communities.

Gary C. Rosenfeld, Ph.D.
Director, Summer Research Program
Associate Dean for Educational Programs

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Acknowledgements

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Lab Research Ownership

Publication and/or Disclosure

Each student participating in this program is required to read, agree to, and sign this disclosure form. The original signed copy is on file in the Summer Research Program office; the student and their faculty mentors are each furnished with a copy.

"In reference to the laboratory research you will perform this coming summer through The University of Texas Medical School at Houston's Summer Research Program, you are required to comply with the standard restrictions regarding participation in the Summer Research Program:

"All of your laboratory research is *CONFIDENTIAL* and although your abstract will be available through our website, you cannot independently disclose or publish any research findings or data in any form (including at meetings or conferences) without the express prior written approval of The University of Texas Medical School at Houston. If you wish to submit your abstract to any third party, you must first contact your faculty mentor no less than three (3) weeks prior to any deadlines in order to obtain the necessary written approvals.

"Because your research was generated from ideas and funds that originated with your faculty mentor and The University of Texas Medical School at Houston, ownership of any data generated by you during the Summer Research Program belongs to The University of Texas Medical School at Houston or the Principle Investigator (PI)."

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ABSTRACT

Debriefing: The Forgotten Phase of the Surgical Safety Checklist

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Sponsored by: KuoJen Tsao, MD, Department of Pediatric Surgery
Supported by: KuoJen Tsao, MD, Department of Pediatric Surgery
Key Words: Post-operative debrief, Surgical Safety Checklist (SSC)

Introduction
The post-operative debrief of the World Health Organization Surgical Safety Checklist (SSC) provides surgery, anesthesiology, and nursing team members the opportunity to share pertinent information with one another to improve communication, efficiency, and patient safety. Additionally, the debrief phase allows teams to acknowledge errors that occurred during procedures to prevent similar problems in later cases. Little research has been done on compliance to the debrief portion of the SCC. The purpose of this study is to evaluate the debrief checklist to find areas for improvement.

Methods
A direct observational study was conducted from May-July of 2014-2016 in an academic children's hospital. Convenience sampling was performed across all 9 pediatric surgical specialties. During the 8-point debrief checklist, trained observers documented team members' implementation of SSC criteria (adherence to all checkpoints). These checkpoints review critical points in the operating room and future concerns for the patient. Regression analysis was used to evaluate the association between events and performance of the checklist (adherence). Inter-rater reliability (kappa) was performed for checklist adherence.

Results
In 2014, 91% of teams performed at least one checkpoint of the debrief checklist (n=205). In 2015, 91% of teams performed the debrief checklist (n=198). In 2016, 95% of teams performed the debrief checklist (n=251). The average number of checkpoints completed in 2014 to 2016 was 6.2, 6.5, and 6.6 respectively ($p<0.05$). From 2014-2016, there has been improvement in ensuring the surgery attending remains in the room during the checklist (85%; 89%; 94% respectively), in confirming the procedure performed and the site of the procedure (88%; 88%; 91% respectively), in using blood transfusions and total blood loss (78%; 83%; 91%), in discussing the wound class (82%, 83%, 83% respectively) and any equipment problems that occurred (73%, 79%, 79% respectively), and in vocalizing concerns from anesthesia and surgery (69%; 70%; 72% respectively). There has been a decrease in adherence of identifying and labelling of specimens from a procedure (81%; 78%; 72% respectively). The inter-rater reliability kappa value for the debrief portion of the SSC was 0.646.

Conclusion
There has been an increase in adherence to the post-operative debrief from 2014-2016, however much work needs to be done to improve performance to all checklist items. Continued monitoring of the execution of the debrief phase can identify specific checkpoints to focus improvement efforts and minimize surgical errors. When performed with high adherence, the debrief portion of the SSC can illuminate areas for focused intervention in yearly safety trainings that may otherwise be forgotten.

ABSTRACT

Horizontal vs. Vertical Positioning of the iCare Rebound Tonometer and Effects on Intraocular Pressure Readings

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Sponsored by: Robert Feldman, M.D., Department of Ophthalmology and Visual Science

Key Words: intraocular pressure, iCare, pneumatonometer, orientation, accuracy

Background: Glaucoma is the second most common cause of vision loss in the world. Unlike increased age and family history, elevated intraocular pressure (IOP) is the only risk factor for glaucoma that can be modified by therapeutic interventions. The accurate and precise assessment of IOP is therefore critical, and IOP is measured at every ophthalmologic exam. The iCare rebound tonometer was developed as a quick and minimally invasive IOP measurement device, ideal for routine IOP screenings. The purpose of this project is to evaluate the agreement between intraocular pressure (IOP) measurements taken by holding the iCare rebound tonometer vertically versus horizontally. iCare measurements will be compared against those taken by a pneumatonometer to assess for accuracy.

Patients and Methods: Prospective study involving 113 patients, 18 years of age or older, with healthy corneas showing no evidence of defects or pathology. Patients with prior corneal surgery, use of topical ocular medications, ocular infection within thirty days prior to enrollment, or an allergy to proparacaine were excluded. Central corneal thickness was determined, and IOPs were measured with the iCare held vertically and held horizontally. Lastly, IOP was measured using a pneumatonometer.

Results: The study eyes were classified into three central corneal thickness groups: normal cornea (between 511 to 580 μm), thick cornea (> 580 μm), and thin cornea (≤ 510 μm). The mean difference between IOP readings taken by holding iCare horizontally versus vertically was on average found to be 0.37 mmHg. Similarly, agreement between IOP measurements taken with each iCare position and with the pneumatonometer was analyzed. iCare, held in either orientation, on average presented an IOP reading approximately 2 mmHg lower than the pneumatonometer. In the thin cornea group, iCare measurements were 3.61 mmHg lower than measurements taken with the pneumatonometer. Bland-Altman plots were used to assess the agreement visually.

Conclusions: While holding the iCare vertically or horizontally has some effect on IOP readings, the difference is statistically insignificant. However, the iCare did underestimate IOP measurements taken by the pneumatonometer, most significantly so in patients with thin corneas.

ABSTRACT

Assessment of Adult db/db Mouse as a Model of Bone Abnormalities in Type 2 Diabetes Mellitus

Andrea Baker   McGovern Medical School at UTHealth   *Class of 2019*

Introduction: Diabetic animal models have led to discoveries that have significantly improved the quality of care for patients with both type 1 and type 2 diabetes. However, the detrimental effect of diabetes on bone strength is not well understood, and it is not clear which animal models may be most beneficial in studying this complication. This study focuses on assessing the extrinsic and intrinsic mechanical properties of one model of diabetes: the leptin receptor-deficient (db/db) mouse. The db/db diabetic mouse model has a homozygous defect in the leptin receptor gene, with a characteristic phenotype of obesity, hyperinsulinemia, and hyperglycemia, which results in a deficiency in the leptin receptor and an increase in circulating leptin. The heterozygous db/+ mouse and the WT were included as controls. Mechanical properties as measured by three-point bending test and microarchitecture as assessed by micro CT were performed on the db/db mice, the heterozygous lean control (db/+), and the littermate wild type control (WT) in order to determine the functional difference between diabetic and normal bone.

Methods: Here we present the results from the 20-week age group, which included six WT, six db/+ and seven db/db animals. Micro CT trabecular (distal femur) and cortical (midshaft) analysis and three-point bending tests were performed on one femur from each mouse. Femurs were tested wet at room temperature on an Instron 5848 MicroTester. Loading was performed in an anterior-posterior (AP) direction using a span of 6.96mm to 4.63mm depending on femur length. Preload was applied at a rate of 3N/min to a preload of 0.2N, and then the femurs were tested to failure at a rate of 0.1mm/s. Subsequently, ANOVA with Tukey HSD post-hoc analysis were performed to compare the groups.

Results: ANOVA demonstrated a statistically significant difference for the yield load (p=0.009), fracture load (p=0.043), stiffness (p=0.013), plastic energy (p=0.001), plastic displacement (p=0.002), plastic toughness (p=0.008), plastic strain (p=0.003), and trabecular thickness (Tb.Th., p=0.029). In addition, there was a statistical trend in the elastic modulus (p=0.095). Post-hoc analysis revealed that db/db mice had decreased strength, stiffness, and trabecular thickness, when compared to either WT or db/+ mice. The db/db mice also had significantly lower plastic parameters compared to the db/+ animals, indicating increased brittleness of the diabetic bone. The db/+ and WT controls were similar for most mechanical measures, indicating that they can both be used as controls for the db/db mice, except in the cases of plastic properties. Plastic energy, plastic displacement, plastic toughness, and plastic strain of the db/db and WT were significantly lower than the db/+ indicating that the heterozygotes have significantly increased ductility.

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Discussion: As has been shown in other studies, the load and stiffness properties decreased in the db/db mice. Most cortical and trabecular microCT variables (except Tb.Sp.) decreased in the db/db mice, but only Tb.Th. was significantly different. Many research groups use db/+ as controls for db/db as the db/+ animals do not become obese nor do they display impaired glucose tolerance. However, our results may indicate that, at 20 weeks of age, the db/+ are not suitable as controls as they have significantly increased ductility, whereas the WT and db/db mice are similarly brittle. This indicates that the db/+ and WT controls cannot be combined together and compared to the db/db mice when investigating the bone phenotype. In summary, this study demonstrated significant differences in the intrinsic and extrinsic mechanical properties from db/db, db/+, and WT mice. This provides further evidence that the db/db mouse has decreased biomechanical strength that may lead to the increased fracture risk seen in diabetic patients.

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ABSTRACT

Using Connectomics to Investigate Retinal Ganglion Cells

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Sriram Navuluri

Sponsored by: David W Marshak, PhD. Department of Neurobiology and Anatomy

Key Words: Retinal ganglion cells, amacrine cells, connectomics, electron microscopy, motion detection

Introduction: Retinal ganglion cells integrate and transmit visual information from the retina to the brain. There are many distinct types of retinal ganglion cells whose functions vary along with their morphology. The focus of this study was on parasol ganglion cells, also known as M cells, which contribute to many aspects of vision and particularly to the perception of motion. Though parasol cells have long been studied by a variety of methods, retinal connectomics provides a novel way to study their synaptic connections. In particular, we propose that inputs from amacrine cells, inhibitory local circuit neurons of the inner retina, impart motion sensitivity to parasol cells. Previous work predicted these synaptic connections, and this project tested this hypothesis.

Methods: Serial blockface scanning electron microscopy was used to generate a series of images through the entire thickness of a piece of central macaque retina. The project utilized Viking software to navigate and annotate these sections. Ultimately, these annotations will be compiled to generate three-dimensional reconstructions of the cells and a high resolution map of their synaptic connections, also known as a connectome. Cells were classified based on their soma size and characteristics of their dendrites, including: field diameter, morphology and depth of stratification in the inner plexiform layer. Criteria for synaptic connections included pre-synaptic vesicle clouds and post-synaptic densities.

Results: We completed annotations of five ON parasol ganglion cells and one midget ganglion cell. Additionally, we partially annotated six other ganglion cells that are not yet classified. A subset of the somas in the ganglion cell layer appeared to be displaced amacrine cells. We investigated the morphology of twenty-four amacrine cells and have begun analysis of their synaptic connections with our completed parasol ganglion cells. We have observed inputs to ON parasol cells from both starburst and wiry morphological types of amacrine cells.

Discussion: Previous studies utilized light microscopy and electrophysiology to predict the existence of synapses from starburst and wiry amacrine cells to ON parasol ganglion cells. Our annotations provided direct, ultrastructural evidence for these synapses. Further analysis of the connectome will provide a more complete description of the retinal circuitry for motion detection.

ABSTRACT

Optical Apparatus for Coupled Shape and Force Sensing

Kaitlyn Brooks   *McGovern Medical School at UTHealth*   *Class of 2019*

Sponsored by: Richard W. Smalling, MD, PhD, Department of Internal Medicine

Supported by: Richard W. Smalling, MD, PhD, Department of Internal Medicine
David Volk, PhD, Department of Nanomedicine and Biomedical Engineering

Key Words: Guidewires, force measurement, optical strain sensors

Background: Surgical mitral valve repair or replacement are the standard for management of heart failure secondary to severe mitral regurgitation. The risks, recovery time, and morbidity associated with open heart surgery, however, have led to investigational efforts in creating minimally invasive techniques. In general, transcatheter procedures are somewhat limited by a lack of haptic feedback and difficulty maneuvering the device over a guidewire to the target location. Force misalignment between guidewire pathways and tracking devices can result in the guidewire moving out of position, impairing device delivery. Additionally, excessive forces on driven delivery systems can potentially harm cardiac structures, causing serious adverse events.

Primary aim: Design a method/device for measuring external forces acting on and the shape assumed by guidewires during transcatheter procedures

Methods: Fiber bragg grating sensors (FBGs) are arranged serially to yield sensing arrays, which are embedded into 0.035" Amplatz Super Stiff guidewires to detect strain. FBG reflective wavelengths are varied along the array for sensor identification. The array arrangement enables computation of forces in three dimensions and shape rendering based upon a Frenet-Serret algorithm.

Results: A macroscale prototype successfully identified tension and compression on opposing arrays due to bending. Curvature and torsion were computed for each sensor triad. Interrogating by sensor reflective wavelength yielded device strain location.

Conclusion: It is recommended that this technology be further explored by developing and testing a shape and force sensing function. A "smart" guidewire capable of real-time combination force and shape sensing may enhance pre-procedural planning as well as intraoperative feedback for clinicians; such a device would facilitate delivery success and significantly increase procedure safety.

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ABSTRACT

The Role of Skeletal Muscle SIK1 in Catabolic Physiology

Tanner Brown   *McGovern Medical School at UTHealth*   *Class of 2019*

Introduction: Skeletal muscles are one of the largest consumers of glucose and work to regulate blood glucose levels in normal physiology. During a catabolic state, skeletal muscle becomes transiently insulin resistant in order to conserve blood glucose for other tissues. In long-term fasting, skeletal muscle proteins are broken down into amino acids used as gluconeogenic substrates by the liver.

Background: SIK1 is a serine threonine kinase that is present in all tissues including the liver, adipose tissue, and skeletal muscle. The Berdeaux Lab has previously found that mice lacking the *Sik1* gene have muscle hypertrophy and improved insulin sensitivity after high fat diet. Because SIK1 is normally induced in skeletal muscle after long term fasting or during pathological insulin resistance, we hypothesized that increased expression of SIK1 in skeletal muscle promotes transient physiologic insulin resistance and protein turnover during the catabolic state of fasting to spare glucose and contribute amino acids to the liver for gluconeogenesis and maintenance of blood glucose levels.

Methods: To test the contribution of SIK1 in muscle catabolism, we compared markers of protein degradation (enzymatic assays of plasma alanine and lactate and intramuscular protein: nucleic acid ratio) and insulin action (tissue glycogen content) in SIK1-KO mice against wild-type mice under *ad libitum* fed and 24 hour fasted conditions. We also examined incorporation and release rates of radioactive 3H-Phenylalanine to estimate protein synthesis and degradation rates in WT and SIK1-KO cultured muscle cells. Finally, we tested insulin signaling in cultured SIK1-KO muscle cells.

Results: In skeletal muscle, we found that *Sik1* mRNA increases with 24 hours of fasting. In the fed state, we observed a trend toward increased protein: DNA ratio in SIK1-KO mice compared to WT controls, indicating a net increase in protein abundance. In vitro, protein synthesis rate was increased in cultured SIK1-KO when compared to WT ($p < 0.05$ by linear regression) but the protein degradation rate was not affected. This result indicates that the observed hypertrophy in SIK1-KO mice is most likely due to increased protein synthesis. Analysis of plasma markers of protein breakdown (alanine and lactate) is ongoing. There was no statistically significant difference in skeletal muscle or hepatic insulin-stimulated AKT phosphorylation or glycogen levels between WT mice to SIK1-KO in the fasted or fed states, indicating that SIK1 does not affect glycogen storage.

Conclusion: SIK1 expression is induced in muscle during fasting. During fasting, SIK1 most likely inhibits protein synthesis in skeletal muscle fibers, contributing to net protein loss. SIK1 does not directly regulate insulin signaling or glucose storage as glycogen, so it remains to be determined how SIK1 contributes to fasting muscle glucose metabolism in lean animals.

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ABSTRACT

Epidemiologic Differences in Young versus Elderly with Traumatic Spinal Cord Injury

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Sponsored by: Sasha D. Adams, MD, Department of Surgery
Supported by: Center for Translational Injury Research (CeTIR)
Key Words: Traumatic Spinal Cord Injury, Young, Elderly

Introduction: Approximately 12,500 spinal cord injuries (SCI) occur annually in the USA, and as of 2014, about 276,000 people living in the US with SCI. These numbers will likely increase due to the growing number and proportion of active elderly individuals who have an increased rate of falls due to sensory loss, muscular weakness and dementia, and are more susceptible to fractures and SCI due to osteoporosis, osteopenia, and degenerative cervical stenosis. The morbidity and mortality following SCI in the elderly far exceeds that of younger adults, and there is significant debate in current literature over the necessity of spinal immobilization and imaging of the elderly after low level mechanisms of injury. We aim to denote differences in the location and type of blunt SCI between the elderly and young. We hypothesize that the elderly have a higher incidence and risk of cervical SCI despite low force mechanism injuries.

Methods: We queried the Memorial Hermann Hospital (MHH) Trauma database for adult (>15 years old) trauma patients with any level of SCI by blunt mechanism over a 5-year period (1/1/2011 - 12/31/2015). SCI was defined by AIS scores to include complete and incomplete cord injury, cord contusion, and ligamentous spinal injuries. Elderly was (defined as $\geq$ 55 years old. We analyzed mortality, length of stay (LOS), ICU days, and non ICU days with Chi square analysis, Fisher's exact test, and Kruskal-Wallis to determine statistical significance.

Results: 23,410 adult trauma patients were admitted to MHH, of which 961 were diagnosed with SCI. 36% were Elderly, and 73% were Male. Mortality following SCI was 9.4% (13% Elderly, 7% Young). Cervical SCI was seen in 69% (n=664), thoracic in 21% (n=205), and lumbar in 10% (n=92), with 7.5% overall having multiple levels of SCI. There was significantly more cervical SCI in both age groups (65% Young, 76% Elderly). Falls led to 44% of the Elderly cervical SCI which was significantly higher than the 16% of the Young with cervical SCI ($p<0.001$). Thoracic and Lumbar SCI were more common following MVCs in both age groups, however were not significant. LOS, ICU days and non-ICU days medians were not significantly different in the Elderly. The median LOS was 9 days in the Elderly vs 8 for Young. Median days in the ICU days was 3 for Elderly and 2 for Young, while median non-ICU days were 26 for the Elderly vs 27 for the Young.

Conclusion: The cervical spine is the most common location of SCI in both the Young and Elderly, however Elderly were more than twice as likely to incur a cervical SCI from a fall. This information has clinical use in determining the need for neck stabilization spinal precautions and imaging in elderly patients after falls. While elderly patients had longer LOS and ICU days these were not statistically significant in our study. Further delineation will require separation of SCI severity and correlation with additional injuries and overall ISS.

ABSTRACT

Optimization of Vimentin-binding Aptamers for siRNA Treatment of Ovarian Cancer

Andrea Costello   McGovern Medical School at UTHealth   *Class of 2019*

Sponsored by: Hongyu Wang, MD, PhD, Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases (IMM), McGovern Medical School, David E Volk, PhD, Department of Nanomedicine and Biomedical Engineering, McGovern Medical School and the IMM

Supported by: David E Volk, PhD, Department of Nanomedicine and Biomedical Engineering

Key Words: Vimentin, aptamer, ovarian cancer

The application of aptamers in biomedicine is emerging as an essential technology in the field of cancer research. As small single-stranded DNA or RNA ligands with high specificity for their targets, aptamers provide many advantages over protein-based molecules, such as antibodies, that are currently used in cancer therapeutics. Vimentin is an intermediate filament protein that is overexpressed in endothelial cells of cancerous tissue. High expression levels of vimentin have been associated with increased capacity for migration and invasion of the tumor cells. Monothioate aptamers (thioaptamers) with high specificity for vimentin have been selected with the intention of optimizing a single vimentin-binding aptamer for the treatment of ovarian cancer. This work focused on the synthesis and purification of two selected vimentin-binding thioaptamers, which were labeled with biotin to aid in the characterization process. Purification of the synthesized aptamers was achieved through High Performance Liquid Chromatography (HPLC) and gel electrophoresis. To confirm specific binding between the two aptamers and vimentin, a tissue binding assay was conducted with both normal and tumor ovarian tissue. The cell surface marker CD31 is expressed on endothelial cells in the vessels of ovarian tissue. Using fluorescence microscopy, we demonstrated that the two aptamers bound to tumor vasculature and co-localized with the CD31 endothelial cell surface marker. It suggests that the synthesized aptamers were successfully targeting vimentin on the surface of the endothelial cells in the vessels of the ovarian tissue. Future objectives for this project include conducting binding assays in live tumor cells, as well as characterizing the kinetics of the binding relationship between vimentin and the aptamers. The ultimate direction of this project will be to conjugate the selected aptamers with nanoparticles containing siRNA to target ovarian cancer.

ABSTRACT

Sensitivity of the human papillomavirus (HPV) mRNA assay to identify women with history of previous HPV infections

Sarah Cotton   McGovern Medical School at UTHealth   *Class of 2019*

Sponsored by: Judith A. Smith, Pharm.D., Robert Brown, M.D., Elizabeth K. Nugent, M.D., Sonia C. Robazetti, M.D., Pamela D. Berens, M.D., Department of Obstetrics, Gynecology, and Reproductive Sciences

Key Words: HPV, DNA Assay, E6/E7 mRNA Assay, Cervical Cancer

Introduction: The recently FDA-approved E6/E7 mRNA assay is known to be highly specific for detecting HPV active infections intended for supplementary screening to DNA assay. It has been used as primary screening tool in many clinical settings but it is unclear how it has impacted detection of HPV latent infections. The objective of this study was to determine the consistency between HPV mRNA testing in women with history of previous HPV infections and potential impact change in follow up screening.

Methods: Data from the LabPath database was reviewed from November 2014-June 2016 to identify patients with one or more HPV positive results. American Congress of Obstetrics and Gynecology (ACOG) cervical cancer screening guidelines were used to compare the follow up recommendations for positive HPV DNA results versus HPV mRNA results and then analyzed by age, history of abnormal cytology, or colposcopy.

Results: A total of 425 patients were identified with at least 1 or more prior HPV infections by DNA assay. There was an overall 69.3% difference in HPV results. There was a potential change in follow up for 71.9% of patients with 1 prior HPV+ result and 60% of patients with at least 2 or more prior HPV+ results. Overall, 19.4% of these patients also had a history of abnormal colposcopy results that included 15.6% with 1 HPV+ results and 36.5% with at least 2 prior or more HPV+ results.

Conclusion: The data suggests HPV mRNA assays does not appear to be as sensitive for detection of latent HPV infections. Based on the data and the potential change in follow up care, the HPV mRNA assay may not be appropriate to use for a primary screening tool for cervical cancer.

ABSTRACT

Postprandial Free Fatty Acid Signaling and Antidiabetic Medications in Prediabetic Patients

Sara Coverdale   McGovern Medical School at UTHealth   *Class of 2019*

Sponsored by: Absalon Gutierrez, MD, Department of Internal Medicine, Department of
                 Endocrinology, Diabetes, and Metabolism
Supported by: NIH Grant 2T35DK007676-23
Key Words: Prediabetes, type 2 diabetes, diabetes mellitus, endothelial dysfunction,
                 cardiovascular disease

Type 2 diabetes mellitus (T2DM) is associated with an increase in the risk of death from coronary heart disease. Recent data shows that glucagon-like peptide 1 (GLP-1) receptor agonists not only regulate hyperglycemia, but may also reduce major adverse cardiovascular events in T2DM. The mechanisms of these effects remain unknown. The GLP-1 receptor agonist exenatide reduces levels of postprandial lipids, lipoproteins and free fatty acids (FFA). Exenatide also improves postprandial endothelial function in individuals with impaired glucose tolerance and recent-onset type 2 diabetes. Finally, exenatide exerts an anti-inflammatory effect in the fasting state. However, it is unknown if this drug affects postprandial free fatty acid signaling. The related drug saxagliptin (a dipeptidyl peptidase-IV inhibitor) may target some of the FFA signaling and inflammatory pathways related to exenatide, but more data is needed.

This randomized, crossover, placebo-controlled, double-blinded prospective trial examines the of efficacy of exenatide and saxagliptin in reducing postprandial FFA signaling, ameliorating postprandial hyperlipidemia, and improving endothelial function in humans with prediabetes. Each subject is studied three times in daylong outpatient visits, each representing one of the three study arms – placebo, exenatide, and saxagliptin. In each visit, baseline labs are drawn and forearm blood flow testing (to quantify endothelial function) is performed. Then study medication is given. Then the patient is fed a high-fat standardized meal. For the six-hour postprandial period, blood is drawn every 2 hours and forearm blood flow is measured every 3 hours. The primary outcome measure is change in monocyte NFkB, which is a key mediator of FFA signaling. Other outcome measures include changes in TLR4 and SOCS3 (mediators of FFA signaling), FFA levels, plasma triglycerides, plasma total cholesterol, and peak forearm blood flow.

Preliminary data suggest that at least one of the medications positively affects free fatting acid signaling, triglyceride levels, and endothelial function. Based on relevant literature, this may be exenatide. However, as per design of the study, treatment arms will not be identified until study completion.

ABSTRACT

Retrospective Chart Review of Renal Transplant Patients and Hyperglycemia

Dewar, Emily   McGovern Medical School at UTHealth   *Class of 2019*

Sponsored by: Sridhar, Srikanth M.D., and Pivalizza, Evan, MD., Department of
                Anesthesiology
Supported by: Sridhar, Srikanth M.D., and Pivalizza, Evan, MD., Department of
                Anesthesiology

Hyperglycemia can be a clinical management problem following renal transplantation. Approximately 23% of patients receiving a kidney transplant have end-stage renal disease (ESRD) due to complications from diabetes mellitus, and are at an increased risk for postoperative hyperglycemia[3]. Hyperglycemia in transplant patients is complicated by the need for corticosteroid immunosuppressive therapy, which has been shown to contribute to the condition by increasing gluconeogenesis and insulin resistance. In addition, impaired or fluctuating renal function after transplant may make maintaining glycemic levels in these patients difficult[1]. The effects of hyperglycemia in kidney transplant patients can be immediate or long-term. Inpatient hyperglycemia immediately following transplant surgery has been shown to increase the incidence of wound infection, while long term inpatient hyperglycemia may affect both graft and patient outcomes[2]. Perioperative glycemic control has been shown in some studies to independently predict acute graft rejection in diabetic patients[4,5].

Due to the need to identify several groups, including patients with type 1 or 2 diabetes, those without diabetes, those with intraoperative hyperglycemia, and those receiving intraoperative insulin/dextrose treatment, groups of sufficient size would be required to afford statistical validity. Given that 80-100 kidney transplants are performed annually at Memorial Hermann Hospital, we retrospectively evaluated three years of transplants to meet the above criteria. Once groups were identified, appropriate non-parametric analysis was performed on group composition in Excel, with the anticipation of unequal group sizes. Correlation was sought between intraoperative glucose findings and postoperative hyperglycemia, with the use of appropriate tests to validate findings.

After analysis, we found that patients with diabetes mellitus (DM) had pre-operative hyperglycemia 27.1% of the time, while patients without DM (non-DM) only had pre-operative hyperglycemia 5% of the time. The mean pre-operative blood glucose level in DM patients was significantly higher than that of non-DM patients ($p=0.002$). In addition, DM patients had post-operative hyperglycemia 85.4% of the time, while non-DM patients only had post-operative hyperglycemia 46.5% of the time. The mean post-operate blood glucose level in DM patients was significantly higher than that of non-DM patients ($p=4.7E-6$). This suggests that DM is a direct risk factor for hyperglycemia in renal transplant patients. There was no significant difference in average post-operative blood glucose level in DM patients who received post-operative dextrose versus those who did not, nor was there a significant difference in post-operative blood glucose level between non-DM patients who received post-operative dextrose

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versus those who did not. In addition, there was no significant difference in average post-operative blood glucose level in DM patients who received post-operative insulin versus those who did not, nor was there a significant difference in post-operative blood glucose level in non-DM patients who received post-operative dextrose versus those who did not. This suggests that post-operative dextrose and insulin did not have a significant effect on post-operative blood glucose level. With further analysis, we hope to find more correlations between peri-operative factors and renal transplant patient post-operative blood glucose level.

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ABSTRACT

Trends of postpartum bilateral tubal ligation after initiation of immediate postpartum placement of long-acting reversible contraception

ChanAn Duong   *McGovern Medical School at UTHealth*   *Class of 2019*

Background: Half of pregnancies in the U.S. are unintended. Long-acting reversible contraceptives (LARC), such as intrauterine devices and the implant, and permanent sterilization with bilateral tubal ligation have similarly high rates of efficacy in preventing pregnancy, but differ in their associated costs and risks. Thus, understanding the trends of LARC uptake versus tubal ligation may minimize costs and aid in determining the best contraceptive strategies to lower unintended pregnancies. Our objective is to determine a correlation between the frequency of postpartum patients receiving tubal sterilization and the frequency of patients receiving immediate postpartum LARC placement, and if there is a significant preference for either method or other predictable trend.

Methods: A retrospective chart review was performed of all patients who received either tubal ligation or immediate postpartum LARC at delivery at a publicly funded hospital between September 2014 and December 2015 (n=3613). The rates of postpartum tubal ligations and LARCs per live birth were calculated. A student's t-test was used for comparison. Demographic information such as parity, ethnicity, age, and funding was collected.

Results: Although the number of patients choosing immediate postpartum LARC was significantly higher than the number choosing bilateral tubal ligation (31% v. 9.4%, $p < 0.001$), when given the option of either there is no significant reduction in the number of patients who choose tubal ligation overall.

Conclusion: Patients at highest risk of unintended pregnancy may benefit if immediate postpartum LARC is offered, as there is evidence of demand. Tubal sterilization remains a consistent choice for some women. More data is being collected to determine what factors influence a woman's decisions about postpartum LARC and tubal sterilization.

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ABSTRACT

Intestinal dendritic cells modulated by *Lactobacillus reuteri* DSM 17938 demonstrate immune functions on T cells in the mouse model of neonatal necrotizing enterocolitis

Devan Evans   *McGovern Medical School at UTHealth*   *Class of 2019*

Sponsored by: Marc J.Rhoads, MD, Yuying Liu, PhD, Department of Pediatrics Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, 2T35DK007676-22

Key Words: Necrotizing enterocolitis, Lactobacillus reuteri, Dendritic cell, prematurity

Background: Necrotizing enterocolitis (NEC) is the most common and severe gastrointestinal disorder affecting premature infants, with a mortality rate exceeding 20%. While prognosis of infants diagnosed with NEC has generally improved in the past few years, treatment options are still fairly limited. Recent evidence has strongly suggested that probiotic supplementation of preterm infants reduces the incidence and severity of NEC. More specifically, *Lactobacillus reuteri* (LR) is known to reduce destructive gastrointestinal pathology in mouse/ rat models. While the mechanism of action has not been identified, research has indicated human-derived LR17938 prevents NEC in newborn rats and mice.

Purpose: To characterize the nature of stimulation of dendritic cells with *Lactobacillus reuteri* as pro-inflammatory or anti-inflammatory as determined by cytokine production

Methods: WT mice were separated into 3 different groups:1) a dam-fed control group, 2) an MRS-fed group and 3) an LR-fed group. The MRS (a media used to facilitate anaerobic bacteria growth) group and LR group were exposed to hypoxic conditions and cold-stress. Spleens, the ileum of the small intestine and mesenteric lymph nodes were harvested on the seventh day of life. Dendritic cells were isolated using CD1lc+ and PanDC Microbeads. Naive T cells were isolated a CD4+ Isolation Kit. Dendritic cells were then either cultured alone or co-cultured with naive T-cells. Anti-CD3 antibody was added into co-cultures immediately after addition of T-cells. TGF-beta was also added to all co-cultures. All cultures were stimulated with PMA/ Ionomycin/ Brefeldin A. Quantity of cytokines were assessed by ELISA.

Results: CD11c+-isolated and Pan-DC isolated dendritic cells both showed increases in RANTES in response to Lipopolysaccharide (LPS) but no response to peptidoglycan (PGN), a common component of the bacterial cell wall, or LR. In addition, co-cultures produced from dam-fed mice showed increased IL-10 and IL-15. Finally, co-cultures produced from LR-fed NEC mice showed increased levels of IL-12p70, decreased levels

ABSTRACT

A lack of correlation between BMI and severity of lymphedema

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Sponsored by: Eva M. Sevick-Muraca, PhD, Melissa B. Aldrich, PhD, John C. Rasmussen, PhD, Center for Molecular Imaging at the Institute of Molecular Medicine

Supported by: 5T35DK007676-23

Key Words: Obesity, lymphedema, NIRFLI, indocyanine green

Background: Lymphedema (LE) is a prevalent medical condition where fluid collects in tissues due to abnormal fluid drainage from the lymphatics. This condition is largely seen in patients that undergo surgical removal of lymph nodes and radiation, but can also be congenital or arise from parasitic infections. LE typically presents as painful swelling of the affected area and pitting edema, where skin remains indented when pressed down. Over time, the affected area accumulates a deposition of adipose tissue. Recent LE rat-model studies have shown that certain cytokines pooled in these affected areas may be responsible for adipose tissue enlargement and proliferation. In the past it has been assumed that body-mass index (BMI) has provided a prognostic indication for LE and the treatment response. This retrospective analysis and continuing imaging study at the Center for Molecular Imaging sought to determine whether or not there was a correlation between BMI and disease severity seen in LE subjects.

Methods: From 2009-16, 30 subjects (n=10 controls, n=20 LE subjects) underwent near-infrared-fluorescence-lymphatic imaging (NIRFLI) of their arms using indocyanine green dye. A LE severity index scoring system was developed to account for multiple characteristics seen in abnormal lymphatic vasculature including: tortuosity, asymmetry, reverse flow, pumping capability, pulse frequency (pulse/min), and extravascular dye from dermal backflow. Paired t-tests were performed to determine if there was a difference in pulse frequencies between control and LE subjects. For the LE subjects, the unaffected arm was used as the paired control; only unilateral LE subjects were used in this analysis (n=18). A correlational analysis between LE severity and BMI including all subjects was used to determine if a relationship is present between the two factors.

Results: Contralateral pulse frequencies were found to be different in LE subjects (p-value<0.0006), but not in the control subjects (p-value<0.723), nor was a correlational relationship observed (p-value<0.127).

Conclusions: Although lymphedema involves the proliferation and enlargement of subcutaneous adipose tissue in the affected limbs, these results suggest that BMI is not a reliable factor in determining disease severity and treatment options available to patients.

ABSTRACT

Predictors of Recurrent Emergency Department Visits in Patients with Benign Biliary Disease

Braden Goldberg   *McGovern Medical School at UTHealth*   *Class of 2019*

Sponsored by: Lillian Kao, MD, MS, Department of General Surgery; Krislynn Mueck, MD, MPH, Department of General Surgery

Introduction: Benign biliary disease accounts for a disproportionate amount of recurrent emergency department (ED) visits and readmissions. It is unknown what factors present at ED consultation predict subsequent readmission with more severe disease such as acute cholecystitis, choledocholithiasis, gallstone pancreatitis, or ascending cholangitis. The aim of this study was to determine if there are patient or radiologic factors which predict recurrent ED visits, readmission with complicated biliary disease, and worse outcomes.

Methods: This was a retrospective cohort study of all patients presenting to a single safety-net hospital ED June 2014-2016 who received an abdominal ultrasound (US) for benign biliary disease. Demographic, admission, and outcome data were recorded. Univariate and logistic regression analyses were performed to identify factors associated with readmission with complicated biliary disease.

Results: Of 288 patients, 189 (66%) were admitted for surgery, and 99 (34%) were discharged. Of those discharged, 71 (72%) were not evaluated by a surgeon at index ED visit. There was no difference in age, gender, race/ethnicity, language, or ASA score between the groups. Discharged patients were more likely to have diabetes (10% vs 19%, p=0.03), heart disease (3% vs 10%, p=0.01), cancer (1% vs 6%, p=0.02), or chronic liver disease (3% vs 9%, p=0.02). 15 (15%) patients underwent elective outpatient cholecystectomy, and 15 (15%) were readmitted with complicated biliary disease. There was no difference in age, gender, race/ethnicity, language preference, ASA score, or comorbidities between the readmitted and non-readmitted groups. Readmitted patients had more prior ED visits (p=0.02) and hospitalizations (p<0.01). They were more likely to have an impacted stone (40% vs 0%, p=0.02), or a stone in the gallbladder neck (p<0.01). Rates of postoperative complications, reoperation, and conversion to open were similar between patients undergoing elective versus urgent surgery, while postoperative readmission rate was higher in the latter group (31% vs 7%, p=0.02).

Conclusion: Patients with comorbidities, with sonographic findings of complicated biliary disease, and without surgical consultation were more likely to be readmitted after discharge from the emergency department. Further study is necessary to determine what factors contributed to discharge and to assess whether admission on index presentation would have resulted in improved outcomes.

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ABSTRACT

Effects of nitrofen on pulmonary artery smooth muscle cells

Sushanth Gouni   *McGovern Medical School at UTHealth*   *Class of 2019*

Background: Congenital diaphragmatic hernia (CDH) is a disorder where incomplete diaphragmatic development results in herniation of abdominal organs into the thoracic cavity. Due to underlying pathophysiologic changes and/or abdominal visceral mass effect, pulmonary organogenesis is stifled, resulting in a combination of severe pulmonary hypoplasia and pulmonary artery hypertension (PAH). An animal model of CDH utilizing enteral administration of the herbicide Nitrofen to pregnant rats leads to diaphragmatic defects and pulmonary vascular remodeling in offspring analogous to human disease. An analysis of the vascular smooth muscle cells (VSMC) in the fetuses identified abnormal relaxation and contractile properties with evidence that the morphogenetic changes occurred before the diaphragm failed to close. We wish to understand the direct effects of nitrofen on the VSMC's by evaluating its effects on VSMC cell viability, mRNA expression profiles and protein expression.

Methods: Human Pulmonary Artery Smooth Muscle Cells (HPASMCs; Lonza) were seeded in 6-well plates, (100,000 cells/well) with or without nitrofen treatment (0, 0.1 and 0.25 mg/mL respectively) for twenty four hours. Cell viability was then assessed by propidium iodide staining and evaluated via Flow Cytometry. To determine the effects of Nitrofen on CDH specific and inflammatory related genes, HPASMCs were seeded under the same conditions as above, after which RNA was extracted and processed into cDNA. qPCR was performed to assess gene expression and results were analyzed. To evaluate the expression of pulmonary hypertension associated proteins, nitrofen treated cells were lysed and sonicated in RIPA buffer containing protease inhibitors. Twenty micrograms of protein were separated in each lane using 12 or 15% SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked in 5% milk, in 1X TBST, incubated with PAH associated proteins primary antibodies, and then probed with corresponding anti-rabbit or anti-mouse HP-labeled secondary antibodies.

Results: VSMC viability decreased proportionally to increasing nitrofen concentration with the highest dose inducing upto 50% cell death. Nitrofen treatment caused specific perturbation of molecular pathways by upregulating genes involved in the retinoic acid (FOS, TGIF1, and PBX1), cell migration (SLIT2, SNAI2, and ILF3), apoptosis (NFKB1, IL6, TNF, CASP1) and inflammatory pathways while downregulating genes involved in normal diaphragmatic development (GATA6, AKT1 and RUNX1). No changes in protein expression of PAH associated genes were observed with the notable exception of downregulation of MCP-1 across VSCMCs, HPAEC and fetal rodent PA. Future studies will aim to determine the role of MCP-1 in CDH and examine the VSCMC response to extracellular vesicles.

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ABSTRACT

Neuropilin-1 blockade enhances anti-angiogenic therapy in ovarian cancer

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Sponsored by: Dr. Anil K. Sood, M.D., Department of Gynecological Oncology, M.D. Anderson

Supported by: Dr. David Volk, Ph.D., Department of Nanomedicine and Biomedical Engineering

Key Words: Neuropilin-1, Anti-VEGF therapy, PDGF-BB, ovarian cancer

Angiogenesis, the process of creating new blood vessels, is known to be critical for tumor growth and metastasis in ovarian cancer. Anti-angiogenic drug therapies have mainly targeted vascular endothelial growth factor (VEGF) and its tyrosine kinase receptors VEGFR1 and VEGFR2. While these therapies can improve the prognosis for some patients, high toxicity and resistance to the drugs has caused them to be less effective than desired. As the 5 year survival rate for ovarian cancer is only 45%, we must develop more effective treatments. Neuropilin-1 (NRP1) is a transmembrane receptor found on endothelial and tumor cells that interacts with $VEGF_{165}$ and enhances its binding to VEGFR2. NRP1 is also expressed on vascular smooth muscle cells and is involved in their migration when stimulated by platelet derived growth factor BB (PDGF BB). This study seeks to determine if targeting NRP1 can augment anti-VEGF treatment to decrease angiogenesis, tumor growth and metastasis in ovarian cancer. RT- PCR and Western blot were used to determine that HeyA8 ovarian cancer cells highly express NRP1 and PDGF BB while A2780 ovarian cancer cells do no express NRP1 or PDGF BB. NRP1 was also expressed on epithelial and pericyte-like cell lines. An *in vivo* experiment was conducted by injecting nude mice with NRP1-null A2780ip2-LC cells and treating them with PBS (control), anti-VEGF antibody, anti-NRP1 Ab, or anti-VEGF and anti-NRP1 combined. This experiment showed promising results with the combination treatment decreasing tumor growth by 96% compared to control mice and 61% compared to VEGF therapy alone. Immunohistochemistry was performed for CD31 and ki67 to determine the microvessel density (MVD) and proliferation. Anti-NRP1 treatment led to a 71% decrease in MVD while combination treatent led to a 67% decrease in MVD and a 25% decrease in tumor cell proliferation compared to control groups. A TUNEL assay showed that anti-NRP1 treatment did not affect apoptosis. A sandwhich ELISA determined that PDGF-BB binds to NRP1 at 1 nM concentration *in vitro*. Anti-NRP1 treatment decreased pericyte-like cell migration by 63% in the presence of PDGF BB and decreased SKOV3 tumor cell migration by 46% in the presence of VEGF but did not have any effect on SKOV3 invasion. SiRNA transfection was used to knockdown NRP1 (85% at 48 hours) and PDGF BB (50% at 48 hours) and proliferation was measured in the presence and absence of recombinant PDGF BB using flow cytometric analysis. siNRP1 transfection caused a 20% decrease in HeyA8 proliferation but the presence of recombinant PDGF-BB did not affect proliferation. Cell cycle analysis performed in the presence and absence of recombinant PDGF-BB and after transfection with siPDGF-BB and siNRP1 confirmed the results from the proliferation experiment. Ongoing experiments include migration and invasion assays after siPDGF-BB and siNRP1 transfection and an *in vivo* experiment treating mice with siNRP1 and anti-VEGF antibody. These results

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demonstrate that targeting NRP1 can lead to decreased tumor growth, proliferation and migration and that these effects may be mediated by PDGF BB. By administering anti-NRP1 treatment alongside anti-VEGF treatment, we could increase the efficacy of anti-VEGF treatment and possibly reduce toxicity by lowering the necessary dosage.

ABSTRACT

Using Biomechanical Force to Improve Bone Marrow Mesenchymal Stromal Cell Engraftment Potential and Immunomodulatory Function

Liang Gu   *McGovern Medical School at UTHealth*   *Class of 2019*

Sponsored by: Pamela Wenzel, PhD, Department of Pediatric Surgery, the Brown Foundation Institute of Molecular Medicine

Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, 2T35 DK007676-23

Key Words: Stem cell therapy, mesenchymal stromal cell, wall shear stress, bone marrow, engraftment, immune regulation, hematopoiesis, traumatic brain injury

BACKGROUND: Traumatic brain injury (TBI) both causes local neuronal inflammation and disturbs bone marrow homeostasis. A family of stem cells collectively referred to as mesenchymal stromal cells (MSCs) have shown tremendous promise to regulate both inflammation and maintain the bone marrow niche. Furthermore, MSCs are believed to mobilize from the bone marrow to travel to sites of injury, exposing them to the fluid flow of the vasculature. While there are ongoing phase 1 and phase 2 clinical trials using MSCs to treat brain or spinal cord injury, activating MSCs with biomechanical force is a relatively new technology.

PRIMARY AIM: To establish the beneficial effects of licensing MSCs via wall-shear stress (WSS) in terms of their immunomodulatory function and engraftment potential *in vivo*.

METHODS: Bone marrow MSCs were derived from human donors and cultured in MEM-α, fetal bovine serum, penicillin, streptomycin, and L-glutamine. MSCs were then seeded on microfluidic platforms and shear stress was then applied via a peristaltic pump or a programmable syringe pump at 15 dyne/cm^2. TBI model was generated by subjecting rats to controlled cortical impact, and MSCs administered via tail vein injection 24 hours post-injury. Rats were divided into four groups, sham (no injury), CCI (injury, no MSC injection), static (non-sheared MSC injection), and WSS (wall shear stress activated MSC injection). Brain, lung, spleen, and tibia sections were collected 24 hours post-injection. Brain sections were stained for pro-inflammatory vs anti-inflammatory microglia. Tibia sections were stained for CD105 (MSC marker), COX2 activity (inflammation signal), apoptosis, and the presence of human nuclear antigen (engraftment signal). Spleen and lung tissue were stained for human nuclear antigen.

RESULTS/DISCUSSION: CD105 signal in the bone marrow was tremendously decreased following TBI. When compared to static and CCI, WSS significantly increased the ratio of anti-inflammatory to pro-inflammatory microglia in the brain, and overall decreased levels of COX2 in the marrow. This suggests that WSS improves MSC treatment efficacy. The human nuclear antigen stain is still ongoing and has not yielded conclusive results. The MSCs may be lodged in the lungs following injection, and carrying out their systemic function via paracrine modulation of lung macrophages. Future work will involve PCR to screen for the presence of human MSCs in rat bone marrow and lung tissue following injection.

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ABSTRACT

The Effects of Gene Targeting on PCSK9 Expression

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Sponsored by: Ba-Bie Teng, PhD, Center for Human Genetics,
                 The Brown Foundation Institute of Molecular Medicine(IMM)
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases,
                 2T35DK007676-23
Key Words: PCSK9, Autophagy, Gene Targeting, CRISPR-Cas9

Background: Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) is involved with the removal of the LDLR, which mitigates the lowering of LDL in the blood. As a result, increased levels of PCSK9 contribute to the development of plaque and inflammation in the vasculature, leading to atherosclerotic lesions. Interestingly, autophagy dysregulation in the cell can compound inflammation. This dysregulation is particularly dangerous in the liver, potentially contributing to hepatocellular carcinoma. Recently, there has been a purported link between PCSK9, apoptosis, and autophagy in vascular smooth muscle cells via mitochondrial stress, supporting the notion that factors involved in autophagy may be affected by the expression of PCSK9. Therefore, the aim of this study was to explore the effect of PCSK9 expression and cell biological function when different regions of the PCSK9 gene were targeted in cardiac and liver cells. This in turn could lead to further understanding of PCSK9 effects, and resultant targeted measures could be taken to address the issues of inflammation, autophagy, and apoptosis in order to better understand and treat disease pathology via PCSK9 regulation.

Methods: DNA sequences homologous for specific regions of the PCSK9 gene were designed to attain eight annealed genetic constructs, which were inserted into the px330 plasmid expressing the CRISPR-Cas9 system via BbsI restriction enzyme. Proper insertion of the constructs into the vector was verified through sequencing. The plasmid was transformed into E.Coli (mycoplasma-free) for replication. The correct sequence constructs were transfected into HepG2 (human liver cancer cell line) and Mouse Cardiac Endothelial Cells (MCEC). The Green Fluorescent Protein (GFP) construct was used to estimate the efficiency of the transfection method. At 48 hours post-transfection, cells were collected and DNA was extracted from the collected cells. PCR was conducted to amplify the DNA by using primers that flanked the edited site. To identify whether the edited sequences embedded into the cell genome, we used T7 Endonuclease I (T7E1) to elucidate mismatch base pairing. Another set of transfection was carried out to determine the biological effects that the insert had on PCSK9 gene expression. We collected cell media to measure levels of secreted PCSK9 protein, and we extracted RNA to analyze the gene expression levels using quantitative PCR (qPCR). cDNA was generated from the total RNA via reverse transcription and mRNA concentrations were determined using real-time PCR. The Western blot technique was used to determine any effect on secretion of PCSK9 due to the genetic constructs.

Results: There was a significant decrease in PCSK9 expression with two of the mouse genetic constructs compared to that of the controls (mg2 vs. GFP/px330 controls, p=0.0018; mg4 vs. GFP/px330 controls, p=<0.0001) and a significant increase in PCSK9 with one human genetic construct compared to that of the controls (hg5 vs. GFP/px330 controls, p=0.0390). However, it appeared that none of the constructs decreased PCSK9 protein levels by Western blot analysis.

Conclusion: Further work will have to be conducted to create better genetic constructs to target regions that will have a greater effect on the PCSK9 regulation. Furthermore, utilization of another CRIPSR method that will increase expression of the gene of interest will allow for better observation of effects, due to low transfection rate in the cell lines used that could otherwise offset any observable decrease in PSCK9 expression. With a better approach, improved evaluation of intracellular and extracellular effects of PCSK9 regulation can be achieved.

ABSTRACT

The Association of Medications on Disease Activity, Functional Impairment in Chinese and American Patients with Ankylosing Spondylitis (AS)

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Sponsored by: Dr. John D Reveille, Department of Rheumatology

Supported by: Dr. John D Reveille

Key Words: Ankylosing Spondylitis, Medications, Chinese, Shenzhen

Objective: To compare clinical, disease severity and medication usage parameters in ankylosing spondylitis (AS) in Chinese and American patients.

Methods: We assessed 450 patients followed since 2005 at Peking Union University Hospital, Shenzhen and compared with 961 AS patients (38 Blacks, 770 Whites, 67 Latinos, 65 Asian and 17 mixed race) enrolled in a longitudinal outcome study for parameters associated with functional impairment, disease activity and medication utilization. For certain clinical features where data form the Shenzhen cohort were still being collected, we used variables from two other AS cohorts from Shanghai whose medication utilization was similar. Univariable cross-sectional associations of clinical characteristics were conducted using Chi-square tests for categorical variables and Student's t-tests for continuous variables or their non-parametric counterparts when necessary.

Results: Shenzhen Chinese AS patients were younger than the Americans at baseline visit 28.3 versus 43.1 years ($p<0.0001$) , were more likely to be male (81.1% versus 73.6%, p=0.0006), and were slightly more likely to be HLA-B27 positive (92.4% versus 87.3%, p=0.009). Chinese AS patients had lesser functional impairment (Bath Ankylosing Spondylitis Functional Index) (median 8.0 vs. 27.7 in the American patients; $p<0.0001$); similar subjective disease activity (Bath Ankylosing Spondylitis Disease Activity Index), (median 2.3 vs 3.9 in the Americans; p<n.s.), lower frequencies of abnormal erythrocyte sedimentation rate (35.5% versus 49.9%, $p<0.0001$) and higher frequencies of elevated C-reactive protein (55.3% versus 38.0%, $p<0.0001$) compared to Americans. Shenzhen Chinese AS patients were more likely to be using nonsteroidal anti-inflammatory agents (NSAIDs), disease modifying anti-rheumatic drugs (DMARDS) and corticosteroids and less likely to be using anti-TNF agents, opioids, anti-hypertensive and anti-depressant drugs and statins than the American AS patients ($p<0.0001$ for all comparisons)

Conclusions: These data suggest important differences in clinical features and medication utilization between Chinese and American AS patients. Of particular interest is the lower functional impairment in the Chinese and similar disease activity between the Chinese and American cohorts despite lack of biologic therapy in the Chinese. Possible reasons for this (shorter disease duration, younger age, the use of different medications in China-i.e. thalidomide and *Tripterygium wilfordii*, etc) are being examined.

ABSTRACT

Effective Timing of Placement of Percutaneous Endoscopic Gastrostomy in Patients with Traumatic Brain Injury

William S. Jones   *McGovern Medical School at UTHealth*   *Class of 2019*

Sponsored by: George W. Williams, II, MD, FCCP
Supported by: Carin A. Hagberg, MD; McGovern Medical School Dept. of Anesthesiology
Key Words: TBI, Percutaneous Endoscopic Gastrostomy, Enteral Nutrition

Background: Previous studies have shown percutaneous endoscopic gastrostomy (PEG) placement may be detrimental to patient health, leading to increased in-hospital mortality. However, our previous work indicates PEG placement and enteral nutrition support in traumatic brain injury (TBI) patients may lead to an overall decrease in mortality. This study aims to evaluate the time at which PEG placement in TBI patients leads to the best overall outcome and lowest in-hospital mortality rate.

Significance: Early enteral national support has been shown to improve patient morbidity and mortality in TBI patients. Early enteral support with PEG is preferred in some cases, but has yet to be confirmed as the best option in provided adequate nutrition in TBI patients presenting with dysphagia requiring enteral nutrition.

Hypothesis: PEG placement within the first 7 days of hospitalization of TBI patients will lead to an overall decrease of in-hospital mortality in the patient population.

Experimental Design: The US Nationwide Inpatient Sample (NIS) was used to analyze data from all hospitalizations in 2008 with ICD9 diagnostic and procedure codes to identify patients with TBI who received PEG. Bivariate and multivariate logistic regression analyses were performed using demographic and clinical variables to identify in-hospital mortality of early PEG (0-6 days post-arrival), standard PEG (7-14 days), and late PEG (>14 days) patient groups.

Results/Data: Mean length of stay (LOS) was 27 days for the early PEG group, 28 days for the standard PEG group, and 39 days for the late PEG group. A 5.74% in-hospital mortality was shown in the early PEG group; with subgroups of 3.69% mortality in 8-14 days, 0.82% mortality in 15-30 days, and 1.23% mortality after 30 days. Additionally, a 7.39% in-hospital mortality was shown in the standard PEG group; with subgroups of 1.30% mortality in 8-14 days, 3.70% mortality in 15-30 days, and 2.39% mortality after 30 days. Finally, a 7.68% in-hospital mortality was shown in the late PEG group; with subgroups of 0.66% mortality in 8-14 days, 2.41% mortality in 15-30 days, and 4.61% mortality after 30 days. No patients in any of the populations died within 7 days of admission.

Conclusions: Although there is no statistically significant difference in the mean LOS between the early and standard PEG groups, there is a large increase in LOS of the late PEG group, demonstrating the need for early consideration for enteral nutrition support in TBI patients. However, overall in-hospital mortality is lowest in the early PEG group, showing early PEG placement can reduce in-hospital mortality of TBI patients.

Medical Student

ABSTRACT

The Impact of Racial/Ethnic Disparities on Survival for Children and Young Adults with Chest Wall Sarcoma: a Population-Based Study

Michael Joseph   *McGovern Medical School at UTHealth*   *Class of 2019*

Purpose: To determine whether there are racial/ethnic disparities in disease presentation and survival outcomes among children and young adults with chest wall sarcomas.

Methods: The Surveillance, Epidemiology and End Results (SEER) database from 1973-2013 was analyzed for all patients less than 24 years old and diagnosed with chest wall based on primary tumor histology and primary tumor site. Tumor histology was categorized as skeletal for histologic subtypes arising from bone and extra-skeletal for histologic subtypes arising from soft tissues. Tumor sites included thorax, clavicle, sternum and ribs. We performed multivariate logistic regression to investigate the association of race/ethnicity with advanced stage of disease at presentation and likelihood of undergoing surgical resection after adjusting for age, sex, treatment, tumor size and sarcoma type. Overall survival (OS) was evaluated using Cox regression to estimate hazard ratios with 95% confidence intervals. All statistics were calculated with SPSS Statistics Version 23.

Results: A total of 598 patients were identified and included 363 non-Hispanic whites (61%), 129 Hispanics (22%), 57 non-Hispanic blacks (10%), and 49 other race/ethnicity (8%). The mean age at diagnosis was 14 ± 6.6 years. Most patients presented with advanced stage disease defined as regional or distant disease (393, 66%). Race/ethnicity was not associated with stage of disease at presentation. However, patients with advanced stage disease were more likely to have a skeletal sarcoma (OR= 2.55, 95% CI: 1.71-3.80), tumor size ≥8 cm (OR= 3.66, 95% CI: 2.35-5.71) and undergone radiation therapy (OR= 1.80, 95% CI: 1.22-2.67). Those who underwent surgical resection were less likely to present with advanced disease (OR=0.34; 95% CI: 0.20-0.59). The 5- and 10-year OS for the entire cohort were 62% and 58%, respectively. Non-Hispanic blacks had a worse 5-year and 10-year OS compared to Non-Hispanic whites (54% versus 65%, 48% versus 60%, respectively). In the univariate analysis, non-Hispanic Blacks were 63% more likely to die than non-Hispanic whites (95% CI 1.07-2.49); however, this association was mitigated after controlling for age at diagnosis, sex, tumor type, tumor size, disease stage, surgical resection and radiation treatment in the multivariate analysis. In the multivariate analysis, predictors of worse OS included older age at diagnosis (HR 1.05, 95% CI 1.03-1.07), tumor size $\geq$ 8cm (HR 2.15, 95% CI 1.50-3.10), regional disease (HR 1.79, 95% CI 1.19-2.69), distant disease (HR 3.99, 95% CI 2.67-5.96), and failure to undergo surgical resection (HR 2.08, 95% CI 1.55-2.81). Most patients (79%) underwent surgical resection. However, non-Hispanic blacks were less likely than non-Hispanic whites to undergo surgical resection even after controlling for sex, age at diagnosis, tumor type, tumor size, disease stage, and radiation therapy (OR 0.49, 95% CI 0.25-0.97).

Conclusions: Non-Hispanic black children and young adults with chest wall sarcomas have decreased overall survival compared to non-Hispanic whites. In addition, Non-Hispanic

blacks are less likely to undergo surgical resection of their tumors which may contribute to the survival disparities identified in this study.

ABSTRACT

Adaptive behavioral dysfunction is associated with an increased risk for maladaptive behavioral and emotional function in toddlers with Tuberous Sclerosis Complex

Martha Kellems   *McGovern Medical School at UTHealth*   *Class of 2019*

Adaptive behavior deficits are associated with suboptimal developmental outcomes. Behavioral problems (e.g., inattention, social withdrawal), can significantly undermine adaptive functioning—which in turn undermines long-term educational, social, and occupational adjustment. Although children with TSC are at high risk for behavioral and emotional problems, and their adaptive function has been noted to decline over time, little is known about the relationship between adaptive function and psychopathology in children with TSC—especially very young children. Objective: The goal of this project was to determine if lower adaptive function is associated with a higher levels of behavioral and emotional problems in toddlers with TSC. Method: Sample consisted of 66 toddlers (35 boys) participating in the TSC Autism Center of Excellence Research Network (TACERN). Mean age was 24.2 months, mean MSEL Composite was 79.6, and 14 had been diagnosed with Autism Spectrum Disorder (ASD). Adaptive function was assessed using the Vineland Adaptive Behavior Scale (VABS-II). Behavioral/emotional problems were assessed using the Child Behavior Checklist (CBCL, ages 1.5-5 years). Parents served as the informants for both instruments. Correlational analyses assessed the relationship between adaptive function and behavioral/emotional function. Results: Toddlers with TSC with lower levels of adaptive function had significantly higher levels of a behavioral and emotional concerns. Weaker adaptive function was associated with higher levels of internalizing problems (p<.001) and externalizing problems (p<.001). Specific areas of concern included affective problems (p=.002), pervasive developmental problems (p<.001), emotional reactivity (p=.022), social withdrawal (p<.001), and attention problems (p=.005). When toddlers with ASD were removed from the sample and correlations re-run, weaker adaptive function was still associated with pervasive developmental problems (p=.001) and social withdrawal (p<.001), suggesting that these findings were not driven by toddlers with ASD. However, it is possible that ASD had not yet been diagnosed in some of these 24-month olds. Conclusion: Even as early as 24 months old, patients with TSC who have weak practical living skills are at high risk for behavioral concerns, underscoring the need for early behavioral intervention for these toddlers in order to optimize developmental outcomes.

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ABSTRACT

McGrath MAC Video Laryngoscope – To View or Not to View?

James Klugh   *McGovern Medical School at UTHealth*   *Class of 2019*

Sponsored by: Carin A. Hagberg, MD, Department of Anesthesiology
Supported by: Carin A. Hagberg, MD, Department of Anesthesiology; The University of
                Texas Health Science Center at Houston McGovern School of Medicine –
                Dean's Office
Key Words: Direct versus Indirect Laryngoscopy, Difficult Airway, McGrath
                Laryngoscope

Introduction: An anesthesiologist's ability to perform laryngoscopy and intubation are essential skills that are necessary when securing an airway. Visualization of the airway is achieved utilizing a tool called a laryngoscope. Traditionally, the laryngoscope allows the anesthesiologist to visualize and possibly manipulate the airway anatomy in order to provide a direct view of the vocal cords through the mouth. Certain anatomical features such as a small mouth opening or a short, thick neck have been shown to increase the difficulty of direct laryngoscopy, therefore leading to further development of the laryngoscope. In this study, we investigated the effectiveness of performing indirect (video) laryngoscopy versus direct (traditional) laryngoscopy on patients with predicted difficult airways. Video laryngoscopes allow for an indirect visualization of laryngeal structures through the added features of a camera and digital monitor or screen.

Method: After IRB approval and informed patient consent, 100 adult patients, > 18 years of age, requiring general anesthesia for an elective surgery were enrolled into this study. Prior to enrollment, patients were assessed for inclusion and considerable difficulty of their airway based on certain mesomorphic features. Only patients with two or more of the following difficult airway predictors were included in this study: Neck Circumference > 40 cm for females or > 43 cm for males, Interincisor distance < 4 cm, Thyromental distance < 6 cm, Sternomental distance < 12 cm, Mallampatti Class III or IV, and a ratio of the patient's height to thyromental distance ≥ 23.5. The McGrath® MAC video laryngoscope was the designated instrument used for performing both direct and indirect laryngoscopy. If a Cormack-Lehane (C-L) grade view of 1-3 was obtained, the anesthesiologist then proceeded with the placement of an endotracheal tube (ETT) using the direct view. If a C-L grade view of 4 was initially obtained, the ETT was placed with the indirect view. The direct and indirect C-L grade view, the time required to achieve a C-L grade view and ETT placement, along with any necessary lifting force, and subjective assessments, such as *Ease of Laryngoscopy*, were all recorded.

Results: The data showed an overall 94% first attempt success rate. When performing indirect laryngoscopy, the McGrath® MAC video laryngoscope demonstrated either an equal or improved C-L grade view in 93% of cases when compared to direct laryngoscopy. However, overall intubation time utilizing the direct view was 27.2 seconds opposed to 34.0 seconds utilizing the indirect view. An initial C-L grade view of 4 was the primary reason for performing indirect laryngoscopy during the first attempt. Also, the *Ease of Laryngoscopy* was described as very easy or easy 54% of the time, and the *Ease of ETT Placement* was described as very easy or easy 60% of the time.

Discussion: Our data further supports the ability and importance of video laryngoscopy in providing a better C-L grade view and an *easier placement of the ETT* when securing an airway. In patients with predicted difficult airways, indirect laryngoscopy should be considered as a

viable alternative that may possibly increase the likelihood of successful first attempt endotracheal intubation versus traditional methods.

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ABSTRACT

Ultrasound in Extraglottic Airway Device Placement

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Sponsored by: Davide Cattano, MD, PhD, Department of Anesthesiology

Supported by: Davide Cattano, MD, PhD, Department of Anesthesiology; The University of Texas Medical School at Houston- Office of the Dean

Key Words: Airway, EAD, Ultrasound

Background: Ultrasound is a versatile way of visualizing internal anatomy noninvasively. In anesthesia, ultrasound has been growing in use for procedures such as peripheral nerve blocks and for anatomical landmark recognition in airway assessment. In this regard, placement of an extraglottic device might modify airway anatomy and assuming ultrasound will be able to detect the modification of the anatomy, we aim to utilize ultrasound to assess internal landmarks before and after extraglottic device placement and to correlate with external anatomy landmarks.

Methods: A total of 6 healthy, non-obese, adult patients (5 male and 1 female) were recruited. External airway landmarks (thyroid width- TW, thyroid height- TH, hyoid-mental distance- HMD, hyoid-thyroid distance- HTD, thyroid-cricoid distance- TCD, sternomental distance- SMD, and thyromental distance- TMD) were gently palpated and measured (in cm) with a disposable tape measure. Then, the same neck landmarks were assessed using a 12MHz linear transducer (Sonosite M-Turbo). Pictures were taken before and after extraglottic airway device (laryngeal mask airway- LMA) placement. Data was assessed utilizing ImageJ software and the ultrasound machine program's caliper as a standard.

Results: HMD, TCD, TH, and TW in ultrasound were found to be significantly ($p < 0.05$) overestimated using a tape measure with TW being the most overestimated. Using a linear regression a patient's predicted internal neck measurement is equal to -0.4 + 0.9 (external neck measurement) cm when external anatomy is measured in cm. The internal neck measurement is increased by 0.9 cm for each cm of external neck measurement. HMD, TMD, SMD, and TCD significantly decreased ($p < 0.05$) after placement of an LMA. Although not significant, TW was the only landmark to consistently show an increase in size after placement of an LMA ($p = 0.067$).

Conclusion: This preliminary study has confirmed the overestimation of external anatomy using a tape measure compared to internal anatomy using ultrasound. Also LMA placement has been shown to alter the anatomy of the neck. Such alteration could be explained by the LMA shortening the linear distance between vertical neck landmarks such as HMD as well as the LMA distorting the thyroid cartilage. Based on this preliminary study, a correlation index could help correct the overestimation of internal anatomy and be used in EAD sizing.

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ABSTRACT

Evaluation of AirSPACE™ Technology in a Clinical Setting

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Supported by: Carin A. Hagberg, M.D., Department of Anesthesiology; McGovern Medical
                School at the University of Texas Health Science Center at Houston – Dean's
                Office
Key Words: Anesthesia device, Airway management, Laryngoscopy, Intubation

Background: The AirSPACE™ Device is a positioning device used to enhance airway management during surgeries. The device assists in adjustable head and neck positioning, which involves head elevation, neck flexion, and atlanto-occipital extension. In current clinical practice, the patient is placed in sniffing position prior to laryngoscopy and intubation by layering blankets and pillows underneath the patient's head. However, the use of blankets and pillows may produce inconsistent positioning and may be burdensome for intraoperative changes in the positioning of the patient. The AirSPACE™ Device facilitates patient positioning by mechanically placing the patient in sniffing position and improving the visualization of laryngeal structures during laryngoscopy, which could lead to an increase in facilitating successful intubations, thereby decreasing the number of complications due to multiple unsuccessful intubation attempts.

Objective: To evaluate the performance of the AirSPACE™ Device, as measured by the average grade of the glottis view (Modified Cormack-Lehane (C-L) classification) and/or improvement in grade III or IV glottis view after repositioning with the device, and ease of use, as determined by the provider performing the laryngoscopy and endotracheal intubation.

Methods: The study was an observational prospective descriptive study. Thirty adult (>18 y/o) patients with American Society of Anesthesiology (ASA) Physical Status Classifications I-III scheduled for elective surgery requiring general anesthesia and tracheal intubation were included in the study. Study practitioners included attending anesthesiologists, residents, anesthesiologist assistants, and medical students. To assess the feasibility and performance of the device, we collected measurements including time required for AirSPACE™ Device setup, time required for patient positioning (if positioning was needed), minimum oxygen saturation (SpO_2) during the intubation procedure, number of attempts required to intubate, initial C-L grade view, C-L grade view after repositioning, and time taken to obtain an optimal view and CO_2 detection.

Results: The average time required to set up the AirSPACE™ Device was 257.16 ± 108.71 sec. Of the 30 patients enrolled in the study, 10 patients needed repositioning during laryngoscopy and intubation; the average repositioning time was 22.84 ± 11.44 sec. The average minimum SpO_2 during intubation was 99.73 ± 0.64%. Six of the patients required more than one intubation attempt. The initial C-L grade view and frequency for the patients were (1, 50%), (2a, 16.67%), (2b, 23.33%), (3, 6.67%), and (4, 3.33%). Of the patients who needed repositioning, 80% of the cases had an improvement in the C-L view, 20% of the cases had no improvement, and none of

the cases showed a decrease in the view. The times required to obtain an optimal view and for CO_2 detection were 19.69 ± 13.80 sec and 42.60 ± 21.95 sec, respectively.

Conclusion: In this study, the AirSPACE™ Device initially positioned the patients in a desirable manner that further repositioning was not required in 20 out of the 30 patients (66.67%). We understand that under common clinical practices, most patients are not positioned in the neutral position when the anesthesia provider is performing laryngoscopy. However, with the AirSPACE™ Device, patients were initially placed in neutral position during laryngoscopy until further movement was deemed necessary. Nevertheless, the AirSPACE™ Device provides real-time airway manipulation during laryngoscopy just from a push of a button when repositioning is needed, allowing for a noticeable improvement in C-L grade view.

ABSTRACT

AAST multi-center prospective, observational study on immune dysfunction in subjects who present with traumatic brain injury (TBI) and receive beta adrenergic receptor blockers

Samuel Leonard   *McGovern Medical School at UTHealth*   *Class of 2019*

Sponsored by: Bryan A. Cotton, MD, MPH, Department of Surgery, CeTIR

Supported by: Bryan A. Cotton, MD, MPH, Department of Surgery, CeTIR

Key Words: Surgery, trauma, traumatic brain injury, beta-blockers

Background: Head injury remains the leading cause of death among trauma patients. One of the causes of death in these patients is believed to be non-neurologic organ dysfunction. This is related to sympathetic hyperactivity following head injury, often referred to as paroxysmal sympathetic storm (PSS). PSS after traumatic brain injury (TBI) is associated with a hypermetabolic state and increased cerebral blood volume leading to increased intracranial pressure. While previous data have shown significant decreases in mortality with beta blocker in TBI patients, blood product resuscitation of trauma patients and the care of TBI has radically changed in the last decade.

Hypothesis: We hypothesized that despite advances in care of the head injured patient and the evolution of hemostatic resuscitation and, receipt of beta-blockers in TBI patients would be associated with improved outcomes.

Methods: Institutional Review Board approval was obtained. Prospective review of all blunt TBI patients with CT evidence of injury requiring ICU admission, >17 years old, and admitted between 01/01/2016 and 07/31/2016. We excluded transfer patients with an injury time >12 hours prior to MHH admission. We focused on in-hospital mortality with secondary analysis of discharge disposition and complications. A logistic regression model was used to control for patient-level characteristics and beta-blocker use on both mortality and Glasgow Outcome Score (GOS). GOS 1=death, 2=vegetative, 3=lower severe disability, 4=upper severe disability, 5=lower moderate disability, 6=upper moderate disability, 7=lower good recovery, 8=upper good recovery

Results: Previous investigators have demonstrated an improvement in survival among TBI patients receiving beta-blockers during their hospital stay. This appears to mediated through damping of the sympathetic hyperactivity following head injury. However, dramatic changes in TBI care and trauma resuscitation have occurred that may diminish the impact of beta-blocker exposure following TBI. This study demonstrates that beta-blocker administration during acute hospitalization may still be associated with a reduction in in-patient mortality (p=0.66). Possible sources of error are single center, non-randomized, and small sample size

with type II error possibility. In addition, beta-blocker exposure in these patients may be associated with a higher functional status on discharge, dramatically impacting their discharge disposition (rehabilitation center versus skilled-nursing facility) and, thereby, their long-term recovery (p=.069).

Table 4. Multivariate logistic regression for discharge GOS of 4 or greater

	OR	95% C.I.	p-value
Age in years	0.97	0.947-0.995	0.021
Injury severity score	0.88	0.835-0.934	<0.001
Treatment intensity	0.40	0.144-1.118	0.081
Mechanism of injury, fall	1.42	0.482-4.189	0.523
Arrival SBP, mmHg	0.99	0.984-1.013	0.865
Beta-blocker exposure	2.48	0.930-6.644	0.069

GOS: Glasgow outcome score; OR: odds ratio; 95% C.I.: 95% confidence interval; SBP: systolic blood pressure

Table 4. Multivariate logistic regression for death

	OR	95% C.I.	p-value
Age in years	1.02	1.004-1.056	0.021
Injury severity score	1.13	1.070-1.196	<0.001
Treatment intensity	2.50	0.900-6.957	0.078
Mechanism of injury, fall	0.70	0.238-2.079	0.526
Arrival SBP, mmHg	1.00	0.965-1.015	0.868
Beta-blocker exposure	0.39	0.149-1.062	0.066

OR: odds ratio; 95% C.I.: 95% confidence interval; SBP: systolic blood pressure

ABSTRACT

The Glycolytic Intermediate Glucose 6-Phosphate as Regulator of Cardiac Growth: A Study in Differentiated iPSCs

Joseph R Martin   *McGovern Medical School at UTHealth*   *Class of 2019*

Introduction: Metabolic dysregulation is the central factor in pathological conditions ranging from type 2 diabetes and obesity to cardiomyopathies. The mechanistic Target of Rapamycin Complex 1 (mTORC1) constitutes a key control point in these metabolic cascades. It functions as a kinase downstream of insulin and other surface receptors which link extra and intracellular signals to growth and proliferation. In heart muscle, mTORC1 promotes left ventricular hypertrophy. Studies from the lab have shown that Glucose 6-Phosphate (G6P) is required for mTORC1 activation in heart muscle in response to both insulin and to an increase in hemodynamic stress. However, it remains unclear if the observed accumulation of G6P in cardiac tissue is the cause of, or only a "bystander" in generalized metabolic remodeling and the progression of hypertrophy. Demonstration of a causative role could provide a new therapeutic target or serve as a predictive marker of pathology in heart failure, type two diabetes, and beyond.

Hypothesis: G6P is an essential metabolic signal for mTORC1 activation and cardiac growth.

Methods: I interrogated the mTORC1 pathway in two induced pluripotent stem cell (iPSC) derived cardiomyocyte lines. Differentiation of iPSCs was pursued with a bilaminar Matrigel protocol using growth factor induction (Activin A, BMP4, and bFGF) until spontaneously contracting cardiomyocytes were observed. Cells were starved of glucose for maturation and then hypertrophied using the inotrope, isoproterenol. Glucose, or the glucose analogues, 2-deoxyglucose (2DG) and 3-O-methylglucose (3OMG), were then applied to the cell cultures. The following assays were used: immunofluorescence to measure cell area, Diaphorase/Resazurin fluorescence for G6P concentration, western blotting for mTORC1 and its phosphorylation of downstream targets, and proximity ligation to assess mTOR/Hexokinase association.

Results: iPSC conversion to cardiomyocytes was verified via spontaneous physiological beating as well as α-actinin scaffold organization. Cardiomyocyte hypertrophic response to isoproterenol concentrations followed a dose response linear relationship at 20μM ($R^2 = 0.99$). Diaphorase/Resazurin amplification of G6P levels provided a highly sensitive assay, limit of detection 1nM $R^2 = 0.996$, for G6P. In the experimental trials, G6P accumulates in response to

isoproterenol treatment. Glucose analogue treatment revealed that 3OMG with isoproterenol exhibited the greatest degree of hypertrophy surpassing all groups including the hypertrophic control.

Conclusion: I have been able to demonstrate the successful differentiation of iPSCs to cardiomyocytes. The isoproterenol induction mimics hypertrophy seen *in vivo* showing the applicability of this cell type in the study of metabolic dysregulation. These preliminary findings reveal that cardiomyocyte hypertrophy is independent of G6P accumulation. Further trials are ongoing.

ABSTRACT

Survey of Techniques Utilized to Access Ventricular Shunts and Reservoirs

Claudia I. Martinez   *McGovern Medical School at UTHealth*   *Class of 2019*

Introduction:
This study assessed variations in pediatric neurosurgical techniques when accessing shunts and ventricular access devices (VAD's).

Methods:
A 12-question survey was developed and sent to members of the American Association of Neurological Surgeons (AANS) whose self-identified subspecialty was pediatric neurosurgery. The survey assessed the following practices when accessing shunts or VAD's: sterile gown, facemask, sterile gloves, sterile drapes, site shaving, who performs the procedure, presence of attending neurosurgeon, assistant holding the patient's head, type of prep solution and wait time for solution to dry, type of needle used, and frequency with which CSF is sent for analysis.

Results:
149 responses were received (35.5% response rate). 95.3% of respondents always use sterile gloves, 55.0% never use a sterile gown and 69.8% always have a member of the neurosurgery team perform the procedure. The majority of respondents answered "sometimes" for use of a facemask (38.3%), sterile drapes (39.6%), site shaving (45.6%), having an attending present (68.5%), and having an assistant hold the patient's head (78.5%). The majority (96.6%) reported using either a 23 or 25 gauge butterfly needle. Betadine and Chloraprep were the most common prep solutions (32.2% each). Frequency in which CSF is sent for analysis is not standardized (31.5%), and wait time for prep solution to dry is not standardized in 62.4%, respectively.

Conclusion:
There is great variation in technique for accessing shunts and VAD's. Future studies are needed to assess whether these discrepancies adversely affect infection rates.

ABSTRACT

Part of the Team: Parent Engagement in the Surgical Safety Checklist

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Supported by: Kuojen Tsao, MD, Department of Pediatric Surgery; The University of Texas
                Health Science Center at Houston Medical School – Office of the Dean

Key Words: Parent engagement, surgical safety checklist, patient safety, quality of care

Introduction: The pre-induction portion of the surgical safety checklist (PI-SSC) provides the perioperative team (anesthesia and nursing) the last opportunity to exchange and confirm critical information with parents of pediatric patients prior to surgery. Few, if any, studies have investigated the manner in which parents are included in this process. We aimed to describe the current state of parent engagement in the PI-SSC at Children's Memorial Hermann Hospital.

Methods: An eight week prospective, observational study was performed by four trained observers. Cases were a convenience sample of nine pediatric surgical specialties. Six PI-SSC items were considered: patient identification, procedure, site of surgery marked, weight, allergies and NPO status. Observers measured the perioperative teams' inclusion of parents for these items through observations of: 1) staff performing and noting checklist item 2) staff directly confirming the items with the parents and 3) parents reporting item was asked. Observers conducted parent interviews to determine if the team asked them about the items at any time during the pre-operative period. Cohen's kappa was used to determine correlation between staff performance, observer report of parent-staff confirmation, and parent report. Kappa was also employed to evaluate inter-rater reliability amongst observers.

Results: 255 cases were observed and 70% of parents (n=178) were interviewed. The inter-rater reliability of observers was 0.85 (95% CI 0.79-0.88). At least one item on the PI-SSC was completed in 95% of the cases. Of those cases, the checklist was completed 89% of the time with both anesthesia and nursing present and 87% of the time in the patient's room. The perioperative team directly confirmed 33% of items during their joint PI-SSC (65% patient identification, 33% procedure, 12% site of surgery marked, 5% weight, 41% allergies, 39% NPO status). Parents reported the team confirmed 95% of items at some point; only confirmation of weight occurred less frequently, 76% of the time. Cohen's kappa for the correlation was <0.09 for all checkpoints.

Conclusion: While parents confirm the perioperative team asks them about PI-SSC items sometime during their preoperative experience, the team could still do much more to include the parents during the PI-SSC. The perioperative team is not explicitly required to confirm items with parents, but the opportunity to acquire new information that can fundamentally affect the patient's care should encourage the team to engage the parents as active participants. Future efforts will include targeted interventions such as encouraging anesthesia and nursing to complete the checklist in the room with verbal confirmation from parents for each of the items.

ABSTRACT

EPHARNA Pharmacokinetics in Patients with Ovarian Cancer

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Sponsored by: Anil K Sood, MD, M.D. Anderson Cancer Center Department of Gynecologic Oncology; David E Volk, PhD, Department of Nanomedicine and Biomedical Engineering
Supported by: David E Volk, PhD, Department of Nanomedicine and Biomedical Engineering
Key Words: Ovarian cancer, EphA2, pharmacokinetics

Background: As the second most common gynecological malignancy with a mean survival time of about four years, ovarian cancer is a major focus in cancer research. EphA2 is a receptor tyrosine kinase that is known to play a part in the angiogenesis necessary for the survival of cancer cells. EphA2 overexpression in ovarian tumors is associated with a poor prognosis in patients. siRNA can be used to down-regulate the deleterious effects of oncogenic proteins such as EphA2. In a clinical trial, seven patients with ovarian cancer were administered 450 ug/m^2 EPHARNA (a nanoliposomal formulation containing siEphA2) and blood samples were taken at 12 different time points for analysis of siEphA2 levels in the blood. Core tumor biopsies were also obtained for analysis of gene knockdown at the mRNA and protein levels. It was observed that siEphA2 levels decrease as time post-administration increases. Tumor core biopsies will be analyzed for EphA2 mRNA levels and protein expression and it is expected that both will decrease after administration of siEphA2.

Methods: Seven patients with ovarian cancer were treated with 450 ug/m^2 siEphA2. Plasma was collected at 12 different time points, including 0, 15, 30, 60, 90, 180, and 360 minutes post-siEphA2 infusion. qPCR was used to analyze the quantity of siEphA2 present in the patients' plasma at different time points post-infusion. These measurements were controlled for using a known miRNA added to the patient sample before RNA extraction. In addition, known quantities of siEphA2 were analyzed in parallel to this experiment. A proof-of-concept experiment was carried out using mouse serum with known quantities of siEphA2 and miRNA added. Successful processing of these samples was necessary for the handling of the clinical trial plasma samples.

Results: Of the 3 mL of plasma that was collected for each time point, it was determined 500 uL was optimal given the RNA extraction methods. Preliminary data shows that siEphA2 is most abundant in patient plasma immediately post-siEphA2 infusion. In addition, the positive control miRNA levels indicate that the RNA extraction methods did not affect siEphA2 quantification.

Discussion: It is expected that the trend of decreasing levels of siEphA2 correlated with increasing time post-infusion will be comparable between the seven patients in the clinical trial. Using a quantification standard of known quantities of siEphA2, exact concentrations and amounts of siEphA2 will be calculated for each time point that blood was collected during the clinical trial. Future experiments include the quantification of EphA2 mRNA and protein from tumor core biopsies of patients to determine whether siEphA2 achieved down-regulation within the tumor cells. In addition, an increased dose of siEphA2 will be administered to the following round of patients in the clinical trial. These patient samples will be compared to the samples analyzed in this study.

ABSTRACT

Longitudinal Study of Erythrocyte Cytochrome b Reductase 1 Level in Sickle Cell Disease Patients

Kathryn McElhinney   *McGovern Medical School at UTHealth*   *Class of 2019*

Background: Sickle cell disease (SCD) pathology results from intermittent ischemia/reperfusion events that generate reactive oxygen species (ROS). Research on antioxidant therapies to counter long-term, ROS-linked complications has become pertinent as improved treatment has increased patients' life spans. Plasma ascorbate is the major antioxidant for neutralizing circulating ROS. Oxidized ascorbate is recycled to its active state by cytochrome b reductase 1 (CYBRD1) in the red blood cell (RBC) membrane. Thus, a deficiency of CYBRD1 in RBCs might promote oxidative stress. Our lab is conducting a study to test if RBC membranes of SCD patients have lower levels of CYBRD1 than RBCs from healthy controls. Initial results (7 patients, 10 controls) suggest a lower mean CYBRD1 content ($p<0.0013$; Mann-Whitney-Wilcoxon) in patients' RBC (0.33 ± 0.09 ng/µg membrane protein; 95% CI: 0.24-0.43) than in controls (0.61 ± 0.06 ng/µg; 95% CI: 0.54-0.66). This project addresses the questions of whether patients' RBC CYBRD1 levels vary between clinic visits, and if observed variations correlate with changes in clinical parameters. **Methods:** RBC membranes were isolated from blood samples of four homozygous SCD subjects (22-year-old female; 24-, 33-, and 39-year-old males) at the UT Comprehensive Sickle Cell Clinic during five regular visits over 7-9 months. Membrane samples and a mixed standard (bovine serum albumin + histidine-tagged recombinant human CYBRD1) were electrophoresed on parallel denaturing polyacrylamide gels. One gel was Coomassie stained for quantitation of membrane protein ≥ 17 kDa (excludes membrane bound hemoglobin). Resolved proteins in the other gel were immunoblotted with CYBRD1 antibody, peroxidase-conjugated secondary antibody and chromogenic substrate. Gels and blots were scanned (transmittance) alongside a calibrated density step tablet and analyzed for membrane protein or CYBRD1 concentration. Tests for correlation between CYBRD1 content (normalized for membrane protein) and clinical parameters used the Pearson r correlation. **Results**: All four patients' RBC CYBRD1 content changed little during the study (mean ± SD; ng/µg protein): Subject 1 = 0.32 ± 0.04; Subject 2 = 0.29 ± 0.07; Subject 3 = 0.26 ± 0.03; Subject 4 = 0.65 ± 0.07. Except for Subject 4, the RBC CYBRD1 content measured at separate visits fell consistently within the 95% CI of the mean from earlier SCD patients. No statistically significant correlations were found between RBC CYBRD1 content and blood lab parameters, and clinical events such as transfusion or pain crisis (> 1 month prior) did not have a marked impact on CYBRD1 content. However, inter-visit changes in RBC CYBRD1 content did seem to oppose changes in mean corpuscle volume (MCV) in 3 patients and to oppose changes in plasma lactate dehydrogenase (LDH), reticulocyte count, and bilirubin in 2 patients. **Conclusions:** Only modest changes in RBC membrane CYBRD1 content occur between patients' clinic visits, supporting the sufficiency of single blood samples in cross-sectional studies. MCV, LDH, reticulocyte count, and bilirubin levels should be prioritized for testing for association between RBC CYBRD1 content and disease severity in larger patient samples.

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ABSTRACT

Medical Student

A Pilot Study to Investigate Grey and White Matter Changes in Patients with Traumatic Brain Injury

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Sponsored by: Dr. Paul E. Schulz, MD, Department of Neurology

Supported by: Dr. Paul E. Schulz, MD, Department of Neurology
Key Words: MRI, DTI Fiber Mapping, Traumatic Brain Injury

Traumatic brain injury (TBI) occurs commonly in the population, whether from sports injury, falls, motor vehicle accidents, or many other reasons, yet the long-term effects of TBI on the brain are not well understood. Some patients seem to suffer no lasting effects from TBI while others experience profound deficits that take a long time to fully heal, and some never fully recover. In this study, we sought to understand and quantify specific parameters on MRI that can predict patient outcomes and allow for tailored treatment in the future.

We hypothesized that white matter tracts would be disrupted in patients with sustained deficits from TBI, and that there would be a change in grey matter and white matter volumes. Using DTI Studio Tractography, we analyzed MRIs of patients with varying degrees of TBI and impairment to ascertain whether there was a correlation between study variables and patient outcomes.

We seeded several areas of interest (caudate nucleus, putamen, anterior limb of internal capsule, posterior limb of internal capsule, and lateral ventricles) to determine white matter tract coherence at those locations, using several markers of white matter tract and microstructural integrity (Fractional Anisotropy (FA), Mean Diffusivity (MD), Axial Diffusivity (AD), Radial Diffusivity (RD)). We then seeded white matter tracts on DTI Studio by placing a region of interest (ROI) at an area where the tract runs and locating all fibers that go through that area. We tracked the anterior and posterior corpus callosum, as well as the corticospinal tracts and somatosensory tracts using this method and found the same measures of tract integrity on these as before (FA, MD, AD, RD). We also measured intracranial volume (ICV), whole brain CSF volume, whole brain white matter volume, and whole brain grey matter volume on MRIcro/MRIcron to investigate whether there was a noticeable pattern of changes in TBI patients.

9 TBI cases were analyzed and compared to normal values, while several other patients were recruited for scans. The study is still underway, as we expect to recruit at least 40 patients (20 with permanent post-concussive symptoms and 20 patients with TBI and no permanent symptoms). In the 9 cases analyzed, we found evidence of missing or thin fibers in certain regions that often corresponded with the location of the TBI. We expect to compare the data from our 40 TBI patients with age-matched normals to determine whether there is a change in white matter tract integrity and ratio of grey to white matter volume across the three groups (mild TBI with persistent symptoms, mild TBI with no persistent symptoms, and no TBI).

ABSTRACT

Using Connectomics to Investigate Retinal Ganglion Cells

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Sriram Navuluri

Introduction: Retinal ganglion cells integrate and transmit visual information from the retina to the brain. There are many distinct types of retinal ganglion cells whose functions vary along with their morphology. The focus of this study was on parasol ganglion cells, also known as M cells, which contribute to many aspects of vision and particularly to the perception of motion. Though parasol cells have long been studied by a variety of methods, retinal connectomics provides a novel way to study their synaptic connections. In particular, we propose that inputs from amacrine cells, inhibitory local circuit neurons of the inner retina, impart motion sensitivity to parasol cells. Previous work predicted these synaptic connections, and this project tested this hypothesis.

Methods: Serial blockface scanning electron microscopy was used to generate a series of images through the entire thickness of a piece of central macaque retina. The project utilized Viking software to navigate and annotate these sections. Ultimately, these annotations will be compiled to generate three-dimensional reconstructions of the cells and a high resolution map of their synaptic connections, also known as a connectome. Cells were classified based on their soma size and characteristics of their dendrites, including: field diameter, morphology and depth of stratification in the inner plexiform layer. Criteria for synaptic connections included pre-synaptic vesicle clouds and post-synaptic densities.

Results: We completed annotations of five ON parasol ganglion cells and one midget ganglion cell. Additionally, we partially annotated six other ganglion cells that are not yet classified. A subset of the somas in the ganglion cell layer appeared to be displaced amacrine cells. We investigated the morphology of twenty-four amacrine cells and have begun analysis of their synaptic connections with our completed parasol ganglion cells. We have observed inputs to ON parasol cells from both starburst and wiry morphological types of amacrine cells.

Discussion: Previous studies utilized light microscopy and electrophysiology to predict the existence of synapses from starburst and wiry amacrine cells to ON parasol ganglion cells. Our annotations provided direct, ultrastructural evidence for these synapses. Further analysis of the connectome will provide a more complete description of the retinal circuitry for motion detection.

ABSTRACT

For older adults with undifferentiated complaints and vague symptoms, how often is there an infectious etiology?

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Sponsored by: Kimberly A. Chambers, MD, Department of Emergency Medicine

Key Words: Geriatric, older adult, sepsis, occult sepsis, emergency medicine

Background: With age, asymptomatic infections become more prevalent. Emergency physicians face a dilemma when deciding how extensive a workup to perform when the patient's symptoms are vague. This decision is made more complex when there is dementia or delirium present, and an accurate history is difficult to obtain.

Significance: Roughly 500,000 older adult patients develop sepsis each year. The number of octogenarians is expected to double by 2030, making sepsis a major health concern, and the prevalence of dementia increases significantly between the ages of 70 and 90.

Hypothesis: A significant number of patients presenting with vague complaints, such as generalized weakness, and no overt signs of infection, such as fever, will have an underlying infection. Patients with AMS will be more likely to have delays in diagnostic testing that rely on patient cooperation, such as urinalysis testing, and treating presumptively with antibiotics to avoid delays may lead to numerous cases of overtreatment.

Experimental Design: This was a retrospective cross sectional study of patients ≥ 60 years old who presented to the Memorial Hermann Emergency Center between January 1st and January 26th 2016 with undifferentiated chief complaints. Patients were excluded if they presented with cardiac arrest or a clearly non-infectious chief complaint such as ST-elevation myocardial infarction. A total of 1021 medical records were screened. 448 patients met inclusion criteria and were included in the analysis.

Results/Data: Of the 142 patients without classic signs of infections, 37 (26.1%) were treated with antibiotics. There were only 6 (4.2%) patients who received antibiotics in whom diagnostic testing later revealed no infection.

Conclusions: 31 (21.8%) of the 142 older adults who presented without signs or symptoms specific for infection ultimately had an infection that required antibiotic therapy, so it is reasonable to screen for infection in this older patient population when nonspecific symptoms are present.

Medical Student

ABSTRACT

Aldh1a1: Friend or Foe in Pancreatic Regeneration and Pancreatitis?

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Sponsored by: Jennifer M. Bailey, M.A., PhD, Department of Internal Medicine, Division of
                Gastroenterology, Hepatology and Nutrition

Background: Inflammatory diseases of the pancreas are painful, difficult to manage, and affect millions of Americans every year. Onset of chronic pancreatitis and pancreatic intraepithelial neoplasia (panIN) are known to be risk factors for the development of pancreatic cancer, which exhibits an extreme resistance to treatment and a very low survival rate. Aldehyde dehydrogenase 1 (Aldh1) has been shown to be expressed in high levels within pancreatic cancer stem cells. Through previous discovery that cells with high levels of Aldh1 are stem cells, we hypothesize that loss of Aldh1a1 will inhibit pancreatic regeneration and the development of premalignant pancreatic disease.

Methods: In our lab, we would like to determine if loss of Aldh1a1 will prevent the development of panIN and progression to pancreatic cancer. In order to test this, we have generated *Pdx1:cre; LsL-Kras^{G12D}; Aldh1a1$^{KO/KO}$ (KP1a1$^{KO/KO}$)* mice to determine their incidence of pancreatitis and panIN formation, as KC mice develop tumor formation by the activation of Kras^{G12D} in Pdx1+ multipotent pancreatic progenitors. We are also determining if different Aldh isoforms are compensating for the lass of Aldh1a1. In order to perform this, we have isolated RNA from *KP1a1$^{WT/WT}$, KP1a1$^{KO/WT}$*, and *KP1a1$^{KO/KO}$* mice at 4.5 months of age. We have primers for murine Aldh1a1, Aldh1a2, and Aldh1a7 and we will perform experiments to determine if isoform compensation is a potential mechanism for accelerated panIN formation in the Aldh1a1 knockout mice. We will perform PCNA staining and immunofluorescence staining to assay for panIN and to stain for activated myofibroblasts.

Results: Though we predicted a reduction in Kras-driven pancreatitis, panIN, and pancreatic cancer in Aldh1a1 knockout mice in comparison to Aldh1a1 wild-type mice, our tissue samples following PCNA and immunofluorescence staining suggest that the loss of Aldh1a1 in KC mice increases the incidence of panIN lesions and stromal proliferation. In the future, we will attempt to determine if the increase in panIN lesions in Aldh1a1 knockout mice is independent of Kras activation.

ABSTRACT

Ubiquitination of Toll-Like Receptor 4 through Surfactant Protein-A Regulation of E3 Ligase Triad3A Protein Activity

Esther Osuji   *McGovern Medical School at UTHealth*   *Class of 2019*

Sponsored by: Joseph L. Alcorn, PhD, Department of Pediatrics

Necrotizing enterocolitis (NEC) is a gastrointestinal disease of preterm infants that results in inflammation and destruction of part or all of the bowel. Hypoxia, formula feeding, and inappropriate bacterial colonization have been shown to be a major cause of the disease. Activation of intestinal toll-like receptor 4 (TLR4), is key in inflammatory processes that lead to NEC. Surfactant protein A (SP-A), a protein that is largely expressed in the lungs, functions in innate immunity and has been shown to mediate inflammation in the lung and gut. SP-A has been shown to reduce levels of intestinal TLR4 in animal models of NEC and in intestinal epithelial cell lines. We hypothesize that SP-A reduces TLR4 expression in gastrointestinal epithelial cells by stimulating ubiquitination of TLR4 via Triad3A, an E3 ubiquitin-protein ligase that regulates Toll-like receptors. Using human colonic (HT-29) and mouse intestinal (IEC-6) cell lines, the effect of SP-A on Triad3A levels was determined by western analysis on the incubated cells. The cells that expressed SP-A had no effect on Triad3A levels, suggesting that SP-A may alter the activity of Triad3A. To demonstrate a role of Triad3A in regulation of TLR4 in these cells, siRNA directed against Triad3A was transfected into both cell lines and TLR4 levels measured, with the prediction of no change in TLR4 concentration. However, no change in Triad3A levels were detected. We are currently evaluating various types of siRNA and transfection methods overcome the lack of knockdown of Triad3A. Additionally, we were to use plasmids expressing HA-ubiquitin to demonstrate that SP-A increased ubiquitination of TLR4. The plasmids we purchased displayed the expected restriction mapping characteristics, but we are having difficulty detecting HA-ubiquitin expression in transfected HT-29 and IEC—6 cells. We are currently evaluating various antibodies specific for HA before embarking on this endeavor.

ABSTRACT

Effects of Missed or Off Schedule Doses of Antibiotics on Patient Outcomes

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Sponsored by: John Holcomb, MD, Center for Translational Injury Research
Supported by: John Holcomb, MD, Center for Translational Injury Research
Key Words: Antibiotics, Missed, Length of stay in hospital

Introduction: When delivered according to the appropriate schedule, antibiotics (Abs) improve outcomes. Missing doses of Abs is a well described but an inadequately recognized issue. We hypothesized that missing doses of Abs decreases quality of care.

Methods: A retrospective study on all patients admitted to the Shock Trauma ICU from February to June 2015 was performed. Patients prescribed a course of Abs were evaluated, those given prophylactic or one dose were excluded. A missed Ab dose was one planned but never given (a completely missed dose) or a dose that was not given within an hour (before or after) the planned time (an off-schedule missed dose). Abs given ± one hour is the standard ICU guideline. There were valid and non-valid reasons for completely missing a dose. Valid examples included, a change in the order, doses held by an MD, high drug levels or a dosing conflict. Non valid examples included off unit and unknown. Patient outcomes include a positive culture, sepsis, ventilator, ICU and hospital days and mortality. Multiple statistical methods were used as appropriate, significance was set as $p < 0.05$.

Results: 280 patients were admitted, 200 met inclusion criteria and 8167 doses of Abs were ordered. 8% of patients (16/200) did not miss any doses of antibiotics, 38% (77/200) had off-schedule missed doses, 43% (86/200) missed a dose for non-valid reasons and 10% (21/200) missed doses for valid reasons. The median Ab doses ordered for those who did not miss doses was 4 (3, 6), while 26 (9, 53) were ordered for those who did miss doses (p<.0001). All demographic data (age, BMI, ISS) were similar between patients who did and did not miss doses of Abs. 8167 total doses of Abs were ordered and 25% were missed. 21% of doses (1729/8167) were off-schedule, 2.3% (189/8167) were completely missed for non-valid reasons, and 1.3% (113/8167) were completely missed for valid reasons. Among off schedule doses (1729/8167), the median number of hours off schedule was 2 (2, 2) for both late doses and early doses. Unadjusted analysis showed that patients who missed Abs had a higher rate of sepsis (P=0.01), while those who missed a dose of Abs for non-valid reasons spent more days on a ventilator (P=.03) and in the hospital (P<.0001) than patients who did not miss any doses. Adjusting for age, gender, BMI, ISS and doses of Abs showed that those who completely missed a dose for non-valid reasons spent 50% more days in the hospital (P=.01) than patients who did not miss any doses of Abs, while patients who only missed Ab doses off-schedule spent 54% more days in the hospital (P=.004). Sepsis, mortality, days on ventilator, and days in the STICU were not significant when adjusted for covariates.

Conclusion: Missing doses of antibiotics (both completely and off-schedule) correlated with a substantial increase in length of hospital stay. To optimize quality of care, methods to improve compliance with antibiotic dosing schedules should be investigated.

Medical Student

ABSTRACT

Menisci tears associated with full thickness ACL tears

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Sponsored by: Dr. Manickam Kumaravel, MD, Diagnostic and Interventional Imaging
Key Words: Prevalence of meniscal tears in patients with ACL tears

BACKGROUND: ACL tears and their treatment are among the most common athletic injuries. Often, ACL injury is coupled with other ligament injuries and meniscal tears. ACL tears are commonly associated with meniscal tears. Establishing a correlation between acute ACL tears with the location and type of secondary meniscal tears allows for appropriate clinical management. Clinical diagnosis of meniscal tears is provided by MRI imaging. Clinicians should be more aware of patterns in acute ACL tears and secondary meniscal injuries to provide effective treatment. Patents chosen for this study had established acute full thickness tears of the ACL with available MRI imaging. A subset of patients with meniscal injuries were evaluated for location and type of meniscal injury.

METHODS: A list of 161 patients who underwent ACL reconstruction was obtained from the surgical register. These patients were retrospectively evaluated. MRI images, radiology notes and post-operative notes were evaluated for occurrence of the varying injuries to their meniscus. The grade of meniscal injury, location, and type of tear were recorded. Some patients had multiple and each injury and their respective classification was noted. Using arthroscopy as a "gold standard". The incidence of each injury was recorded, and corroborated with the preoperative imaging. The observations were done by the orthopedic surgeon, upper level musculoskeletal radiology resident, and senior musculoskeletal radiologist.

RESULTS: Of the 161 patients in the study, 60% of patients had either a medial or lateral meniscal tear. Of the 60%, 26.09% of patients had only medial meniscus tears, 22.36% of patients only had lateral meniscus tears, and 10.56% of patients had both medial and lateral menisci tears. Both medial and lateral menisci tears were commonly in the posterior horn with a prevalence of 68.97% and 69.23% respectively. In the lateral meniscus the second most common location of a tear is in the anterior horn with a prevalence of 15.38%. In the medial meniscus the second most common location of a tear is the body (13.79%). With the grade 3 ACL patients, the most common lateral meniscus type of tear among ACL reconstructive patients is radial at 44.2% prevalence. The most common medial meniscus type of tear was longitudinal of 18.9% prevalence.

CONCLUSION: Injury patterns are important to note during evaluation of acute trauma. Patients with full thickness ACL tears have a propensity to have an associated meniscal injury. 60% of ACL tear patients exhibited meniscal tears, with an even distribution between both medial and lateral menisci. Interestingly, the lateral meniscal tear had a significant preference of radial tear with 44% and a significantly higher prevalence for anterior horn compared to medial meniscus tears. Awareness of correlation between ACL tears and meniscal injury facilitates improved diagnosis by both clinicians and radiologists, particularly as appropriate identification directs treatment.

ABSTRACT

Elevated Polyreactive Antibody Levels in a Rat Model of Hypertensive Renal Injury

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Background: Previous research has been performed on a rat model of hypertension called the spontaneously hypertensive rat (SHR). This strain was produced by selection on the trait of elevated blood pressure and represents an inbred genetic model of disease. SHR exists in several related, but genetically distinct hypertensive lines. The SHR-A3 line develops hypertensive renal injury. The SHR-B2 strain serves as a hypertensive renal injury-resistant control. In SHR-A3, hypertensive renal disease is effectively prevented by pharmacological immunosuppression (mycophenolate). This project seeks to test whether polyreactive IgM antibodies may be involved in renal disease. Polyreactive antibodies bind with low affinity to a variety of structurally dissimilar antigens. They serve to protect against pathogens in young animals prior to the development of IgG affinity maturational processes and they may also aid in phagocytosis of damaged cells. They are characterized as having broad specificity, but low affinity. Serum levels of these antibodies can be measured with a dinitrophenol assay.

Significance: Hypertension affects as much as 37% of the global population and associated with 18% (9.4 million) of annual deaths. Hypertension is a leading cause of chronic kidney disease (CKD), which affects 10% of the world's population and is associated with 1 million annual deaths. This project aims to increase our understanding of the mechanisms involved in the evolution of renal injury in hypertension.

Hypothesis: Genetic differences in the immunoglobulin heavy chain locus may result in different levels of polyreactive antibodies in SHR-A3 relative to the SHR-B2 control.

Experimental Design: Serum samples were obtained from four groups: (1) SHR-A3 rats at 18 weeks, (2) SHR-A3 rats at 40 or greater weeks, (3) SHR-B2 rats at 18 weeks and (4) SHR-B2 rats at 40 weeks. Eight samples were obtained from each group and a dinitrophenol ELISA assay was performed on each sample to quantify the level of polyreactive antibodies.

Results: The samples from the SHR-A3 rats at 18 weeks exhibited significantly higher levels of polyreactive antibodies relative to the SHR-B2 rats ($p=0.02$). The levels of polyreactive antibodies after 40 weeks (during end stage kidney disease) were elevated in the SHR-A3 rats but not to a statistically significant degree.

Conclusions: The significantly elevated levels of polyreactive antibodies in the SHR-A3 rats at 18 weeks suggest they may be involved in the initial development of autoimmune mediated renal injury. Their role in progressive kidney disease remains inconclusive. More research is needed to establish a causal link between these antibodies and the resulting disease.

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The University of Texas Health Science Center at Houston

ABSTRACT

Morphoproteomic Treatment of Chemotherapy Resistant Colon Cancer using Natural Based Therapies

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Sponsored by: Dr. Priya Weerasinghe, MD, MSc, PhD, Department of Pathology and Laboratory Medicine

Supported by: Dr. Robert Brown and Dr. Priya Weerasinghe

Key Words: Colon Cancer, Therapeutics, Morphoproteomics

Background: Colorectal cancer was estimated to have the fourth newest incident rate and estimated to have the second highest death rate in 2015 according to the American Cancer Society. There were an estimated 132,700 new diagnoses of colorectal cancer made and approximately 69,000 deaths in 2015 attributing to this form of cancer. 5-fluorouracil (5-FU) or 5-FU with oxaliplatin (FOLFOX) continues to be the standard treatment protocol for patients presenting with colorectal cancer. However, treatment with FOLFOX and conventional chemotherapy has shown to lead to cancer recurrence and a number of toxic side effects. Alternative therapies based on natural ingredients show promise in combating cancer by inhibiting immune pathways, such as mTORC, c-MYC oncogene, and the COX-2 pathway. Using these nature based therapies and targeted morphoproteomic analysis, the goal of this study was to evaluate the effectiveness of targeted therapies in four of our colon cancer patients with chemotherapy resistant cancers.

Objective: The objective of this study was to evaluate the effectiveness of nature based therapies using morphoproteomics to determine the necessary cellular targets.

Method: Four colon cancer patients were evaluated by the morphoproteomics lab at UT Houston Medical School for targeted cancer therapy all within the past year. The attending physicians were contacted to obtain a brief history and current status of the patients. If able, the patients were contacted afterwards to ask how effective the treatment had been and if there were any noticeable side effects.

Results: Of the four patients to be interviewed, one passed away shortly after receiving news of the morphoproteomic recommendations, one is still trying to be contacted, and two have been contacted and evaluated. Of the two that have been contacted, one admitted non-compliance that had colon cancer metastasizing to the lymph nodes, ovaries, and left fallopian tube. The other had a non-metastasizing colorectal cancer that showed "minimal treatment effect" with chemotherapy and was compliant. As of May 2016, CT scans and colonoscopy confirmed that the cancer was in remission after that patient had been compliant with therapy for nine months.

Discussion: There is some evidence that targeted therapies using natural based medicine could help in concurrent treatment with chemotherapy and radiation or as a stand-alone treatment. The patient that was compliant was given melatonin, metformin, curcumin, celecoxib (patient replaced with skull cap), sulforaphane, and vitamin D3, each having a unique cellular target in this patient. Morphoproteomics has great application in creating personalized medicine available to cancer patients and when combined with nature-based therapy can create a viable alternative to chemotherapy resulting in reduced toxic side effects.

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ABSTRACT

mHealth Use Amongst Caregivers of Pediatric Surgical Patients

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Sponsored by: Mary T. Austin, MD, MPH, Department of Pediatric Surgery
Supported by: Mary T. Austin, MD, MPH, Department of Pediatric Surgery
                Emma Hamilton, MD, Department of Pediatric Surgery
Key Words: eHealth, mHealth, Pediatric Surgery, smartphone, apps

Introduction: eHealth is an umbrella term that includes various forms of digital information and communication technology involved in improving healthcare. mHealth is a subset of eHealth that includes mobile phones and other wireless technology in the process of healthcare delivery. mHealth technology is cost effective and can cross language barriers as well as help increase access to health care in underserved areas. Despite this utility, little is known about the general interest in using mHealth technology like mobile apps, for caregivers of pediatric surgical patients. Our purpose was twofold; first of all to analyze the prevalence of smartphone and eHealth usage amongst our pediatric caregiver population, and second to evaluate interest in utilizing a mobile app for perioperative care as well long term management of chronic conditions.

Methods: We performed a cross-sectional study in which we administered a modified version of the 2012 mobile health survey from the Pew Research Center to caregivers of children <18 years of age presenting to the Pediatric Surgical Outpatient Clinic from June – July of 2016. The survey had an English and Spanish version, with 98 English respondents and 31 Spanish respondents. The survey was split up into 12 modules that looked at parents' current use of technology, phones/smartphones, and smartphone apps, and smartphone fluency. The survey also asked specific questions about usage of mHealth as well as specific questions about mHealth and smartphones. The survey finally asked about interest and ability in using apps for perioperative care and management of long term conditions.

Results: 92% of survey participants owned smartphones, with the other 8% being predominantly over that age of 50, less than high a high school graduate, with an annual income less than $30,000, Hispanic, and Spanish speaking only. 90% of participants used their phones to look up health information online and almost 50% received some sort of health information by text. Similarly, 40% used health related apps, including apps like exercise/fitness apps, diet/calorie counters, weight, blood pressure, and blood sugar. Between English speaking and Spanish speaking participants, Spanish speaking patients were 3.5 times less likely to get text health updates and 3.2 times less likely to use health related apps than non-hispanic whites. Language and race were not significant indicators of interest level in trying new apps, with 75% of parents displaying interest and 92% being confident in their abilities to use a new app

Conclusion: Over 90% of our caregiver population owns smartphones. Though usage characteristics do vary based on language and race, interest in trying new healthcare

management apps as well as confidence remain high. This allows a knowledge base for development of apps that can help our caregiver population and increase access to healthcare.

ABSTRACT

Frailty or Age? Mortality and Disposition after Traumatic Spinal Cord Injury in the Elderly

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Sponsored by: Sasha D. Adams, MD, The Center for Translational Injury

Supported by: The Center for Translational Injury Research (CeTIR)

Key Words: Frailty, Elderly, Trauma, modified Frailty Index (mFI), Spinal Cord Injury (SCI)

Introduction: Predicting outcome and discharge disposition in geriatric patients with traumatic spinal cord injury (SCI) is imperative to appropriately direct hospital resources and to coordinate the extensive post-injury care required by these patients. Age is commonly used to predict outcomes in these individuals, however it does not account for the altered physiologic reserve in the geriatric population and therefore may not accurately predict a patient's response to injury. The modified Frailty Index (mFI) assesses frailty based on accumulated deficits, or comorbidities present prior to injury, and has demonstrated predictive utility for outcome and disposition in various surgical populations. The mFI has yet to be investigated in traumatic SCI. We calculated the mFI to determine if it is more reliable than chronological age at predicting in-hospital mortality or unfavorable discharge disposition in elderly patients with traumatic SCI.

Methods: The Memorial Hermann Hospital (MHH) Trauma database was queried over a 5 year period (1/1/2011-12/31/2015) to identify patients ≥ 55 years old with blunt spinal cord injury. SCI was defined by AIS code, including complete/incomplete cord injury and cord contusions. Pre-admission comorbidity data was obtained by chart review to calculate mFI. In-hospital mortality and discharge disposition were determined. Discharge to home or rehabilitation hospital was considered favorable, and unfavorable discharge was defined as skilled nursing facility, long term acute care, hospice or death. Logistic regression evaluated the relationship between age, mFI, mortality and disposition. ROC curve analysis determined the relative performance of age and mFI in predicting mortality and unfavorable disposition. Patients lacking adequate documentation of pre-admission comorbidities were excluded (n=17).

Results: Of 807 blunt SCI patients that met the injury criteria, 299 were ≥ 55 years of age and 281 had sufficient pre-admission data for inclusion. Median age was 68 years (IQR 61 to 76), 72% were male, median ISS was 18 (IQR 14 to 26), and median mFI was 0.09 (IQR 0 to 0.18). There was no correlation between chronological age and frailty (R^2=0.06). After adjusting for age and ISS with multiple logistic regression analysis, mFI was not significantly associated with unfavorable discharge disposition (p=0.5), while age (p<0.001) and ISS (p<0.001) were. Upon ROC curve analysis, mFI did not add significant predictive value to a model already including age and ISS (AUC 0.7617 vs. 0.7616).

Conclusion: Chronological age may be a better predictor than mFI of in-hospital mortality and unfavorable discharge disposition in elderly patients with traumatic SCI. Further analysis of specific levels or severity of SCI are warranted.

ABSTRACT

EPHARNA Pharmacokinetics in Patients with Ovarian Cancer

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Sponsored by: Akemi Kawaguchi, MD, Department of Pediatric Surgery
Supported By: Akemi Kawaguchi, MD, Department of Pediatric Surgery
Key Words: Teamwork, Observational Teamwork Assessment for Surgery (OTAS), Patient
Safety, Quality Care, Pediatric Surgery

BACKGROUND: Effective operative teamwork behaviors are fundamental to providing quality surgical patient care. Lapses in communication, cooperation, awareness, or other related behaviors may result in avoidable surgical complications and adverse events. However, little work has been done to assess the differences between behavioral interactions in the operating room and their predicted impact on patient safety. This study was performed to characterize teamwork behaviors in pediatric operating room team members to be able to identify areas for improvement.

METHODS: Teamwork scores for randomly selected, live pediatric surgical procedures were prospectively measured using the Observational Teamwork Assessment for Surgery (OTAS) scale. This is a previously validated rating system with 15 items on a 7-point Likert Scale (0-6, with 3 representing behavior that neither hinders nor enhances team function). Observers were standardized for accuracy using 5 OTAS standardization video assessments. The surgical, anesthetic, nursing, and scrub technician teams received a composite score for each behavior (communication, coordination, cooperation/back-up behavior, leadership, and situational awareness) in the preoperative, intraoperative and postoperative phase for a total of 60 data points per procedure. Statistical variance analysis was then performed, followed by post-hoc mean   analysis.

RESULTS: A total of 496 cases were observed (214 from June 1-July 28, 2015 and 281 from June 1-July 22, 2016). Of the 496 cases, 440 are included in this analysis. Observer standardization showed 83.9% of the assigned scores were within ± 1 range relative to the standard (threshold for validity ≥ 70%). Across all teams, operative periods, and behaviors the mean OTAS score was 3.65 ± 0.75 (95% CI, 3.51-3.75; $P < 0.001$). Overall, surgical teams had the highest mean (+/- standard deviation) teamwork score, followed by nursing and anesthesia (3.76 ± 0.82, 3.70 ± 0.74, 3.62 ± 0.72; $P < 0.001$). Post-hoc analysis showed that surgical teams exhibited the best communication (3.92 ± 0.88) and leadership (3.70 ± 0.86) with the poorest overall cooperation/back-up score (3.60 ± 0.77) ($P < 0.001$); nursing teams exhibited the highest amount of cooperation/back-up behavior (3.86 ± 0.76, $P < 0.001$). Leadership was much lower among nursing and anesthetic teams (3.47 ± 0.67, $P < 0.001$ and 3.41 ± 0.64, $P < 0.05$ respectively). OTAS scores also varied significantly with operative period, revealing the preoperative period to have the highest overall score (3.74 ± 0.77, $P < 0.002$), followed by the intraoperative (3.68 ± 0.76, $P < 0.001$) period. Continued investigation showed nursing and anesthetic teams to have the highest relative scores preoperatively (3.88 ± 0.74 (nursing) and 3.83 ± 0.75 (anesthesia); $P < 0.001$) and surgical teams the highest scores

intraoperatively (4.00 ± 0.84, $P < 0.001$). Mean OTAS scores recorded for scrub technicians, during the post-operative period, and for situational awareness (behavior 5) (3.53 ± 0.69, 3.52 ± 0.68, and 3.64 ± 0.73) did not show significant variance.

CONCLUSIONS: Our analysis of teamwork behaviors in the pediatric operating rooms shows overall strong teamwork behaviors. Areas for improvement include leadership among nursing and anesthetic teams, surgical team cooperation, and preoperative teamwork behaviors of the surgical team. The lack of significance for scrub technicians, the post-operative period, and situational awareness can be attributed to consistent teamwork scores for these groups across procedures and with respect to other teams, operative periods, and behaviors. This should not discount their consideration in the scope of quality improvement. Future research will focus on using medical record analysis to retrospectively correlate these observed teamwork behaviors with pediatric surgical patient outcomes.

ABSTRACT

Enzyme-Sensitive Liposomal Payload Delivery Systems

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Sponsored by: Dr. Patrick Kee, MD/PhD, Internal Medicine/Cardiology
                Dr. Delia Danila, PhD, Internal Medicine/Cardiology
Supported by: Dr. Patrick Kee, MD/PhD, Internal Medicine/Cardiology
                The University of Texas Health Science Center at Houston – Office of the
                Dean
Key Words: Atherosclerosis, Liposomes, Macrophages

Background: Atherosclerosis is a disease of the arterial wall that can introduce chronic inflammation from local macrophages, characterized by the formation of plaques, as well as a local environment rich in cathepsin K. The current imaging technology, including the "gold standard" of invasive angiography, is limited to the detection of luminal narrowing. Tests that detect flow-limiting lesions may be unreliable, and characterizing plaque composition itself will likely improve the ability to predict future vascular events or the likelihood of plaque rupture. Liposomes are attractive agents for the molecular imaging of these atheromas as they allow for imaging via the delivery of contrast agents and also have the ability to deliver specific therapeutic payloads for treatment. Our goal is to create a liposomal delivery platform that can sense the elastolytic activity of cathepsin K and use a cleavable PEG to efficiently deliver a payload, via a receptor independent mechanism, using a membrane penetrating peptide.

Methods: During this research, pNP-PEG-DOPE cleavable lipid was synthesized to be incorporated into liposomes. Liposomes containing 1% pNP-PEG-DOPE, DPPC, and 30% cholesterol were prepared. Multi-laminar vesicles were loaded with calcein, extruded with a 0.2 micron filter, and coated with membrane penetrating protein, TAT. The liposomes were tested for their stability at 4^0C, 25^0C, 37^0C, and again at 37^0C while inoculated with 20% human serum. A macrophage cell line (variably activated with TNF-alpha) was grown and tested for cathepsin K release and used for internalization experiments.

Results: This project is ongoing. Activated macrophage cell lysate illustrated the release of cathepsin K and showed an increase in cathepsin K levels when compared to non-inflamed cells. DLS analysis of liposomes illustrated a size of 140 nm with a polydispersity index (PDI) of 0.075. Preliminary liposome stability tests illustrated the percent of liposomal content released at various temperatures. Liposomes at 4^0 C showed a 2.51% release of contents after 24 hours; at 25^0C, it showed a 9.48% content release after 24 hours; at 37^0 C, a 0.68% release after 1 hour, 1.63% at 2 hours, 5.16% at 4 hours, and a 20.77% release after 24 hours. The liposomes at 37^0 C inoculated with human serum illustrated an improved stability, releasing only 0.37% after 1 hour, 0.42% at 2 hours, 0.51% at 4 hours, and 2.51% after 24 hours.

Conclusions: TNF-alpha activated macrophages secreted higher levels of cathepsin K than non-inflamed macrophages, indicating the contribution of cathepsin K to an inflammatory macrophage environment. The payload retention of the liposomes is promising, especially in the presence of human serum proteins, and suggestive of the stability of liposomes *in vivo*. At this time, research is ongoing to determine the interaction between TAT coated liposomes and macrophage cells, as well as the cellular internalization of these liposomes.

ABSTRACT

Intracranial Hypertension and Association with Clinicoradiographic Variables in Patients with ICH

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Sponsored by: Nancy J. Edwards, MD, Department of Neurology, Department of Neurosurgery

Supported by: Nancy J. Edwards, MD, Department of Neurology, Department of Neurosurgery.

Key Words: Intracerebral hemorrhage, Intracranial pressure, Intracranial hypertension

Background: A subset of patients with intracerebral hemorrhage (ICH) will develop elevated intracranial pressure (ICP), a potentially treatable cause of secondary brain injury. Though intracranial hypertension is often seen in patients with extensive intraventricular hemorrhage (IVH), there is minimal literature on the determinants of intracranial hypertension in patients with primarily parenchymal hemorrhages, with or without intraventricular extension.

Methods: From a prospective ICH database, 71 patients with intraparenchymal hemorrhages (with or without IVH) and serial ICP monitoring were identified. Patients with primary IVH without parenchymal hemorrhage were excluded. Several variables including hematoma volumes, IVH volumes, perihematomal edema (PHE), hemisphere volumes, midline shift (MLS), and presence of herniation were analyzed to determine whether any of these variables were associated with elevated ICP. We also examined via regression analysis whether intracranial hypertension was associated with short- or long-term neurological outcomes.

Results: Of the patients included in our study, a minority of patients – only 41% – had an ICP reading ≥ 20 throughout the duration of monitoring. The relative frequency of ICP readings ≥ 20 averaged 5.8%. Peak ICP and relative frequency of ICP readings ≥ 20 were not significantly associated with PHE volume, hemisphere volume, degree of MLS, or presence of herniation; there was a trend toward an association of peak ICP with peak hematoma volume (p = 0.05). Of the patients with clinical or radiographic uncal herniation, an ICP ≥ 20 was documented in only 22.5%. Of all the radiographic variables analyzed, intracranial hypertension was significantly associated only with volume of IVH (p = 0.04). We did not find a statistically significant association between ICP and early or delayed neurologic deterioration or 90 day functional outcomes.

Conclusions: Intracranial hypertension occurred in a minority of the parenchymal hemorrhage patients studied here. Though there was a trend of higher ICP in patients with larger hematomas, the only significant association was with the extent of intraventricular hemorrhage. As previously described in ischemic stroke patients, clinicoradiographic herniation was evident with an ICP of less than 20 in a substantial number of ICH patients. Further studies with larger patient cohorts are needed to confirm these findings.

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ABSTRACT

Comprehensive Clinical Assessment Tool for Axillary Burn Contractures

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Sponsored by: David J. Wainwright, MD, Department of Plastic Surgery
Supported by: David J. Wainwright, MD, Department of Plastic Surgery
Key Words: Axillary Burn Contracture Release Evaluation

Introduction: Axillary burn contractures are one of the most common reconstructive surgery cases seen in burn centers. Despite this fact, a comprehensive tool to gauge axillary burns and the progress of surgery departments in dealing with axillary burns patients does not currently exist. There are some general forms that look at different aspects of burns including scars, shoulder range of motion, and pain. The purpose of this study was to design and implement an assessment tool for axillary burns contractures by looking at how doctors have assessed and evaluated axillary burn contractures, the treatment and surgical methods employed to address the burns and the outcomes of the treatment. The findings were subsequently organized into an assessment tool that is comprehensive, objective, user friendly and reproducible. This is the first stage of a future "whole body" form for burns that will include a wide range of assessments, specific to different regions of the body. The creation of these standardized forms is necessary because they will permit standardized care and allow for outcomes research to be conducted in the future.

Methods: A literature search for assessment modalities including range of motion and scar scales was conducted to determine what are the present day standards and options for evaluating burns, and more specifically axillary burns contractures. The literature was also reviewed for functional testing of the axilla. After conducting a thorough review of current contracture classification systems, outcomes assessment tools, questionnaires that are general and burn specific, a single assessment was created that can be used as a standard by burn centers.

Results: We were able to make both a printed hard copy of the axillary burn contractures evaluation form that can be used by hospitals as a standard for assessing burns and a writable pdf that can be added onto the electronic medical records of axillary burns contracture patients.

Conclusion: The evaluation form includes a comprehensive assessment that has specific fields with respect to range of motion, classification of deformities/contractures, a diagram of the specific region affected, scar characteristics, functional tests and a questionnaire. The form also has a systematic review of treatment methods and a review of patients' hospital experiences. There is also an outcome questionnaire that has basic information and specific application to the clinical problem/anatomical area. This is a comprehensive, all inclusive form that can become a standard tool of management with this type of burn contracture.

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ABSTRACT

What is Safe Anesthesia Induction for Infants with Pyloric Stenosis?

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Sponsored by: Maria E. Matuszczak, MD, Department of Anesthesiology
Supported by: Maria E. Matuszczak, MD, Department of Anesthesiology
Key Words: Controlled Rapid Sequence Induction, Pediatrics, Pyloromyotomy, Pyloric Stenosis, Anesthesia

Background: Despite being relatively rare, pulmonary aspiration of gastric contents is a perioperative complication with high morbidity and requires specific anesthetic strategies to avoid. One technique used in patients with increased risk of aspiration due to having a full stomach is known as rapid sequence induction and intubation (RSII). A RSII includes preoxygenation, use of succinylcholine or a high dose (1+ mg/kg) of rocuronium, cricoid pressure, and no mask ventilation until the muscle relaxant has taken full effect and the patient is ready for intubation. However, there is little to no evidence to support the efficacy of RSII in preventing pulmonary aspiration. Also, the technique inherently presents extra risks including hypoxemia and related adverse hemodynamic events in the pediatric (especially neonatal and infant) population. An alternate technique known as controlled rapid sequence induction and intubation (cRSII) has been developed which should reduce those risks associated with RSII in the pediatric population. During cRSII there is no use of cricoid pressure, and the infant is gently ventilated with a maximum peak pressure of 10-12 cm Hg until medication has taken full effect. However, more data are needed to confirm that cRSII does indeed provide better outcomes when compared to RSII in pediatric patients. A QI project was developed to examine complications involving patients with pyloric stenosis in order to prepare for an eventual prospective study.

Methods: We examined the charts of 141 infants who received an open or laparoscopic pyloromyotomy for correction of pyloric stenosis at Children's Memorial Hermann Hospital between June 2010 and September 2015. For all cases it was determined from the chart whether the anesthesiologist used RSII or cRSII, whether there was aspiration, hypoxemia (<94% O_2 saturation for 2+ minutes), or hypotension (<50 mmHg systolic pressure within 10 minutes of anesthesia induction). We also recorded the medications used for induction, and the use of succinylcholine or a high dose rocuronium was an indication that a RSII was performed.

Results: 5 charts were eliminated from the data analysis due to insufficient documentation. Of the remaining 136 cases, in 25/8/56/47 cases succinylcholine/rocuronium high dose/normal induction dose/no paralytic was used, respectively. No aspirations were noted, and there were 5 documented hypoxemic events and 10 hypotensive events.

Conclusion: The limitation of the retrospective view of our data collection does not allow any conclusions to be made concerning a difference in complications between cRSII and RSII. A prospective, descriptive study regarding this topic is being prepared for submission to IRB in order to obtain better data and confirm the safety of cRSII in the pediatric population.

ABSTRACT

Maternal Anesthesia Management for In Utero Myelomeningocele Repair

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Sponsored by: Ranu Jain, MD, Department of Anesthesiology
Supported by: Ranu Jain, MD, Department of Anesthesiology
Key Words: In utero myelomeningocele repair, volatile anesthetics, phenylephrine

Background: Corrective surgery for myelomeningocele used to occur immediately after birth, but a multicenter study revealed that in utero repairs provided better outcomes for the child. Anesthesia management during in utero myelomeningocele repairs involves decreasing uterine tone without compromising the fetus. Previous data from animal models recommend administering volatile anesthetics with a minimum alveolar concentration (MAC) of 2-3 to provide optimal uterine relaxation during fetal surgery. However, high doses of anesthetics can cause maternal hypotension and thus would require additional drugs to manage blood pressure. We hypothesized that lower doses of sevoflurane would decrease the amount of phenylephrine used and provide uterine relaxation.

Objective: To examine the effects of lower levels of volatile anesthetics on management of maternal hypotension by comparing phenylephrine dosages between patients who have received different dosages of sevoflurane during surgery with optimal uterine relaxation.

Methods: Thirty-two patients who received in utero myelomeningocele repairs between 2012 and 2016 were identified. Seven cases were excluded from the study due to insufficient data. A retrospective chart review was performed on 25 cases. The first 15 cases received sevoflurane levels within the recommended dose, while <2 MAC sevoflurane was administered to the latter 10 cases. Phenylephrine, the pressor used to maintain maternal blood pressure, was analyzed between the two groups.

Results: Patients in the second group had less expired sevoflurane (average: 3.45%, median: 3.34%) than patients of the first group (average: 4.14%, median: 4.15%). Lesser doses of phenylephrine were also administered to the second group (average: 0.44 mcg/kg/min, median: 0.37 mcg/kg/min) than to patients of the first group (average: 0.57 mcg/kg/min, median: 0.6 mcg/kg/min). On average, the second group received 16.7% less sevoflurane and 22.0% less phenylephrine than the first set of cases.

Discussion: Data suggest with a lower dose of sevoflurane the amount of phenylephrine required to provide optimal uterine relaxation was less. More cases need to be followed to determine the relation between sevoflurane and phenylephrine with respect to uterine relaxation during fetal surgery.

ABSTRACT

Pediatric Blunt Cerebrovascular Injury: the McGovern Experience

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Sponsored by: Manish N. Shah, M.D.   Department of Pediatric Neurosurgery
Supported by: Manish N. Shah, M.D.
Key Words: Pediatric BCVI incidence, evaluation, and screening

Background: While the risk factors, screening protocol, and treatment options for blunt cerebrovascular injury (BVCI) have been well described in the adult trauma literature, there has been little research performed regarding this injury in pediatric patients. The purposes of this study are to assess the incidence of BCVI in pediatric trauma patients, retrospectively evaluate risk factors and treatments in pediatric patients diagnosed with BCVI, and validate the "Utah Score", a screening tool for predicting pediatric BCVI.

Methods: This is a retrospective cohort study of pediatric patients (0-16 years) admitted with blunt trauma to Children's Memorial Hermann Hospital/McGovern Medical School, a level 1 pediatric trauma center, between January 2005 and June 2015. Digital Subtraction Angiography (DSA), MR Angiography (MRA) or CT Angiography (CTA) were used to confirm BCVI in patients with blunt trauma.

Results: 12,612 patients with a mean age of 6.6 years were admitted with blunt trauma and followed for an average of 7.7 months. Of those patients, 504 (4.0%) received angiographic imaging based on institutional criteria. Specifically, 329 (65.3%) received a CTA only, 106 (21.0%) received a MRA only, and 6 (1.2%) received DSA. The remaining 63 (12.5%) received a combination of the three imaging modalities. The incidence of BCVI in the total study was 0.17% (21 patients), significantly ($p<0.05$) lower than a comparably large adult blunt trauma group. Of the 21 patients with BCVI, 10 (47.6%) were involved in a motor vehicle accident and 2 (9.5%) presented with focal sensorimotor abnormalities. Additionally, 8 (38.1%)of the BCVI patients, were treated with Aspirin, 12 (57.1%) were given no medication, and 1 was given both Aspirin and Plavix (4.7%). When the Utah score was applied to this cohort, 10 (47.6%) patients were misclassified as "low-risk".

Conclusion: With such a low incidence of BCVI in the pediatric population and a majority of patients not requiring treatment, a more conservative approach to screening and management than is currently recommended under the Denver Scale should be adopted. However, we found a high false negative rate for the previously validated Utah Score in our equally large patient cohort, potentially suggesting that a more accurate predictive screening tool should be developed.

ABSTRACT

Pediatric facial fracture patterns in a Level 1 Trauma center: mechanisms, management, and outcomes

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Sponsored by: Dr. Matthew Greives, MD
Dr. John Teichgraeber, MD

Supported by: The University of Texas at Houston Medical School - Summer Research Program

Key Words: Facial fracture, pediatric, trauma, mechanism, management, cervical spine

Background: Pediatric facial fractures require a much different approach than adult facial fractures with respect to future growth and development, and therefore tend to be poorly understood. Often times, these cases involve a concomitant injury involving the cervical spinal cord or neurologic trauma. This study aims to expand our knowledge of the mechanisms as well as our understanding of how best to treat and manage pediatric facial fractures.

Methods: This is a retrospective analysis of demographics, mechanism, management and outcome of pediatric patients (0-18yrs) that presented to the Memorial Hermann Texas Level 1 Trauma Institute in the last ten years (January 1, 2006 to December 31, 2015) with one or more facial fractures. Charts were reviewed and data collected including, but not limited to: type of fracture, mechanism of trauma, any concomitant brain or cervical spine injuries, method of management and length of stay.

Results: An initial query identified a total of 1298 pediatric patients from above date range. At this point, XXX have been reviewed and data collected, of which XXX were excluded, as they did not meet inclusion criteria or had incomplete records. By looking at demographic information, XXX (XX%) were male while XXX (XX%) were female. The most common fracture site was the _____ (xx pts, XX%), followed by _____ (xxx pts, XX%) and _____ (xxx pts, XX%). The majority of fractures were caused by _____ (xxx pts, XX%), _____ (xxx pts, XX%), or _____ (xxx pts, XX%). XX pts (XX%) presented with an additional c-spine fracture, while xx pts (XX%) suffered from traumatic brain injury. XX (xx%) of patients also fractured their skull. XX% of patients that presented with facial fractures underwent an operative intervention, while the remaining were managed with a nonsurgical approach. In assessing the data thus far, male/female patients between the ages of xx-xx involved in motor vehicle/all-terrain vehicle accidents were the most likely to be treated operatively. Teenage male/female patients made up the majority, xx of xx (xx%), of facial fractures resulting from assault.

Conclusion: Pediatric facial fractures are sustained through various mechanisms, and require a knowledgeable approach which takes age, extent of fracture, concomitant injuries and severity of damage into consideration before deciding to intervene. As more patient charts are reviewed and data is added to our database, we hope to gain additional insight into the best modes of treatment as we improve our understanding of both the causes and outcomes of pediatric facial fractures.

ABSTRACT

A Pilot Study to Investigate a Non-Invasive Method for Diagnosing Patients with Normal Pressure Hydrocephalus

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Normal pressure hydrocephalus (NPH) is a condition that results when an excess of cerebrospinal fluid (CSF) accumulates in the ventricles of the brain. This disorder is typically characterized by urinary incontinence, dementia, and gait disturbances. However, because these symptoms overlap with other conditions, better tools are needed for an accurate, earlier diagnosis. No non-invasive method has been used to detect NPH, as a lumbar puncture is normally involved in the diagnosis of NPH. Because tissue damage usually occurs before symptoms even appear, it would aid in the diagnosis and treatment of NPH if a non-invasive method could be sensitive and specific for detecting NPH in the early stages. It is hypothesized that compression of brain tissue and fiber tracts as a result of NPH can be identified and quantified via magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) analysis. A protocol is currently being developed to utilize MRI and DTI as a non-invasive method to reliably diagnose NPH. Variables of interest were measured and compared between patients with and without NPH. Markers of microstructural integrity such as mean diffusivity, radial diffusivity, axial diffusivity, and fractional anisotropy were measured and ventricular and fiber tract volumes were obtained through DTI and MRI software packages, including DTIStudio, MRIcron, and MRIcro. These values were examined in various regions of the brain, including the caudate, putamen, limbs of the internal capsule, genu and splenium of the corpus callosum, lateral ventricles, corticospinal tracts, and somatosensory tracts. A key part of this task involved developing standard operating procedures to analyze DTI and MRI scans for other studies in the future. This study is currently being conducted and results are pending validation.

ABSTRACT

Infidelity: Cheating on the Pre-incision Surgical Safety Checklist

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Sponsored by: Kuojen Tsao, MD, Department of Pediatric Surgery

Supported by: KuoJen Tsao, MD, Department of Pediatric Surgery; McGovern Medical
                 School – Office of the Dean

Key Words: Fidelity, Patient Safety, Surgical Safety Checklist (SSC)

Background: In 2008, the World Health Organization published guidelines for implementation of a 3 phase Surgical Safety Checklist (SSC) with the goal of reducing medical errors in the operating room. Since then, the pre-incision, or "timeout" phase of the SSC has been the most widely adopted. Children's Memorial Hermann Hospital (CMHH) has implemented all 3 phases- pre-induction, pre-incision and debriefing of the SSC, and holds annual, mandatory checklist and OR safety training with all surgical team members to improve adherence to the SSC. CMHH training has focused on the pre-incision phase during these trainings. We hypothesize that focused, multi-faceted training interventions will improve or maintain high adherence and fidelity (meaningful completion) of the pre-incision checklist over time.

Methods: Four trained observers conducted a direct observational study between May and July 2016, documenting surgical team adherence for the perioperative pre-induction, intraoperative pre-incision, and post-operative debriefing aspects of the SSC. Fidelity - a measure of complex completion of a checklist point requiring inter-team communication and coordination above simple verbalization – was recorded for 11 of the 19-point pre-incision checklist in 2016 and 2015, and 5 of the 19 in 2014. The pre-incision data was statistically analyzed for significant differences to 2014 and 2015 fidelity data, which was identically collected by four trained observers. No fidelity was recorded for the pre-induction and debrief portions of the SSC. Kruskal-Wallis test by ranks was utilized to determine significance between years and inter-rater reliability was performed to insure accurate data collection.

Results: 287 pediatric surgical operation pre-incision checklists were observed across 9 specialties in 2016, 212 operations were observed in 2015, and 206 operations in 2014. Overall pre-incision SSC fidelity in 2016 ranged from 62.1% to 99.6% with an average of 90.9%, which is significantly different from the overall averages of 78.4% in 2015 and 84.4% in 2014. ($p<.01$). Specifically, the "estimated blood loss" (43.3-97.8%, $p<.01$) and "essential imaging displayed" (35.9-62.1%, $p<.01$) displayed the most drastic differences between 2015 and 2016. Noticeable trends were noticed for "All quiet" (66.2-90.6%, $p<.01$) and "All equipment available" (84.5-92.4%, $p<.01$), increasing year on year from 2014-2016. Performance did not decrease significantly in any aspect of the pre-incision SSC. Inter-rater reliability kappa was, 0.70 in 2015, and 0.756 in 2016.

Conclusions: Overall pre-incision SSC performance at CMHH has improved annually from 2014-2016 evaluated through fidelity specifications. However, several key aspects such as ensuring essential imaging is displayed, can still be improved, and targeting them in future interventions will yield the greatest benefits in patient safety and quality of care.

ABSTRACT

Next Generation Sequencing of Biopsy and Surgical Specimens to Create a Tumor-specific Molecular Profile Database

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Sponsored by: Robert Amato, DO, Department of Internal Medicine –Division of Oncology

Supported by: Robert Amato, DO, Department of Internal Medicine –Division of Oncology, The University of Texas at Houston McGovern Medial School-Office of the Dean

Key Words: NGS, solid tumors, precision medicine, biomarkers

Background: Cancer remains the number two cause of death worldwide. A total of 1,685,210 new cancer cases and 595,690 cancer deaths are projected to occur in the United States in 2016. While there has been progress in cancer treatment such as with chemotherapy, surgery, and radiation therapy, new advances in diagnosis and treatment of certain cancer types rely on a more individualized approach. This is based on the shifting perspective of classifying tumors based on their genomic profile instead of site of origin, with the key hypothesis that different tumor types with the same molecular profile will respond to the same pathway specific drug. Currently, researchers use molecular profiling with next-generation sequencing (NGS) to sequence cancer genomes and classify them based on a panel of signature genomic alterations. Previous studies have determined "driver gene" mutations and subsequent cell signaling pathways activated in cancer, leading to the development of pathway-specific drugs. The aim of this project is to create a tumor-specific molecular profile database with the ultimate goal of using cancer biomarkers to select the drug of choice for therapy. This would avoid the trial-and-error approach for cancer treatments and the adverse effects of ineffective therapies, thus improving treatment responses. In addition, newly identified biomarkers can translate into targets for future drug development.

Methods: We collected primary or metastatic tumor biopsies when patients underwent surgical excision or biopsy for cancers ranging from prostate, renal, bladder, lung, pancreatic, uterine, colon, breast, and head and neck. We performed Ion-Torrent™ NGS on fresh tissue using the Oncomine^R Gene Panel, a universal cancer panel that detects cancer-specific genomic variants for 143 unique genes. The genes included are known, actionable mutations (can be matched to an available targeted drug therapy) that are potentially responsive to targeted drug therapies.

Results: We successfully sequenced 36 solid tumor biopsies with NGS. Across all tumor types, we found actionable and nonactionable genomic alterations, including insertions and deletions (indels), copy number variations (CNV), fusions, and synonymous variants. The most common mutation genes detected were ATM, TP53, BRCA2, and EGFR among others.

Conclusion and Future Directions: The Oncomine Comprehensive Cancer Panel is a valid screening assay to detect molecular alterations and can be used as a clinical tool to help clinicians in diagnosis and treatment. We will conduct further experiments such as culturing 3D tissues and circulating tumor cells and applying targeted drug therapies to validate our hypothesis that an actionable variant matched with a targeted therapy results in the same treatment response regardless of cancer type.

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ABSTRACT

Proteolytic mechanism of transthyretin-specific catalytic antibodies

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Transthyretin (TTR) serves as a blood-borne carrier thyroid hormone, retinol binding pigment and Vitamin A. Misfolded forms of TTR (misTTR) are associated with age-related TTR amyloidosis {ATTRm). Amyloidogenic misTTR forms toxic soluble oligomer and fibrillar deposits in multiple organ systems, including kidney, gastrointestinal tract, adrenal gland, gallbladder, bladder, and pancreas. No approved pharmacological agents are available for treating ATTRm. Previous work by Paul and Planque describe polyclonal nucleophilic catabodies from the sera of healthy humans that degraded misTTR but not physiological TTR (phyTTR) or irrelevant proteins. Catalytic antibodies can complete multiple cleavage cycles without forming immune complexes and thus avoid the inflammatory side-effects seen with non-catalytic antibody-antigen binding. Thus, high speed and specific monoclonal antibodies hold potential for therapeutic degradation and dissolution of misTTR amyloid deposits.

In my summer research project, I studied the proteolytic mechanism of two newly-isolated misTTR-directed monoclonal IgMs (clones lOElO and SES). The monoclonal IgMs were obtained by covalent binding of electrophilic misTTR (E-misTTR) to B cells receptors on human B cells, flow cytometric sorting of the E-misTTR labeled cells, and Epstein-Barr virus immortalization of the B cells. Consistent with the selection principle, the catalytic hydrolysis of synthetic misTIR amyloid by both IgMs was inhibited completely by an electrophilic hapten, indicating a serine protease mechanism of the proteolytic reaction. Pepstatin, an inhibitor of the acid protease enzyme family, was without effect on the IgM catalytic activity. Prolonged treatment with the chelating reagents EDTA or 1,10-phenanthroline resulted in inhibition of IgM catalytic activity, indicating divalent metal dependence. No misTTR hydrolysis was evident upon EDTA removal from the EDTA/IgM mixture by dialysis prior to assay of misTIR hydrolysis, indicating that removal of IgM-bound metal rather than misTTR-bound metal was the reason for the EDTA inhibitory effect. The catalytic activity was restored upon reconstitution of the EDTA-treated IgM with low µmolar zn^{2+} concentrations. Interestingly, treatment with iodoacetamide, a reagent that inhibits cysteine proteases by alkylation of the active site -SH group, caused an enhancement of IgM misTIR hydrolytic activity. Disulfide bond exchange reactions between several Cys residues located on the heavy and light chain subunits can result in disruption of the native quartenary IgM structure that maintains IgMs in their physiological pentameric form. Iodacetamide likely alkylates any free -SH groups generated during the exchange reactions, thereby stabilizing the IgM pentameric

structure and expression of improved catalytic activity. From these data, the IgMs can be anticipated to provide therapeutic clearance of misTTR amyloid in environment that contain sufficient Zn2+ and are deficient in serine protease inhibitors.

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ABSTRACT

Quality of Life Comparison of Cervical and Lumbar Radiculopathy

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Sponsored by: Mark Burish, M.D., Ph.D, Department of Neurosurgery
Supported by: Mark Burish, M.D., Ph.D, Department of Neurosurgery
Key Words: Lumbar and cervical radiculopathy

PURPOSE/BACKGROUND: Lumbar and cervical radiculopathy are widely prevalent diseases in the population that can impact day-to-day activities such as the ability to move, mood, and work. A substantial number of patients with these diseases will fail conservative management by primary care providers (such as medications and physical therapy referrals). These patients may require fluoroscopically-guided epidural steroid injections before considering surgery, due to preferences of insurance companies and spine surgeons. Unfortunately, there are a limited amount of pain management physicians and interventional radiologists who are trained to do these injections. The purpose of this study was to see if quality of life differs in patients with cervical radiculopathy versus lumbar radiculopathy, and hence from a population standpoint which group of patients should have preferential access to this limited resource of pain management physicians. In essence, this study was looking to determine which one would be best to treat in order to improve quality of life.

SUBJECTS AND METHODS: All patients scheduled for a lumbar or cervical epidural steroid injection at a single pain clinic over a ten-week period were included for this study. A total of 72 patients were screened and 33 were enrolled. Patients received a survey and physical exam prior to their injection. The survey consisted of questions that were determined to be good indicators of pain level (Numerical Rating Scale and Short Form McGill Pain Questionnaire), disability (Brief Pain Inventory Interference and Pain Self-Efficacy Questionnaire Short Form), mobility (Tampa Scale for Kinesiophobia, Hypermobility Questionnaire), and mood (Patient Health Questionnaire 2 and the General Anxiety Disorder 7). The physical exam consisted of flexibility testing with the Brighton criteria.

RESULTS: With the exception of walking disability being strongly associated with lumbar radiculopathy, no significant differences between lumbar and cervical radiculopathy were found for pain, disability, mobility, or mood in this small study. In a subgroup analysis of 26 lumbar radiculopathy patients, anxiety levels did not correlate with pain level, disability, or mobility.

CONCLUSION: Based on this data, there is no difference in quality of life between cervical and lumbar radiculopathy. This study provides good pilot data for a larger comparison of lumbar and cervical radiculopathy. It also suggests that patients should be referred equally if they have either lumbar or cervical radiculopathy to pain management physicians for an epidural steroid injection.

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ABSTRACT

Defining a Stellate Cell Axis in Liver Disease and HCC

Eric Yoon   *McGovern Medical School at UTHealth*   *Class of 2019*

Chronic liver diseases represent a rising public health concern both in the United States and throughout the world. Non-alcoholic fatty liver disease (NAFLD) affects an estimated 25% of the world population; furthermore, almost 60% of these patients develop non-alcoholic steatohepatitis (NASH) related fibrosis and cirrhosis of the liver. Nearly 30% of patients with cirrhotic livers will subsequently develop hepatocellular carcinoma (HCC). HCC is the fifth most common malignancy in the world, yet it is the second leading cause of cancer death. HCC incidence is steadily increasing each year due to the increase in chronic liver diseases. Of all HCC's, 80-90% arise in cirrhotic livers. Recently, the role of hepatic stellate cells (HSCs) have been a major focus of research. Studies have shown HSCs are activated after injury to the liver. Activated HSCs upregulate pro-inflammatory pathways such as IL-17 and alter the hepatic parenchymal architecture through changes in ECM composition. Additionally, are involved in the initiation and progression of HCC. Malignant potential is increased through various mechanisms such as promoting epithelial to mesenchymal transformation (EMT) of dysplastic and malignant hepatocytes and through altering epithelial-ECM pathways. Our guiding hypothesis is that activated HSCs regulate interactions between the liver stroma and parenchyma through paracrine signals that promote malignant transformation of the liver epithelium. The interaction between hepatic epithelial cells and HSCs was studied using the ATCC Liver Cancer Panel and an immortalized stellate cell line. Supernatant from stellate cell cultures as well as the pro-inflammatory cytokine IL-17 were added to both hepatoma cells and stellate cells, and changes in protein expression and transcription were studied. Additionally, samples of healthy, fibrotic, cirrhotic and malignant livers were obtained from the McGovern Medical School Department of Organ Transplantation. These samples were sectioned and probed for various known markers of malignancy, with a major focus on an uncharacterized role for c-myc and p53 activation in the stroma. Primary cell cultures were also grown using these samples, and the interaction between the HSCs and epithelial cells were studied in a similar manner to the work previously done with the immortalized cell lines. Work is still ongoing, and results are pending.

ABSTRACT

Opposing Effects of TGF-(3 and BMP2 on MicroRNA-200b and Pancreatic Fibrosis

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Sponsored by: Tien C. Ko, MD, Yanna Cao, MD, Department of
Surgery Supported by: NIH grant 2T35 DK007676-23.
Key Words: Chronic Pancreatitis, micro-RNA, Fibrosis

Introduction: MicroRNA (miR)-200b is an anti-fibrogenic regulator in the lungs and liver, but its effects in the pancreas are not clear. We reported that bone morphogenetic protein (BMP)2 opposes the pro-fibrogenic effects of transforming growth factor (TGF)-P in the pancreas. We hypothesize that miR-200b mediates the opposing effects of TGF-P and BMP2 on pancreatic fibrosis; thus miR-200b level may be a defining factor during chronic pancreatitis (CP) progression. To test our hypothesis in this study, we examined miR-200b level in mouse CP in vivo and the fibrogenic response to miR-200b regulation in pancreatic cells in vitro.

Methods: CP was induced in C57BL/6 mice by cerulein (50μg/kg, 5 IP injections/day, 3 days/wk for 4wks). Control mice received normal saline (n=4/group). Pancreatic fibrosis was assessed by Sirius red staining and qPCR for extracellular matrix (ECM) collagen and fibronectin measurement. In vitro, primary human pancreatic stellate cells (hPSCs), the key cells that produce ECM leading to pancreatic fibrosis, were used. The cells were treated with vehicle, TGF-P (Ing/ml), BMP2 (50ng/ml), or TGF- & BMP2 for 24 hrs and harvested for miR-200b measurement by qPCR, for 48 hrs and the conditioned media were collected for fibronectin secretion by Western blotting. In a separate set of experiments, the cells were transfected with miR-200b inhibitors (30nM), mimics (30nM), or control with transfection reagent alone for 48 hrs and the conditioned media were collected.

Results: In CP mice, miR-200b level decreased by 70% compared to control mice and inversely correlated with fibrosis ($p<0.05$). In hPSCs, compared to control, miR-200b level was not altered by TGF-P alone, but increased by BMP2 alone by 150%, and the BMP2's effect was abolished with the combined treatment. Fibronectin secretion was induced by TGF-

p alone by 150%, but unaltered by BMP2, and the TGF-P's effect was abolished with the combined treatment ($p<0.05$). miR-200b inhibitor induced fibronectin secretion by 135%, while miR-200b mimic did not alter secreted fibronectin level, compared to control ($p<0.05$). **Conclusions:** TGF-P and BMP2 have opposing effects on miR-200b and fibronectin secretion in hPSCs. The inversely correlated miR-200b level with pancreatic fibrosis in CP and the stimulating effect of fibronectin secretion by miR-200b inhibitor in vitro suggest that miR- 200b is anti-fibrogenic in the pancreas. However, whether miR-200b mediates the opposing effects of TGF-P and BMP2 on pancreatic fibrosis warrants further investigation.

ABSTRACT

Hypertension More Associated than BMI with Thickened cIMT in Children and Adolescents

Jesse Zamarripa   *McGovern Medical School at UTHealth*   *Class of 2019*

Sponsored by: Joshua A. Samuels, MD, MPH Pediatric Nephrology Pediatric Medicine
Supported by: NIH Grant 2T35 DK007676-23, Joshua A. Samuels, MD, MPH Cynthia Bell
Key Words: cIMT, carotid Intima media thickness, Hypertension, Obesity, Children

Background: Hypertension (HTN) and obesity are risk factors associated with increased carotid intima media thickness (cIMT), a marker of atherosclerosis, stroke and target end damage. cIMT increases with blood pressure (BP) independent events (age and height) and also BP dependent events. The aim of the study was to determine the relationship between BMI, blood pressure, and carotid measurements by comparing children categorized into four groups; normotensive-normal weight, normotensive-obese, hypertensive-normal weight, hypertensive-obese. **Methods:** Systolic or diastolic BP averages greater than the 95th percentile for gender, age and height were used to classify hypertension status. Hypertension status was confirmed through 24-hour ambulatory blood pressure using current AHA criteria. BMI greater than 95th percentile was used to determine obesity. The subjects' cIMT (left, right, or either) was compared to 95th percentile normative values. Demographics, covariates, and outcomes were reported and tested using Kruskal-Wallis test for continuous variables and Fisher exact or Chi squared for categorical variables. **Results:** The study included 79 children aged 7-18 who were matched by gender and height. Distribution was normotensive-normal weight (n=23), normotensive-obese (n=11), hypertensive-normal weight (n=23), hypertensive-obese (n=22). There were more Black subjects in the hypertensive-obese category compared to other groups. Results reveal that HTN is more associated than BMI to increased cIMT. Of 45 with HTN, 33 had increased cIMT (p<0.02). Although obese children were not more likely to have increased cIMT (p=0.23), the presence of obesity with hypertension further increases the cIMT (82% increased cIMT). Girls were wore likely to have increased cIMT compared to boys (83% versus 52%; p=0.01). **Conclusions:** Childhood hypertension is associated with increased cIMT regardless of BMI. The presence of obesity increases the risk of elevated cIMT, but only in hypertensive children.

Undergraduate Students

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Health Science Center at Houston

ABSTRACT

Role of Renin-Angiotensin Receptors in Increased Intraluminal Stress Caused Aortic Remodeling in Mice

Erin Arthur   *Rensselaer Polytechnic Institute*   *Class of 2018*

Background: Thoracic aortic aneurysms and dissections are the primary cause of death for about 10,000 people in the US each year, and contributing factor in nearly 7000 other deaths. This disease has both genetic and environmental causative factors, and the Renin-Angiotensin System (RAS) has been shown to play a pivotal role in aortic remodeling. Angiotensin receptor 1 (AT1R) blockade, losartan, reduces aortic dilatation significantly both in Marfan Syndrome patients and mouse models. Our previous study illustrates how ascending aortic remodeling caused by transverse aortic constriction (TAC) can be attenuated by losartan.

Objective: Our study will pursue the effects of specific RAS receptors, AT1R, AT2R and Mas Receptor (MASR) and possible pathways under this receptors in the mouse TAC model.

Methods: This study establishes induced aortic dilatation in mice through TAC surgery. Mice are grouped into 9 categories: those who undergo wild-type (WT) sham surgery, WT TAC, WT TAC in addition to drug treatment, and Agtr1a null mice who receive both TAC and sham surgery. The drug treated mice undergo treatment pre- and post-surgery with agonists and antagonists in the RAS. The drug-treated groups are Losartan, Captopril, an ACE inhibitor (ACEi), as well as Captopril plus AT2R agonist Compound 21, Captopril plus p53 inhibitor Pifithrin-α, and Captopril plus MASR agonist AVE 0991. These mice are assessed 3 days post-operation through Doppler analysis with VEVO 3100 imaging system to measure relative flow velocities in the left and right carotid arteries. A successful surgery will induce a blood flow ratio between 5 and 10. Two weeks post-operation, the mice are again assessed to measure the relative flow velocities in the left and right carotid arteries, aortic sinus and ascending aorta diameter, intraluminal blood pressure through catheterization via Millar system. The mice are then sacrificed and ascending aortas are excised for histology, qPCR, and Western Blot assay.

Results: AT1R blockade losartan attenuated aortic remodeling in TAC mice models, while ACEi Captopril caused 50%-60% deaths. However, TAC mice treated with Captopril plus p53 inhibitor Pifithrin-α show a lower death rate.

Conclusion: We speculate that AT2R and MASR blocked by Captopril lead to remodeling failure post-TAC. These increased fatalities may be associated with vascular smooth muscle cell apoptosis in the ascending aorta.

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ABSTRACT

Development of Computer Assisted Micro-Neurosurgical Robot

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Sponsored by: Daniel H Kim, MD, Department of Neurosurgery; Dong Suk Shin, PhD, Department of Neurosurgery

Supported by: Daniel H Kim, MD, Department of Neurosurgery

Key Words: Robotic surgery system, Minimally Invasive Surgery (MIS), Microsurgical, Neurosurgery,

Introduction:
Minimally Invasive Surgeries (MIS) decrease postoperative pain, reduce hospital stay, and allow patients to return to normal activities quicker. Computer-assisted micro-neurosurgical robots allow surgeons to perform more complex surgeries with better dexterity and at a microscopic level. Single port surgeries are hard to perform with current robotic surgery systems due to size and limitations of the rigid arms. Current robotic surgery systems have mechanical drawbacks as well (i.e. hysteresis, backlash, and no haptic feedback). The goal of the project is to create the world's first microsurgical robot with flexible snake-like arms for single port surgeries, which can perform complex microsurgeries while overcoming current mechanical drawbacks.

Methods:
To engineer and design this surgical robot, research and analysis were done on previous surgical robots, arm joint types, and activation techniques. Joints and parts are designed and assembled into a working prototype. Each prototype is then tested. Refinement is done based off the results from tension, friction, and performance testing. Design and refinement are still happening.

Results:
Prototypes of different arm joints and types have been manufactured and assembled. Testing of each prototype refines next prototype. During my time here the joint types were tested and redesigned to reduce backlash of the tendons during actuation. New joints and parts are still being tested. New flexible arms with tendon sheath mechanism are being started.

Conclusion:
As of 7/29/16, research and development is ongoing, therefore no conclusions are available at this time.

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2016 Summer Research Program
Office of Educational Programs

Undergraduate Student

ABSTRACT

Oxidative Stress Alters Splicing of Soluble Guanylyl Cyclase

Mohan Bhadriragu   *Rice University*   *Class of 2019*

Sponsored by: Iraida Sharina, PhD, Department of Internal Medicine
Supported by: Iraida Sharina, PhD

Background: Soluble Guanylyl Cyclase (sGC) is a protein that acts as a receptor for nitric oxide (NO). NO is produced by stimulated endothelial cells of the blood vessel. NO diffuses into the smooth muscle cells and activates sGC by binding to the heme moeity, which activates the conversion of GTP to cyclic GMP (cGMP) . Increase of cGMP induces a number of important downstream enzymes and causes the smooth muscle to relax. The molecular mechanisms of this process, known as vasodilation, are being studied because of their implications in cardiovascular disease.

Objective: Previous research has shown that sGC expression and activity can be modulated through changes in alternative splicing. Alternative splice variants of sGC can rise when cells are exposed to oxidative stress. One splice variant, C-a1 sGC, is resistant to oxidative stress. Our experiment attempted to modulate alternative splicing of sGC by treatment with morpholino oligonucleotides targeting the canonical splice site of the sGC gene.

Methods: To establish the effect of oxidative stress on sGC splicing, we used mammalian cells treated with several oxidative reagents. MDA-468 (human adenocarcinoma cells) and BE-2 (human neuroblastoma cells) were used for the experiment because they naturally express both splice variants of our interest. To recreate conditions of oxidative stress, we applied hydrogen peroxide (H202), ODQ (heme oxidant), and glucose oxidase (GO) to cell culture. We identified the concentration and incubation time with H202 treatment which allows us to see a switch from one splice isoform to another. Then we treated cells with morpholino oligonucleotides targeted to the 5'splice site of the sGC gene. This approach allows us to manipulate the artificial switch of sGC splicing towards the oxidation-resistant splice variant. By applying different concentrations of MO and changing incubation time, we identified the optimal conditions for treatment. During the course of the experiment, the cells were cultured to 80% confluence and media was changed right before treatment. After treatment, cells were collected for RNA purification and analysis. RNA was purified with the Ambion RiboPure kit (Thermo-Fisher Scientific). We measured RNA concentration in our samples with a spectrophotometer and confirmed RNA integrity with agarose electrophoresis. RNA samples were then converted to cDNA using reverse transcription reaction (Invitrogen) and expression of splice variant was identified with polymerase chain reaction (PCR).

Results: We confirmed that the treatment with H202 induces the expression of the oxidation-

resistant splice variant in our cell models. We also demonstrated that treatment with MO induces a similar effect and increases the expression of alternative sGC transcript at an oligo concentration of 15 μM and the incubation time of 48 hours. Our results validate the idea that splicing of the sGC gene can be altered by oligonucleotide treatment

ABSTRACT

Jasmine Breaux   *Baylor University*   *Class of 2017*

Sponsored by: Darren Boehning, Ph.D., Department of Biochemistry and Molecular Biology
Supported by: Darren Boehning, Ph.D.
Key Words: Ubiquilin, ALS, Chaperone

BACKGROUND: Amyotrophic lateral sclerosis (ALS), or Lou Gehrig's disease, is a motor neuron disease resulting in muscle atrophy and death. This disease affects nearly 30,000 people and has also been linked to frontotemporal dementia(FTD). A small percentage of ALS or ALS-FTD cases are caused by inherited mutations in genes that affect neuronal proteostasis. Most mutations are thought to lead to aggregation of proteins such as TAR DNA-binding protein 43 (TDP-43) and tau, leading to toxic aggregation and motor neuron death. It has been shown that the protein ubiquilin-1 behaves as a molecular chaperone by binding to unfolded polypeptides and preventing their aggregation. Recently, mutations in a closely related family member, ubiquilin-2, have been shown to cause ALS. Both ubiquilin-1 and ubiquilin-2 belong to the UBL (ubiquitin-like) and UBA (ubiquitin-associated) family of proteins and share a high degree of homology. Based on these findings, we anticipate ubiquilin-2 to also function as a molecular chaperone by preventing protein aggregation and ultimately neuron death. **METHODS:** We tested our hypothesis in two specific aims; the first was to determine whether or not ubiquilin-2 acted as a molecular chaperone by using citrate synthase, a model chaperone client sensitive to thermal denaturation, in an inactivation assay. Our second aim was to observe the effects of ALS-causative mutations P189T and Q425R on ubiquilin-2 chaperone activity. The mutations were made via PCR-based mutagenesis. Wild type and mutant UBQLN2 protein was isolated by metal affinity chromatography from E. coli BL21 cells and checked for purity via gel electrophoresis. Citrate synthase (CS) thermal inactivation curves then monitored via spectroscopic assay for 20 minutes by holding CS constant and increasing ubiquilin-2 in 1:1, 1:5, and 1:10 molar ratios.

RESULTS: There was much less expression and more degradation of mutant protein P189T and Q425R on our gel in comparison to the WT ubiquilin-2. CS inactivation was observed in the presence of WT ubiquilin-2 protein by t=14 in both the 1:1 and the 1:3 assay. Preservation of CS activity in the 1:3 ratio was greater than that in the 1:1 ratio, 100% and 50% respectively. However, the results did not achieve statistical significance.

CONCLUSION: STI1 mutantsP189T and Q425R have a negative effect on protein stability and ability to achieve native structure, and these mutations may lead to lower levels of protein in motor neurons. Ubiquilin-2, unlike ubiquilin-1, does not have intrinsic molecular chaperone activity for citrate synthase. Future studies will need to be conducted to determine whether or not ubiquilin-2 functions as a molecular chaperone for other proteins involved in ALS such as TDP-43.

ABSTRACT

Expression and biochemical characterization of human proteins involved in microcephaly

Karina Cabrera   *University of Houston*   *Class of 2017*

Background: Aminoacyl-tRNA synthetases (aaRS) are responsible for pairing amino acids to their corresponding tRNAs. In the event that there is not efficient aaRS activity it will lead to protein synthesis defects. It has been shown in earlier research that mutations in GlnRS leads to a neurological disease called progressive microcephaly, in which the brain does not fully grow resulting in a small head. In conjunction with GlnRS mutations, mutations in human tryptophanyl-tRNA synthetase (WARS) and tRNA modification enzyme (METTL1) have been found in patients with the same disease. However, how mutations in WARS and METTL1 affect the function and folding of the proteins remain to be characterized.

Hypothesis: Our project focuses on biochemically characterizing the mutations in WARS leading to microcephaly. The mutations in WARS are expected to cause reduced activity in ligating tryptophan to tRNA^{Trp} and therefore decrease overall protein synthesis rates. Likewise mutations in METTL 1 are expected to lead to defects in tRNA modification and protein synthesis.

Methods: The *E. coli* system was used to produce the proteins of interest from either WARS or METTL 1. An optimized hWARS gene or METTL 1 was cloned into a pET28 plasmid and expressed in a Rosetta pLysS competent strain. Two mutations were introduced into the WARS genes in order to compare to the wildtype. The proteins will be purified using a Ni-NTA resin that binds His-tagged proteins. After purification assays to test protein activity will be used on the proteins from each gene and compared to the mutants. One assay will look at aminoacylation activity in order to quantify the amount of charged tRNA. Another assay may quantify protein misfolding.

ABSTRACT

Determining the Predictive Value of Fecal Inflammatory Markers in Recipient Stools for Success and Failure of Cure after Fecal Microbiota Transplantation of Patients with Recurrent *Clostridium difficile* Infection

Amaris Cardenas   *University of Dallas*   *Class of 2017*

Sponsored by: Herbert L DuPont, MD, Center of Infectious Diseases, University of Texas School of Public Health

Supported by: Herbert L DuPont, MD, Center of Infectious Diseases, University of Texas School of Public Health

Key Words: *Clostridium difficile*, fecal microbiota transplantations, cytokines

Introduction: *Clostridium difficile* infection (CDI) is the most common hospital-associated infection causing 70% of all fatalities from a diarrhea pathogen in the U.S. It develops in people with depleted gastrointestinal microbiota and recurs in 25% of patients (RCDI). The treatment of choice for RCDI is microbial restoration in a process called fecal microbiota transplantation (FMT) which cures ~90% of patients treated once with donor stool microbiota. There is currently no biomarker of success to determine which patients with CDI should receive FMT and to predict success in the tens of thousands of patients seen each year with this complication. It has been demonstrated that in CDI immunoloregulatory cytokines increase in stool samples. In this study, we measured Interferon-gamma (IFN-γ), important in innate and adaptive immunity, IL-6, both an pro-inflammatory and anti-inflammatory cytokine which stimulates production of IFN-γ, IL-12, important in natural killer cells and IL-12, part of IL-10 superfamily important in innate immunity, in stools of recipients of FMT pre-and 7 days after FMT. 51 patients with a history of $\geq$ 3 separate bouts of CDI in the past 12 months undergoing FMT were included in the study. Cytokines were measured by Quantikine ELISA kit from R&D Systems using 50mg of stool per subject. Values were expressed in pg/mL.

Results: Median fecal levels of IFN-γ were 214.5 (0-2091.8) in the baseline sample and 0 (0-1148.2) ($P= 0.0003$) in the stool sample obtained 7 days later. IL-6, IL-12, and IL-22 levels were not elevated in the stools pre-or post-FMT. None of the four test cytokines related to response to FMT, cure or failure.

Discussion: Fecal IFN-γ levels appear to be elevated in patients with multiply-recurrent CDI and likely reflect important immune events in these patients. Fecal IFN-γ levels may be used as a marker of success of FMT if studied in a larger sample size. The low failure rate in this study may have explained the lack of significance in comparing the groups. The limitations of this study are small number of subjects included and limited number of cytokines evaluated. We will need to increase this sample size and to follow the IFN-γ response beyond 7 days after FMT to see if failures of FMT can be predicted by a subsequent rise in the cytokine.

Appendix:
Table 1. Selected Cytokines in Pre-FMT and Post FMT Stools in 51 Patients with ≥ 3 Bouts of
Clostridium difficile Infections in the Past 12 Months

Cytokine	Number of Samples	Pre-FMT		Day 7 Post		P Value
		Median	Range	Median	Range	
IL-6	51	0	0	0	0-99.8*	NS
IL-12	48	0	0	0	0	NS
IL-22	48	0	0	0	0	NS
IFN-γ	48	214.5	0-2091.8	0	0-1148.2	0.0003

FMT= Fecal Microbiota Transplantation
*3 patients out of 51 gave a recorded value

Table 2. Cytokine Response Pre-FMT as Predictors of FMT Clinical Response

Cytokine	Median Value		P Value
	Cure	Failure	
IL-6	0	0	NS
IL-12	0	0	NS
IL-22	0	0	NS
IFN-γ	101.4	268.6	0.4627

FMT= Fecal Microbiota Transplantation

ABSTRACT

Pharmacological Effects on the Biochemical and Electrophysiological Properties of Neurons

Amber Darr   *McGovern Medical School*   *Class of 2020*

Two projects investigated the effects of pharmacological agents on the biochemical and electrophysiological properties of neurons. One project sought to further elucidate the actions of doxorubicin (DOX), a chemotherapeutic agent suspected to affect neuronal plasticity and learning. Treatment with DOX is known to cause chemotherapy-induced cognitive impairment, also known as "chemobrain", a side effect resulting from its non-specific actions. Chemobrain affects about one third of cancer patients undergoing treatment however the cellular mechanisms underlying this phenomenon remain largely unknown. It has recently been shown that DOX treatment of sensory neurons results in increased levels of phosphorylated extracellular signal-regulated kinase (pERK) and p38 mitogen-activated protein kinase (p-p38 MAPK), two MAPK isoforms important to long-term memory, during and immediately after treatment. Increased pERK levels persist for 24 h following treatment while p-p38 MAPK levels return to baseline within two hours of treatment. Down-regulation of MAPK phosphatase-1

(MKP-1) has been implicated in this activation of pERK and p-p38 MAPK by DOX. In the present study sensory neurons of Aplysia were used to further examine MKP-1 modulation following DOX treatment. MKP-1 levels were decreased one h after a two-h DOX treatment and returned to baseline within two h of treatment. These results correlate with the time course of p-p38 MAPK activation suggesting immediate increased activation of p-p38 MAPK may be due to a decrease in expression of MKP-1. The finding that pERK levels remain elevated after MKP-1 levels reverse suggests that ERK activation depends upon another mechanism, at least at later times after DOX treatment.

The second project examined the effects of levodopa (L-DOPA), the predominant treatment for Parkinson's disease, on synaptic connections. Although L-DOPA is known to alleviate the symptoms of Parkinson's by increasing dopamine levels in the brain, its specific effects on synaptic connections are not well characterized. Voltage-sensitive dye (VSD) recordings were used to measure the voltage response of neurons before and after L-DOPA treatment. Two synaptic connections were identified using this method. L-DOPA treatment induced an increase in the strength of one the connections and a decrease in strength in the other. A vehicle group was also examined in which one synaptic connection was identified. Vehicle treatment did not induce a change in this synaptic connection. These results suggest that L-DOPA may reduce symptoms of Parkinson's Disease by modulating the strength of specific synaptic connections in

the network, which would in turn increase the likelihood of successful motor programs emerging from neural activity.

ABSTRACT

Metabolic Remodeling Precedes Pressure Overload-Induced Left Ventricular Hypertrophy in Spontaneously Hypertensive Rats

Gina De La Guardia   *Rice University*   *Class of 2019*

Introduction: With long standing hypertension, the heart remodels to sustain its function. Metabolically, the heart muscle switches from fatty acids to glucose as its main source for energy provision. Structurally, it hypertrophies. Whether both processes occur independently, or if they are interrelated is not known. The objective study was to examine the progression of remodeling of hearts subjected to chronic pressure overload. Our hypothesis was that metabolic remodeling precedes and sustains structural remodeling.

Methods: To test our hypothesis, we used three-month-old rats from Charles River Laboratories: Wistar Kyoto Rats (WKY) as the control group and Spontaneously Hypertensive Rats (SHR) as the experimental group. To assess rates of glucose oxidation, cardiac power (CP), myocardial oxygen consumption (MVO_2) and cardiac efficiency (CE) in response to increased workload, hearts were perfused as working hearts. Briefly, rats were anesthetized with chloral hydrate (300 mg/kg), heparin was injected, and their hearts quickly excised and placed in ice cold buffer. The aorta was cannulated and retrogradely perfused with buffer at 37ºC. The perfusate consisted of Krebs-Henseleit buffer equilibrated with 95% O_2, 5% CO_2, glucose (5 mM) and oleate (0.4 mM) dissolved in 1% albumin plus 80 µCi/L [U-14C]-glucose. At baseline, hearts were perfused at a physiological workload (afterload of 100cm H_2O) for 30 minutes after which the hearts were subjected to increased workload (afterload of 140 H_2O plus 1 µM of epinephrine added to the perfusate). The atrial filling pressure was maintained at 15 cm H_2O. At the end of the each perfusion, the hearts were freeze-clamped and their wet and dry weights were determined. The rates of glucose oxidation were measured by determining the content of $^{14}CO_2$ in coronary effluent samples collected at five minute intervals during the perfusion protocol.

Results: Dry heart weights corrected to body weight were not different between the two groups (0.72 ± 0.06 versus 0.84 ± 0.06, $p = 0.22$). The CP was also comparable between the two groups at baseline, but hearts of SHR had a less pronounced increase when subjected to high workload. Glucose oxidation rates were greater in the SHR during baseline and both groups had a similar increase in glucose oxidation rates when subjected to high workload. Consistent with this finding, rates of MVO_2 were lower in the SHR and CE was higher in SHR during baseline.

Conclusion: Hearts of three-month-old SHR exhibit metabolic remodeling changes in glucose metabolism prior to structural remodeling. This suggests that changes in metabolism support structural remodeling that leads to cardiac hypertrophy. This mechanism could provide means to identify patients with high risk of developing hypertension-induced LVH.

ABSTRACT

Role of Heat Shock Protein During Inflammatory Response

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Chronic inflammation is a leading cause of stress, hypotension and associated disorders. Lipopolysaccharides (LPS) induce inflammation and oxidative stress. Corticosterone is a hormone which is released in greater abundance as stress levels increase. Heat-Shock Proteins (HSPs) are thought to play a role in the inflammatory response, specifically through the role of cytokines.

We hypothesize that stress levels in rats will decrease over time after the initial shock of inflammation from LPS. Fifteen male Sprague-Dawley rats were anesthetized with oxygen and isoflurane to implant one catheter into the femoral vein per rat. Each rat was given three days to recover from surgery. After recovery, a blood sample was taken for each rat prior to being given LPS (20 mg/kg IV). Blood samples were taken once a week per rat, and were centrifuged to collect serum. Group 1 (n=5) was sacrificed at one month after LPS using anesthesia and severing of the neck from the spine, and the brain (intact), lung samples, and CSF (Central Spinal Fluid) were taken for further analysis. Group 2 (n=5) will be sacrificed at 2 months after LPS, and Group 3 (n=5) will be sacrificed at 3 months after LPS, with the same samples being taken as Group 1. The serum from Groups 1 and 2 for weeks one through four as well as the baseline sample taken prior to LPS were used to complete a corticosterone assay to measure stress levels weekly after inducing inflammation. The extracted, intact brains will be used for immunostaining and a HSP assay to measure the presence of HSPs due to inflammation. The lung and CSF will be analyzed in the future to measure HSPs and their role in inflammation outside of the brain.

The results of the corticosterone assay on the week 1-4 and baseline serum have not come in/have not been analyzed yet. At this time it cannot be determined if stress levels generally increase or decrease over the course of the month after an initial shock (LPS). The heat-shock protein assay will be completed in the near future, as such results have not been obtained yet. Therefore the role of these stress proteins in chronic inflammation cannot be determined at this time.

ABSTRACT

Pancreatic Enzyme Concentrate, CM-AT, as a Treatment for Autism in Children with All Levels of Chymotrypsin

Madeline Flanagan   *Texas A&M University*   *Class of 2018*

A diagnosis of Autism Spectrum Disorder (ASD) requires the presence of restrictive and repetitive patterns of behavior, deficits in social communication, and problems with social interactions. In addition to these core symptoms of ASD, ASD is often associated with a variety of comorbid conditions, including inattention, poor motor coordination, and gastrointestinal issues. These symptoms can cause difficulties for families of children with autism. Although there are proven behavioral therapies and medications that can target some comorbid conditions (e.g., ADHD), there are no known treatments that address the core symptoms of ASD. Curemark LLC found in previous research that a large subgroup of children with ASD have low levels of chymotrypsin, a serine protease that cleaves select essential amino acids necessary for the synthesis of neurotransmitters and other essential proteins. Curemark's research has indicated that digestive enzyme therapy using CM-AT, a liquid-encapsulated pancreatic enzyme concentrate (PEC) designed to release chymotrypsin in the proximal small intestine, may lead to a reduction in symptoms of autism, improvements in gastrointestinal problems, and increased neurological functioning related to the increased availability of essential amino acids. The purpose of Curemark's clinical trial is to determine the effectiveness of CM-AT in the treatment of ASD in children with all levels of chymotrypsin and to continue to monitor the safety of the drug. Children diagnosed with ASD, ages 3 to 8 years, participated in an initial screening for the study, and those who met study inclusion criteria entered a double-blind phase in which they received either the study drug or placebo for 12 weeks. At every study visit, standardized measures of quality of life, behavior, and health were administered through parent questionnaires/ interviews and a brief physical examination. Additionally, stool samples were collected every other visit to monitor levels of fecal chymotrypsin, occult blood, pH, globin, and microorganisms. Upon completion of the 12-week study, the patients were invited to participate in the open-label extension of the study in which all participants are given the study drug. Data collection for the double-blind and open label studies are currently ongoing. If the clinical study is successful and CM-AT becomes approved and available as a treatment for ASD, children with autism and their families may be able to benefit greatly from improvements in behavioral, social, and cognitive functioning.

ABSTRACT

Functional Recovery After Chronic Spinal Cord Injury By Transplantation of Human iPSC-derived Neural Stem Cells

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The majority of spinal cord injuries (SCI) occur within the thoracic or cervical region, resulting in paralysis as a paraplegic or quadriplegic, respectively. Cervical SCI is especially devastating as most patients lose sensory and motor function below the neck. Currently, there is no effective therapy for SCI patients. Therapeutic intervention(s) to restore even partial function would significantly increase SCI patient's quality of life. Transplantation of neural stem cells (NSCs) could be a novel and promising therapeutic option for SCI patients. Previous studies often used human NSCs from aborted fetal brains and spinal cords or human embryonic stem cells (ESCs). The cell sources raised ethical controversy, but also were not completely effective because of the need to immunosuppress patients. Even though most SCI patients suffer chronic SCIs, the majority of previous studies have used an acute SCI model, with very few investigating a chronic model. The purpose of this study is to test the therapeutic efficacy of NSCs, derived from SCI patients' human inducible pluripotent stem cells (hiPSCs), by using a clinically relevant chronic cervical contusion SCI. The hiPSCs have potential for individual and disease specific therapy, escaping ethical concerns and the need for immunosuppression. We hypothesize that transplantation of hiPSC-derived NSCs will promote functional recovery after chronic SCI by replacing lost neural cells and modifying the injury environment, thus promoting intrinsic repair. This study used nude rats to obviate the need of immunosuppression after human NSC transplantation. Nude rats were trained in behavioral tests of grooming, horizontal ladder, and Treadscan to track forelimb function before injury. The nude rats received a moderate, unilateral C5 contusion injury. Behavioral tests to assess forelimb functions were performed every other week for the entirety of the study. Three months after SCI, the rats were randomly divided into four groups to receive control medium, human fibroblasts, human NSCs or human NSCs plus ablation. We continued with the behavioral tests for three more months following cell transplantation. Histology was used to examine the survival and differentiation of grafted NSCs, as well as the injury sizes. Our results showed grafted NSCs survived and filled in the injured cavity, which significantly decreased the injury size. The grafted NSCs differentiated into mature neurons, astrocytes and oligodendrocytes. No tumorigenesis was observed in any rats receiving hiPSC-NSCs. Importantly, forelimb function significantly increased in the NSC group compared to the medium or fibroblast groups. Whether the functional recovery is directly mediated by grafted NSCs will be confirmed by an ablation experiment, which will be done in the next few weeks. Our results show transplantation of hiPSC-NSCs significantly improve anatomic and

functional outcomes after chronic SCI, suggesting transplantation of hiPSC-NSCs is an effective treatment for chronic SCI.

ABSTRACT

Impaired DNA Repair During Oxidative Growth

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Supported by: Nayun Kim, Ph.D., Department of Microbiology and Molecular Genetics

Key Words: spontaneous mutation, oxidative growth, fermentative growth, BER/NER, cancer

Spontaneous DNA damage occurs during typical cellular metabolism, amounting up to an estimated 10^5 lesions/cell/day in humans. These lesions are repaired primarily through two pathways: base excision repair and nucleotide excision repair (BER & NER respectively). Previous studies conducted in our lab have demonstrated that spontaneous mutation rates in BER-deficient or BER/NER-deficient *Saccharomyces cerevisiae strains* were much greater when grown in oxidative conditions with glucose as carbon source than in fermentative conditions with glycerol and ethanol. In order to confirm that the difference in mutation rates is due to oxidative and fermentative growth, I carried out a fluctuation analysis by growing the *S. cerevisiae* strains in media containing raffinose, another non-fermentable sugar. The Lea-Coulson method of the median was utilized for calculating the mutation rates. Mutation rates of cells grown in raffinose were congruent with those grown in glycerol-ethanol, indicating that the difference is likely due to the metabolic growth pathway, not the particular carbon source.

A potential source of the increased mutagenesis when grown with glycerol/ethanol or raffinose is reactive oxygen species (ROSs) produced by mitochondria during oxidative growth. Further analysis was conducted by creating 'petite' derivative of strains deficient in NER, BER or both. "Petite" strains have severely impaired mitochondrial function and, therefore, do not produce ROSs from their mitochondria. Petite strains are unable to grow oxidatively and rely solely on fermentative growth, regardless of growth conditions. When the petite strains were grown in glucose, fluctuation analysis showed that the mutation rates in each of the strains were similar to that of the same strains grown oxidatively in glycerol/ethanol. This result suggests that mitochondrial ROSs are not likely the cause of elevated mutation rates first observed in cells grown in non-fermentable sugars. We are testing whether various stress-response pathways are involved in directing DNA repair machineries to spontaneously occurring DNA damage. One such oxidative stress response pathway is controlled by the transcription factor YAP1. YAP1 was knocked out from *S. cerevisiae* strains that were DNA repair proficient and BER deficient. Preliminary fluctuation analysis indicates that BER deficient *yap1Δ* strains have mutation rates that are similar to YAP1 strains during oxidative growth. Future trials will explore other stress response pathways.

The findings of this study can contribute to understanding how glycolytic growth influences various DNA repair pathways. They can also lead to the mechanism behind how cancer cells that primarily utilize glycolysis become highly resistant to radiation-induced DNA damage.

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Undergraduate Student

ABSTRACT

The Effects of Chibby Overexpression in *Xenopus laevis* Kidney Development

Hannah Hanania 　　　　*Rice University* 　　　　*Class of 2019*

Sponsored by:　Rachel Miller, PhD, Department of Pediatrics, Pediatric Research Center

Supported by:　Rachel Miller, PhD, Department of Pediatrics, Pediatric Research Center

Key Words:　Wnt, polycystic kidney, nephrogenesis

Introduction: Overexpression of the canonical Wnt signaling pathway component, β-catenin, is associated with the development of cystic kidneys in mice. Cystic kidneys result from a class of diseases called ciliopathies that affect the development of the cilia. We examined the role of the protein Chibby in kidney development. This protein is known to affect the Wnt signaling pathway and cilia. Chibby acts as a β-catenin inhibitor by shuttling β-catenin out of the nucleus preventing it from acting as a transcriptional activator. Additionally, Chibby and β-catenin are found in the transition zone of cilia influencing ciliogenesis. However, the role for β-catenin in ciliogenesis and the role for Chibby in kidney development are unknown.

To test the roles of Chibby and β-catenin, we utilize *Xenopus laeviss* frog embryos. This model organism is beneficial because the embryo develops external to the mother allowing for direct visualization of development, gene expression can be modulated through injection of mRNA's or morpholinos, and many embryos can be used in a single experiment. We hypothesized that overexpression of Chibby in *Xenopus laevis* embryos will disrupt the normal function of β-catenin, leading to irregular tubulogenesis during kidney development.

Methods: mRNA encoding Chibby (Cby1) fused to GFP was injected into the eight cell stage embryos targeting the embryonic kidney. A mutant version of Chibby (Cby1-S20A) that prevents phosphorylation and activation of Cby1 was injected. In addition, embryos were injected with a GFP control. All embryos were fixed at stage 40 and stained with 3G8 and 4A6 antibodies to label the kidney.

Results: Of the embryos injected with wildtype cby1, 87% (n=23) were found to have at least one malformed kidney. Of the Cby1(S20A) injected embryos, 17% (n=18) showed at least one malformed kidney. GFP showed 31% (n=13) with at least one malformed kidney.

Conclusion: Chibby may play a role in the regulation of kidney development. When overexpressed, Chibby inhibits β-catenin, inducing irregular development of *X. laevis* kidneys. Further work is necessary to confirm overexpression effects of Chibby and Cby(S20A) on kidney tubulogenesis.

ABSTRACT

Conditional Deletion of Adora2B from Smooth Muscle Cells Attenuates Pulmonary Hypertension But Not Lung Fibrosis

Ankit Hanmandlu   *The University of Texas at Austin*   *Class of 2019*

Introduction: Pulmonary Hypertension (PH) is a complication that is associated with elevated blood pressures (≥25 mmHg) in the lungs that affects 1-2 million people annually and can lead to right ventricular failure and potential death. Group 1 PH is PH that only affects the vasculature of the lungs and is typically referred to as pulmonary arterial hypertension (PAH). Group III PH is associated with chronic lung diseases such as pulmonary fibrosis. The mechanisms that lead to PH are not fully understood. We have evaluated the role of adenosine A2B receptor (Adora2B) in the pathogenesis of PH using two distinct models of PH. The hypoxia-sugen (Hx-Su) model of PH emulates features of Group I PH while the bleomycin-induced model of lung fibrosis and PH emulates features of Group III PH. Our overall hypothesis is that the conditional deletion of Adora2B from smooth muscle cells will reduce PH.

Methods: In this study, we used Adora2BFloxTransgelinCre mice that lack Adora2B in the smooth muscle using the TaglnCre driver. Under the Hx-Su model, mice were injected with Sugen-5416 (Sugen) once weekly and kept at hypoxia (10% oxygen) for 4 weeks. Control mice were kept at normoxia and received Solutol (vehicle for Sugen). In the BLM model, mice were injected with BLM weekly and control mice received PBS. Surgery was performed to measure RVSP/LVSP (Right and Left Ventricular Systolic Pressure) in the heart. Subsequently, the lungs were collected for histology. Paraffin-embedded lungs were then cut into sections and used for immunohistochemistry (α-SMA) and Massons-Trichome stains. Fibrosis and vascular remodeling were quantified using the modified Ashcroft Score Method and Image-Pro 6.3 Software by measuring the amount of fibrosis, vessel smooth muscle area and vessel circumference.

Results: Adora2BFloxTransgelinCre (Adora2Bf/f-TaglnCre) mice under the BLM and the Hx-Su models both showed a reduction in RVSP (diminished PH) when compared to the TransgelinCre (TaglnCre) mice exposed to the control conditions of PBS and hypoxia, respectively. However, no concrete evidence of any difference in vascular remodeling in the BLM model was shown between the Adora2Bf/f-TaglnCre and TaglnCre mice lines. Moreover, the deletion of A2B in smooth muscle cells had minimal impact on induced lung fibrosis in the BLM model as opposed to the control. Increased vascular remodeling was shown in the Adora2Bf/f-TaglnCre mice under the Hx-Su model up to five fold (5x).

Discussion: Deleting the Adora2B receptor in smooth muscle cells seems to disrupt the response in cells that are involved in the development of PH; however, it does not attenuate the pro-fibrotic response in mice exposed to BLM. Adora2B is still expressed in other cells that contribute to lung fibrosis such as Alternatively Activated Macrophages (AAM), fibroblasts, and epithelial cells that could contribute to the fibrotic response induced by BLM-exposure. However, the difference in vascular remodeling shows that a different signaling pathway could have caused both models to show reduced PH in the Adora2Bf/f-TaglnCre mice. This difference could be attributed to an

inflammatory response in which the consequence is vasoconstriction. Ultimately, discovering a way to treat PH could allow us to develop molecular therapies to treat this fatal condition.

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ABSTRACT

Ganglion Cell Stratification in the Inner Plexiform Layer of Rabbit Retina

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Introduction: The mammalian brain is a vastly complex organ with relatively little known about the variety of cells and ways in which they interact. The retina is a separate part of the brain in charge of receiving and processing light signals in the back of the mammalian eye. Because tl1e retina is separate from the rest of the brain, it can be studied individually to gather information about cell types and interactions which may assist later studies for the rest of the brain. This study investigated the Stratification of ganglion cell dendrites in the mammalian retina to gain a better understanding about the different types of retinal ganglion cells. We hypothesized that different types of ganglion cells would be distinguishable from each other by the depth and width of their stratification.

Methods: A confocal microscope was used to take 2 cha1mel images of the dendrites of a dye-filled ganglion cell and the choline acetyltransferase (ChAT) bands within a rabbit retina. The image stacks were deconvolved using a point-spread function derived from .S☐t fluorescent beads. ImageJ was then used to plot the Z Axis Profile of 4 different sections of the ganglion cell dendrites as well as the ChAT bands. Next, the data points of the Z Ax.is Profiles were imported to Origin where the fluorescent intensity was normalized from [0,1] and X values for stratification were adjusted such that ChAT B was at O and CMT A was at 100. The X value at Y Max and Full Width at Half Max were recorded and used to generate a Gaussian curve showing the location of the ganglion cell in relation to the Amacrine Cell Layer (ACL) and the Ganglion Cell Layer (GCL) of the retina.

Results and Conclusions: Of the 10 different types of cells tested, they could all be distinguished from each other using the criteria of sh·atification depth, width, or presence of bistratification. While certain cells such as the G9 cell and the diving cell were both located approximately 8% of the way from the ACL to the GCL, the diving cell has bish·atified dendrites also present around 75%. There was a similar occurrence around 65% for the ON OFF OS cell, which is bish·atified, and the GlO (ON OS) cell which is not. All other cells, including the GS, OFF Alpha, Ackert, G4, Local Edge Detector (LED) and ON Alpha could clearly be distinguished due to their stratification depth or width. This provides support that dendrite stratification can help classify

retinal ganglion cells which further aids in understanding retinal cell types with possible implications for other neural cells. Future directions could include analyzing the stratification depths and widths of more retinal ganglion cell types. There are gaps in the overall stratification pattern and it is plausible that novel ganglion cell types could be located at those levels, but this would require further investigation.

ABSTRACT

Using a cysteine-reactive cross-linker to investigate redox interactions in proteins

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Sponsored by: Kevin Morano, PhD Microbiology and Molecular Genetics
Supported by: UT Health Graduate School of Biomedical Science-SURP
Key Words: Cysteine, Crosslinker, Oxidative stress, Glutathionylation, Disulfide

Protein damage during oxidative stress primarily occurs on cysteine residues, resulting in disulfide bond formation in particular target proteins. Understanding the target proteins of oxidation is difficult because of the dynamic nature of the disulfide bonds. Previously we have found a cysteine-specific cross-linker, divinyl sulfone (DVSF) traps interactions between thioredoxin and its redox partners. We tested whether DVSF could also be used to probe interactions between other redox-active proteins including proteins prone to forming intramolecular and intermolecular disulfides as well as with the small molecule glutathione. Specifically, in the glutathione peroxidase (Gpx) family these proteins undergo intramolecular or intermolecular cross-linking upon treatment with DVSF. Both Gpx2 and Gpx3 formed intramolecular cross-links in the presence of DVSF whereas Gpx1 formed intermolecular cross-links with other proteins. Upon mutation of the active site cysteines in Gpx3 to alanine, DVSF no longer formed an intramolecular cross-link. While some redox-active proteins form protein disulfides some undergo oxidation to form disulfides with glutathione. We found that DVSF could crosslink glutathione to a variety of proteins and that this cross-linking was decreased in a yeast mutant incapable of producing glutathione. Moreover, several proteins that undergo glutathionylation during oxidative stress including sulfiredoxin 1 (Srx1) and the chaperone Ssa1 were cross-linked to glutathione in cells treated with DVSF. Our results suggest that DVSF can be used to probe redox partnerships within individual proteins and between proteins and glutathione. In the future, DVSF will be used to investigate thiol based redox relays in less studied organelles and organisms.

ABSTRACT

Hyaluronic Acid Hydrogels with IKVAV and LRE for Neuronal Differentiation of Human Induced Pluripotent Stem Cell Derived Neural Stem Cells

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Sponsored by: Laura, Smith-Callahan MD, PhD, Department of Neurosurgery
Supported by: Bentsen Stroke Center, William Stamps Farish Fund
Key Words: Tissue engineering, stem cells, peptide gradient, hydrogel, scaffold

Stem cell transplant therapy is a leading contender for repairing central nervous system injuries; however, developments thus far have shown little effect. The main problem to this approach is finding an environment that can promote differentiation, growth and extension within the transplanted cells without placing patients in harm. Our lab is exploring varying types of minimally invasive, synthetic hydrogels with bioactive peptide signals in order to mimic the extracellular matrix (ECM) of neuronal cell. Two such peptides are Ile-Lys-Val-Ala-Val (IKVAV) and Leu-Arg-Glu (LRE), which are both fragments of s-laminin, a protein in the ECM that promotes cell differentiation, adhesion and migration. We are currently exploring di-functional hyaluronic acid (Diff-HA) as an alternative hydrogel material because its porous nature and high water content. The mechanical properties of hydrogels are known to influence differentiation of stem cells, making Diff-HA optimal for differentiating cells into neurons due to its similar mechanical properties to the brain. Additionally, HA is naturally abundant in human tissue and is known to assist in healing wounds. Our current objective is to determine whether pairing Diff-HA matrix with IKVAV and LRE promotes neural differentiation of neuronal stem cell in a 3D system. Prior to the biological studies, Diff-HA was coupled with IKVAV and LRE, which was later coupled with methacrylated-HA at a 1:2 ratio. Doing so allows us to individually control the concentrations of the two peptides. Once cells were added to the material, APS/TEMED, a chemical initiator, was utilized at a concentration of 5 uM to overcome health and gelation issues that other initiators would instigate in future in vivo studies. The material was then pipetted into 1.5 mL Eppendorf tube caps and allowed to gel for 20 minutes. This process was repeated for Diff-HA without peptides in order to compare the effects of the peptides. The cells were left to grow over one and two weeks, which were then imaged and quantified for ratio of neurites to total cell count. Through this study we saw significant increase in cellular viability and extensions in Diff-HA with LRE and IKVAV in comparison to Diff-HA without peptides, which encourages us to move on to an in vivo study in the near future.

ABSTRACT

Mechanisms of Net1A Cytosolic Localization in Breast Cancer Cells

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Sponsored by: Jeff Frost, PhD, Integrated Biology and Pharmacology

Supported by: Arzu Ulu, PhD, MS

Key Words: Breast Cancer, Net1A, Src, metastasis

Background: Metastatic breast cancer is the most severe type of breast cancer with a five year survival rate of only 22%. Currently there are no specific therapies targeted at metastasis, meaning there is a critical unmet need. The aim of this project is to target metastasis in order to meet this need. One known aspect of metastasis is that it requires cancer cell motility and invasion. RhoGTPases control cell motility and are necessary for metastasis. Due to these reasons, targeting RhoGTPase activation may be a useful therapeutic strategy. RhoGTPases are activated by RhoGEFs. The RhoGEF neuroepithelial cell transforming gene 1A (Net1A) is an oncogene that shuttles between the cytosol and the nucleus and is overexpressed in breast cancer. Its localization dictates its function, such that localization in the cytosol causes cell motility and invasion. This project examines mechanisms that dictate Net1A localization and determined whether these can be targeted for intervention.

Methods: MCF7 breast cancer cells were grown and transfected with Net1A, a 5YF Net1A mutant (Src phosphorylation deficient), or specific mutant Net1A variants (Y126F, Y310F/Y311F, or Y370F/Y373F) in the presence or absence of EGF, Src, or inhibitors of Src or Src regulated kinases (PF-562271, Imatinib, or Bosutinib). Immunofluorescent staining was performed to quantify the cytoplasmic to nucleic ratio of Net1A localization.

Results: EGF caused Net1A to relocalize to the cytosol; however with 5YF mutant failed to relocalize. Src also caused wild type Net1A to relocalize to the cytosol but not the 5YF mutant. Among the Net1A mutant variants, the Y370F/Y373F mutant exhibited the least relocalization. When inhibitors were used, Bosutinib and PF-562271 prevented Net1A relocalization with EGF. Imatinib was ineffective.

Conclusion: The Src phosphorylation sites are important for EGF- or Src-induced Net1A relocalization to the cytosol. The most important sites are the Y370/Y373. Src and FAK are required for Net1A cytosolic relocalization with EGF stimulation.

ABSTRACT

Characterization of Tbr2 Retina Ganglion Cells

Harry Liu   *Rice University*   *Class of 2017*

Sponsored by: Chai-An Mao, PhD, Department of Ophthalmology & Visual Science

Supported by: Chai-An Mao, PhD, Department of Ophthalmology & Visual Science; The University of Texas at Health Science Center at Houston

Key Words: Tbr2, retina, morphology, ganglion

Background: The visual pathway in the retina has been understood and documented for years. After entering the retina, light travels through three layers of cells: ganglion cells, amacrine cells, bipolar cells, horizontal cells, and photoreceptors. The photoreceptors are the most prominent detectors of light and they are the reasons for our color and night vision. However, intrinsically photosensitive retinal ganglion cells (ipRGCs) have been found to also be sensitive to light. The main function of ipRGCs is to regulate circadian photo-entrainment and pupillary light reflex. Unpublished research by Drs. Mao and Massey found that a transcriptional factor T-brain 2/Tbr2 appears to regulate these ipRGCs. The hypothesis is that Tbr2+ RGCs serve as a reservoir or backup for ipRGC cells, only turning on when needed or when there is damage or loss of other ipRGCs.

Methodology: Two genetically modified mice lines were created prior to the experiment by Dr. Mao to examine the morphology and central brain projection of Tbr2+ RGCs. Retinas taken from the Tbr2-CreERT2:R26-iAP mouse line were stained for alkaline phosphatase and viewed using brightfield imaging to examine the morphology of RGCs. Different conditions were tested such as the time of fixation to yield better images. The brain from the Tbr2-TauGFP mouse line was used to conduct immuno-fluorescent staining to examine GFP+ central projections of Tbr2+ RGCs. In parallel, retinas from the Tbr1-CreERT2:Brn3a-CKO mouse line were used to examine the morphology of Tbr1+ RGCs.

Results: Images for both Tbr2- and Tbr1-expressing retina ganglion cells were taken and compared. Tbr1-expressing retinas contained many more RGC's than Tbr2-expressing retinas so no conclusion could be reached until more Tbr2 data was collected. However, a preliminary analysis of the central projection of the Tbr2+ RGCs suggests that the hypothesis presented by Dr. Mao may be correct because most of the Tbr2+ RGCs project to the same region as ipRGCs.

Conclusion: AP staining and brightfield analysis appear to be a sufficient method of determining the morphology of RGCs. Although images were taken for both mouse lines, a more in-depth analysis of the cells is required. Additionally, more experiments with the Tbr2+ cells are needed to provide more data regarding the morphology of Tbr2+ RGCs.

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ABSTRACT

Differential Expression of GTAP and Insulin-Like Growth Factor 1 Receptor in Cancer cells

Kaiwen Ma   *The University of Texas at Austin*   *Class of 2018*

Background: As an evolutionarily conserved enzyme, galactosyltransferase 1-associating protein (GTAP) belongs to the ubiquitin-conjugating (E2) family, which performs the second step in the ubiquitination reaction that targets a protein for proteasome degradation. GTAP participate in multiple biological processes, such as intercellular adhesion and embryonic development (Wassler, Stem Cell, 2008). Primarily existing in embryonic cells, GTAP is upregulated in several tissues or organs with high levels of cell-turnover, such as testes and ovaries. Because GTAP expression is upregulated in fast growing cells in reproductive organs, we hypothesized that GTAP expression is prominently associated with cell growth and expression of receptors for growth factors, such as insulin-like growth factor-1 receptor (IGF-1R) in certain cancer cell lines from the reproductive system. This study was designed to investigate the correlation between GTAP and IGF-1R expression in certain types of cancer cells.

Methods and Results: In order to find a relationship between GTAP and IGF-1R in cancer cells, we performed Western blot analysis. Total proteins were extracted from several cancer cell lines: HeLa (cervical), COV (ovarian), TOV112 (ovarian), IMR-32 (neuroblastoma), SK-N-AS (neuroblastoma), MCF-7 (breast), and MDA-MB (breast). Bradford protein assay was used to determine protein concentrations. After electrophoresis in SDS-PAGE, protein bands were transferred onto a filter membrane, and probed with antibodies against GTAP and IGF-1R. Protein bands reactive towards GTAP and IGF-1R antibodies were visualized by antibodies conjugated with a peroxidase that catalyzed a chemiluminescent reaction. Quantification of protein band intensity was conducted by densitometry. We detected the strongest GTAP expression in the TOV112 cells. However, HeLa, COV, IMR-32, SK-N-AS, MCF-7, MDA-MB had little or no detectable GTAP expression. The IGF-1R expression results were inconclusive and experiments are still ongoing.

Conclusion: Our results indicate that GTAP is expressed at various levels in certain types of cancer cells. Among all the cell lines used in this study, GTAP expression appears predominantly in ovarian cancer cells in comparison with other cancer types. Combined with previous data using normal ovarian tissue, these results suggest that differential GTAP expression occurs because of tissue types, regardless of cancer state. Ongoing western blot experiments involving IGF-1R will allow us to correlate GTAP expression and IGF-1R expression.

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ABSTRACT

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Sponsored by: Walid D. Fakhouri, MSc, PhD, UTHealth School of Dentistry, Department of Diagnostic and Biomedical Sciences

Supported by: Walid D. Fakhouri, MSc, PhD, UTHealth School of Dentistry, Department of Diagnostic and Biomedical Sciences; UT School of Dentistry at Houston Research Office

Key Words: IRF6, mandible, bone, cartilage, micrognathia, cleft palate

Background: Interferon Regulatory Factor 6 (IRF6) and Twist 1 are transcription factors that play critical roles in craniofacial development in mammalian systems. Compound heterozygous mice for Irf6+/- and Twist1+/- have small mandibular processes (micrognathia) that lead to a posterior positioning of the tongue upwards into the palate which then causes cleft palate at birth. The goals of this study are to delineate the nature of the genetic interaction and signaling pathways that play a critical role in mandibular bone development as well as the pathology associated with the knockout of IRF6.

Methods: We performed Next Generation Sequencing for total RNA (RNA-Seq) extracted from mandibular tissue of affected Irf6 null and wildtype littermate embryos. The RNA-Seq data was analyzed bioinformatically and by; textmining, and then validated by real time quantitative PCR (RTqPCR) on the differentially expressed genes. Histological, immunohistochemical, and immunofluorescent staining were also performed on both Irf6 null and wildtype mouse tissues at embryonic ages of 13.5, 15.5, 17.5 days and newly born mice.

Results: According to RNA-Seq analysis, we identified 66 genes that were differentially expressed in Irf6 null mice. RTqPCR validated our gene ontology analysis by identifying Helt and Foxn4 as the most differentially expressed genes. Our lab's modification of Goldner's Trichrome stain identified significant differences in the meckel's cartilage and mandibular bone. The chondrocytes in the Irf6 null were more condensed and had less cellular matrix than the wildtype which leads us to believe the wildtype chondrocytes are more mature and have undergone more remodeling than their mutant counterparts. Irf6 null mice contained fewer osteocytes embedded within the mandibular bone as well as having bone fragments with more vacuolated area in between and fewer osteoblasts surrounding them. This suggests a reduction in mineralization of mandibular bone as well as a delay of osteoblast differentiation in Irf6 null mice compared to wildtype.

Conclusion: Our findings suggest that IRF6 is involved in mandibular bone development and that mutations in this gene may contribute to the risk of mandibular defects in humans. Our future goals are to manipulate the most differentially expressed genes either by knockout or knockdown, and observe any effects this might produce in the phenotype. Ultimately, we seek to translate these findings from the mouse model into the clinic and pioneer a better understanding of these disorders, possibly leading to better treatments in the future.

ABSTRACT

Evidence For Previously Unreported
DNA-Binding Protein Activity in *Bacillus anthracis*

Adam Morris   *Texas A&M University-Corpus Christi*   *Class of 2017*

In *Bacillus anthracis*, virulence genes coding for anthrax toxin, capsule synthesis, and other factors important for pathogenesis are regulated by the master virulence regulator AtxA. Lethal Factor (LF) is one of the three components of anthrax toxin whose gene, *lef*, is positively regulated by AtxA. When DNA containing the promotor for LF (P_{lef}) is isolated and mixed with crude extract from *atxA*-null mutants and subjected to an electrophoretic mobile shift assay (EMSA), results suggest the presence of a protein that associates with P_{lef}. To further investigate the possibility of a novel DNA-binding protein or unreported DNA-binding activity of a known protein, we performed affinity chromatography to isolate the possible protein, followed by SDS-PAGE. The affinity chromatography technique used involved performing PCR on P_{lef} with biotinylated primers, then mixing that biotinylated DNA with streptavidin agar. Streptavidin agar binds with strong affinity to biotin, binding the DNA to the agar. We then added crude extract from lysed *B. anthracis* cells that had been cultured in conditions that promote toxin production. The supernatant fraction was removed, leaving only the agar. SDS buffer was added and the mixture boiled to denature the DNA and release any protein bound to it. The sample was subjected to SDS-PAGE, revealing a prominent band representative of a protein weighing just under 50kD. Further investigation is required to determine the protein's identity and confirm its DNA-binding activity with P_{lef}. Future research include N-terminal sequencing of the protein and location of the associated gene in the *B. anthracis* genome.

Commented [KT1]: Proper to say " code for" or "encoding", Not "encoding for"

Commented [KT2]: Abstract should be one parag.

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ABSTRACT

Flexural, Compressive and Diametral Properties of a Bioactive Cement

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Sponsored by: Joe C Ontiveros DDS, MS, Department of Restorative Dentistry and
                         Prosthodontics,
Supported by: Joe C Ontiveros DDS, MS, Department of Restorative Dentistry and
                         Prosthodontics; The University of Texas School of Dentistry at Houston
Key Words: Bioactive Cements, Luting Cements, Glass-ionomer cements, Resin-based
                         cements

Objectives: Determine the mechanical properties of bioactive cements in relation to glass-ionomer and resin-ionomer cements. Properties tested were flexural strength, flexural modulus, compressive and diametral tensile strength.

Methods: A bioactive cement (Ceramir, Doxa), a resin-ionomer(Fujicem2,GC) and glass-ionomer cement(Ketac, 3M) were tested for flexural strength and modulus according to ISO 4049. Samples for compressive and diametral strength were made in discs according to ISO 9917. Cements were dispensed and activated according to the manufacturer's instructions. Samples were stored in distilled water for 24±1 hours at 37°C before being tested for its mechanical properties. A static load was applied to each specimen with an Instron testing machine at a crosshead speed of 0.5mm/sec. Data was analyzed using ANOVA and Tukey post hoc at $p<0.05$ significance.

Results: Mean (SD) values for Flexural Strength (FS), Flexural Modulus (FM), Compressive Strength (CS), and Diametral Tensile Strength (DTS) are reported below.

Material	FS MPa	FM GPa	CS MPa	DTS MPa
Ceramir	23.3(7.7)	13.9(3.8)	50.8 (26.4)	6.2(2.1)
Ketac	18.2(8.0)	11.3(5.8)	36.5(97)	5.6(1.7)
Fujicem2	9.1(2.2)*	2.9(0.4)*	79.7(10.0)*	11.8(2.6)*

*Significantly different along columns, $p<0.05$

The results of ANOVA reveal a significantly higher flexural strength and modulus for the bioactive cement Ceramir and glass-ionomer Ketac in comparison to resin-ionomer Fujicem2($p<0.05$). However, the results revealed significantly lower compressive and diametral strength for Ceramir and Ketac in comparison to Fujicem2($p<0.05$).

Conclusions: The results of mechanical testing revealed that the strength of materials varied among properties evaluated depended on test performed. Bioactive cement Ceramir did not show a conclusive difference in strength in relation to the other material types.

ABSTRACT

The Role of Trichodysplasia spinulosa polyomavirus (TSPyV) Middle T Antigen in Cell Signaling Pathways

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Sponsored by:　Dr. Stephen K. Tyring, MD, PhD, MBA, Department of Dermatology
Supported by:　Dr. Stephen K. Tyring, Department of Dermatology
Key Words:　　TSPyV mT, PP2A, 4EBP-1, MAPK Pathway

Trichodysplasia spinulosa (TS) is a skin disease that causes follicular-centric popular eruption and keratin spine formation. Recent research in the field of virology has highlighted the link between Trichodysplasia spinulosa polyomavirus (TSPyV) in TS pathogenesis. The genome of TSPyV is divided into early and late regions, with the early region encoding regulatory proteins called tumor antigens, specifically small T, middle T, large T, tiny T, and alternative T antigens. These tumor antigens may play an important role in viral replication, transcription, and cellular transformation and contain protein binding motifs that regulate virus replication.

TSPyV sT and LT have been extensively studied, revealing their functions in the cell signaling pathways. Studying the pathogenic potential of TSPyV mT and understanding how its function differs and resembles that of TSPyV sT will provide a better understanding of the role of T antigens in hyperproliferation that is seen in TS. Additionally, identifying the pathogenic regions of the antigens that cause oncogenesis could reveal methods to target these antigens to provide treatment options for patients.

Using western blotting and coimmunoprecipitation, we identified if mT phosphorylates protein targets downstream of PP2A after it binds to the phosphatase. After conducting coimmunoprecipitation using antibodies against the specific target protein in the signaling pathway, we used Western blot detection to verify that the antigen is in fact mT with an enzyme-labeled antibody. By using phospho-specific antibodies and total antibodies, we checked to see whether the protein was truly phosphorylated or only increased in expression. First, we studied the MAPK pathway (MEK and ERK proteins) to find out if TSPyV mT binding to PP2A phosphorylates and deregulates this signaling pathway. Our data shows that MEK and ERK are hyperphosphorylated in the presence of TSPyV mT. However, total MEK is also slightly upregulated. Next, we studied c-Jun and 4EBP1 phosphorylation in the presence of TSPyV mT to see if mT enhances phosphorylation of these proteins that are known to cause induction of cellular transformation when hyperphosphorylated. Our data shows that c-Jun is hyperphosphorylated and slightly upregulated. In the presence of TSPyV mT, 4EBP1 is not phosphorylated by the presence of TSPyV mT. Additionally, we repeated the study using TSPyV mT truncated in the unique region to prove that altering the Zn binding domain affects its ability to bind to PP2A and regulate the oncogenic signaling pathway. Our results from this study showed that the Zn binding domain deletion caused MEK, ERK, and c-Jun to not phosphorylate in the presence of TSPyV mT.

Thus, we can conclude that TSPyV mT binds to PP2A, causing the phosphorylation and activation of the MAPK pathway.

ABSTRACT

The role of IRF6 in Salivary Gland Development

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Sponsored by: Walid D. Fakhouri, MSc, PhD, UTHealth School of Dentistry, Department of Diagnostic and Biomedical Sciences

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Key Words: IRF6, Salivary Glands, Alcian blue and PAS staining, immunofluorescence staining, Popliteal Pterygium Syndrome, cleft lip and plate, lip pits

Background: Interferon Regulatory Factor 6 (IRF6) encodes for a transcription factor protein, which regulates expression of target genes that are critical for biological processes during craniofacial and ectodermal development as well as in immune functions. Mutations in IRF6 cause Van der Woude and Popliteal Pterygium syndromes, which cause morphological changes in the craniofacial structure such as cleft lip and palate and lip pits. The rational of this project, to investigate the function of Irf6 on the salivary gland's development, is based on the preliminary data from the transgenic mouse model made in our lab, which showed high levels of expression of Irf6 in the salivary glands during embryonic development. This data was confirmed with the detection of a strong signal from an endogenous expression of IRF6 protein. From this finding, we hypothesized that IRF6 is important for the development and function of exocrine glands. The aim of this project is to analyze the pathophysiology of the salivary gland in Irf6 null mice by determining the affected cell types, altered genes, and ultimately regulatory pathways.

Methods: Bioinformatics was performed on 168 differentially expressed genes in mutant salivary gland tissues compared to wild type in order to identify the most differentially and relevant expressed genes for testing with immunofluorescence staining. Histological staining was used to investigate when morphological changes in the mutant emerge and how the salivary gland is being affected during specific embryonic ages.

Results: Histological staining with Alcian blue and Periodic Acid Schiff (PAS) staining and H&E staining showed irregularly shaped acinar cells along with a disruption in the extracellular matrix and disorganization of cells within the mutant salivary glands. When comparing the histological staining of the mutant and wild type tissues, a difference was found in E15.5 aged mice but not at E13.5 leading to the idea that E14.5 is the best time period to perform transcriptional profile to detect differentially altered genes in embryonic mutant compared to wild type.

Conclusion: Our findings have shown that lack of Irf6 remarkably affects the development of salivary glands causing disorganization of acinar cells and disruption of mucus formation that potentially impair the salivary gland's functions. Our ultimate goal is to translate our research into the clinical setting by being able to identify the risk factors in salivary gland disorders.

ABSTRACT

Chronic Exposure to Long Chain Fatty Acids Reverses Intramuscular Lipid Accumulation via a Novel p62-mediated Lipophagy Pathway

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Sponsored by: Heinrich Taegtmeyer, M.D., D.Phil, FACC, FAHA
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Key Words: Intramuscular Lipid Accumulation, Lipophagy

Background: The lab has previously reported that in obese patients undergoing bariatric surgery intramuscular (IM) lipid deposition decreases over time. In hepatocytes, autophagy, a lysosomal-mediated pathway, plays an important role in lipid remodeling. We have also recently reported activation of autophagy in L6 myocytes promotes the clearance of lipid droplets and protects cells from lipotoxicity. Furthermore, cells treated with high fat over a long term activated a new pathway for the removal of IM lipids.

Hypothesis: We therefore proposed that knockdown of p62 in rat L6 myocytes exposed to long chain fatty acids may increase lipid accumulation and lipotoxicity.

Methods and Results: Rat L6 myocytes were treated with fatty acids (oleate/palmitate) at 0 mM (BSA/control), 0.5 mM (physiologic), 1.0 mM (hyperlipidemic) concentrations and incubated for six days. On the fourth day, Rapamycin (1 µM), a known activator of autophagy, Bafilomycin A1 (200 nM), a known inhibitor of autophagy, or a combination of both were added. Autophagic flux, lipid accumulation and colocalization of p62 with lipid droplets and ADRP were assessed. As expected, cells treated with rapamycin reduced lipid accumulation and cells treated with bafilomycin A1 reduced lipid accumulation. However, cells treated with both drugs still showed breakdown of p62, suggesting a novel p62 mediated pathway of autophagy. In order to assess the importance of p62 for autophagy, the same cells were transfected with p62 siRNA. Western blots were done to assess successful knockdown of p62. Oil-Red-O staining and triglyceride assays were done determine lipid accumulation. Further analysis revealed efficient knockdown of p62 in transfected cells. Oil-Red-O staining in cells transfected with p62 siRNA revealed higher lipid content than in the nontransfected cells.

Conclusions and Plans: Chronic exposure to long chain fatty acids reverses intramuscular lipid accumulation via a novel p62-mediated lipophagy pathway. Knockdown of p62 leads to increased lipid accumulation in myocytes, which confirms that p62 is necessary for the removal of lipids in cells treated with high fat long term. Future work assess rates of triglyceride turnover by a pulse-chase experiment using radiolabelled fatty acids. This will also determine the fate of fats processed by the p62 mediated pathway. In addition, knockdown of ATG5 or ATG7 will confirm that cells experiencing chronic lipid overload require a p62 and an autophagy dependent pathway for fat breakdown.

ABSTRACT

Role of Computed Tomography of the Chest in Pediatric Trauma

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Sponsored by: Christine Koerner, MD, Emergency Medicine

Supported by: Christine Koerner, MD, Emergency Medicine

Key Words: Infectious Diseases, Pediatric, Tomography

Background: The lab has previously reported that in obese patients undergoing bariatric surgery intramuscular (IM) lipid deposition decreases over time. In hepatocytes, autophagy, a lysosomal-mediated pathway, plays an important role in lipid remodeling. We have also recently reported activation of autophagy in L6 myocytes promotes the clearance of lipid droplets and protects cells from lipotoxicity. Furthermore, cells treated with high fat over a long term activated a new pathway for the removal of IM lipids.

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ABSTRACT

Testing the role of PNPase – RNase E interactions on sRNA- mediated gene regulation in *E. coli*

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Sponsored by: Nicholas R. De Lay, Ph.D, Department of Microbiology and Molecular
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Supported by: Nicholas R. De Lay, Ph.D
Key Words: PNPase, RNase E, sRNA, protein interactions, gene regulation

Introduction: sRNAs are small RNA molecules that regulate gene expression in many bacteria via base pairing with mRNAs. Gene regulation by sRNAs is an attractive target for future antibiotic development because sRNAs are involved in the virulence and survival processes of many pathogens. This regulation is dependent in part on the exoribonuclease PNPase. Previous studies in *Escherichia coli* have shown that PNPase interacts directly with the endoribonuclease and major degradosome component RNase E. This study examines whether the interaction between PNPase and RNase E plays any role in sRNA-mediated gene regulation.

Methods and Results: Five PNPase mutants were generated with single point mutations in a region previously identified to interact with RNase E. Interactions between each mutant and RNase E were first assessed using a beta-galactosidase-based bacterial two-hybrid assay. Two of the PNPase mutants significantly impaired interactions with RNase E, reducing wild type interactions by 63% and 98%. Expression of these two mutants was next evaluated by western blotting. Both proteins were found at levels close to the wild type protein, averaging at 126% and 117% of the wild type, respectively. Finally, the effect of each PNPase mutant on sRNA-mediated gene regulation was tested. For this assay, downregulation of an *ompX'-'lacZ* translational reporter fusion by the sRNA CyaR was measured using a beta-galactosidase assay. Regulation by CyaR was as effective with the two mutants as with wild type PNPase, but was significantly reduced in a *pnp* deletion mutant.

Discussion: In this study, two PNPase mutations were identified that significantly disrupt interactions between PNPase and RNase E, without resulting in a loss of PNPase protein stability. Despite this loss of interaction between PNPase and RNase E, downregulation of *ompX'-'lacZ* by the sRNA CyaR was unaffected. This suggests that a close association with the degradosome is unnecessary for PNPase to perform its role in sRNA-mediated gene regulation. While sRNA mediated regulation remains an attractive target for future antibiotic development, the results of this study do not support that disruption of the PNPase and RNaseE interaction would be a suitable method for disrupting sRNA regulation.

Adam Saleh

ABSTRACT

Isolation of Wharton's Jelly and Evaluation of In Situ Osteogenic Differentiation Potential of Wharton's Jelly-Embedded Stem Cells

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Sponsored by: Fabio Triolo, DdR, MPhil, PhD, Department of Pediatric Surgery
Supported by: Fabio Triolo, DdR, MPhil, PhD, Department of Pediatric Surgery; The
                University of Texas at Houston Medical School – Office of the Dean
Key Words: Wharton's Jelly, Stem Cells, Osteogenic differentiation

Introduction: Wharton's jelly (WJ), the connective tissue matrix of the umbilical cord (UC), is a gelatinous substance rich in stem cells considered to be a promising natural "tissue engineering" construct for innovative clinical applications of regenerative medicine. WJ is typically discarded as post-delivery medical waste, its use does not pose ethical concerns and its harvest is completely non-invasive. Moreover, it consists of a scaffold derived from the recipient's own molecules, naturally seeded with the recipient's own stem cells, and is thus immunologically inert. WJ-embedded stem cells are particularly appealing for cell-based therapeutic use as they are safe, easy to isolate and expand, and they exhibit high plasticity and proliferative ability as well as immune-evasion and -regulation capacities and antitumoral properties. Collectively, these properties are exciting for regenerative medicine and warrant further investigation in order to explore potential clinical applications of WJ. Specifically within the lab of Dr. Triolo, WJ is currently being investigated as an adjunct to cleft palate repair in a pre-clinical model representative of cleft palate repair in newborns. In this context, a primary goal to achieve is to successfully isolate the WJ from the umbilical cord and assess the *in situ* osteogenic differentiation potential of WJ-embedded stem cells.
Methods: The WJ was isolated through a process in which the UC was initially rinsed in PBS supplemented with gentamycin until complete blood and blood clot removal. The UC was then dipped in 10% povidone-iodine, dabbed dry with a sterile gauze and sectioned into 4-5 cm longitudinal segments, each of which was incised along its length to unravel and lay it flat. Under a dissecting microscope, all three blood vessels were removed, and WJ was scraped off the epithelium using a scalpel. The isolated WJ was then placed in osteogenic and control media and observed for mineralization after fourteen days, using alizarin red staining.
Results: The WJ isolation method proved effective and the WJ cultured in osteogenic medium for fourteen days showed significant mineralization, as indicated by the alizarin red staining. By contrast, the WJ cultured in control medium showed no mineralization.
Conclusions: The results of this experiment shows the *in situ* osteogenic differentiation capability of WJ-embedded stem cells and underscores the potential of WJ in clinical applications involving bone regeneration.

ABSTRACT

Quality Improvement to ensure proper handling and processing of blood

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Sponsored by: Robin, L., Hardwicke, PhD, FNP-C, Department of Internal Medicine

Supported by: Memorial Hermann Hospital System Virology Laboratory

Key Words: HIV-1 RNA, viral loads, PPT tube, EDTA tube

Background Levels of HIV-1 RNA in plasma have been found to be useful markers in disease progression and efficacy of antiretroviral therapy (ART) and used primarily to make decisions to change ART and determine mode of delivery in pregnancy. Detectable viral loads which could be any persistent value >20c/ml may warrant medication change or additional costly resistance testing. Viral loads >1000c/ml will recommend cesarean delivery over natural delivery to reduce risk of mother-to-child (MTCT) transmission of HIV. Beginning October, 2015 patients with previously undetectable HIV RNA (viral loads) began surmounting alarmingly detectable levels. Labs remained elevated after extensive medication adherence counseling. This phenomena has crossed through all Memorial Hermann (MHH) outpatient draw stations and it is suspected to be result of improper processing of PPT tubes which was a change from previous EDTA tubes. Due to VL elevations, C-sections were chosen over natural deliveries in pregnant women to prevent MTCT. In addition, expensive resistance tests have been ordered, and changes have been made to patients' treatment regimens due to the (potential) false positives. Therefore, it is imperative to have accurate, reproducible, reliable, high quality, data on VL to support the appropriate therapeutic actions. We suspect improper processing of the PPT tubes which is resulting in a release of cell-associated virus into the plasma fraction and can in turn result in falsely elevated viral loads. We aim to compare processes applied by collection of PPT tubes versus EDTA tubes. Comparison will be between the MHH lab and an outside commercial lab. If there is a difference among the various processes, then this research will take a further step by looking at the individual processes of each lab, and find the outlier that causes them to be different when compared to the reference lab.

Methods Plasma samples were collected in both PPT and standard EDTA tubes at MHH outpatient draw station, Bellaire over a 3 month period. For blood collected in EDTA tubes, plasma was separated from blood cells within 6 h of collection by centrifugation at 800 x g for 20min at room temperature. EDTA plasma samples were aliquoted into cryovials, and then stored and transported, 1 each to MHH lab and outside commercial lab frozen. PPT tubes were processed within 2 h of collection, centrifugation took place at room temperature at 1100 x g for 10 min. PPT plasma

samples were stored and transported frozen in situ to MHH cytology lab. A total of 20 samples were compared and evaluated.

Results/Data pending

Conclusions pending

ABSTRACT

Antibiotic susceptibility of *Clostridium difficile* to rifaximin, fidaxomicin, vancomycin, and metronidazole in over 200 consecutive isolates at a university hospital in Houston, Texas

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Sponsored by: Herbert L. DuPont, MD, Center for Infectious Diseases, University of Texas Houston School of Public Health

Introduction: *Clostridium difficile* infection (CDI) is the most commonly identified cause of diarrhea-associated deaths in the USA, making up 70% of the total number of deaths from diarrhea. A standard course of therapy for CDI consists of metronidazole or vancomycin, but in about 25% of treated patients, disease recurrence is seen. New therapies are being sought such as rifaximin and fidaxomicin. Rifaximin is a non-systemic, gastrointestinal-selective antibiotic that binds to the β-subunit of bacterial RNA polymerase and prevents transcription by blocking extension of short RNA transcripts. Fidaxomicin is also non-systemic, acting earlier in the transcription initiation pathway by blocking formation of the RNA polymerase open promoter complex. Widespread and chronic use of these antibiotics, especially rifaximin, in gastroenterology has led to concerns about the promotion of resistant strains of CDI, which will limit future efficacy.

Objectives: The aim of the present study was to chronicle the frequency of the development of in vitro resistance by the major drugs used to treat CDI—rifaximin, fidaxomicin, metronidazole, and vancomycin—against *Clostridium difficile* isolates obtained over the past three years.

Methods: For vancomycin and metronidazole Etest strips were used to test susceptibility. Rifaximin and fidaxomicin were tested using agar dilution methodology, established by the Clinical and Laboratory Standards Institute (CLSI). 260 strains of *C. difficile* examined from consecutive patients with CDI at a university hospital in Houston were obtained for the study. Rifaximin was dissolved in acetone before making 2-fold dilutions ranging from 1,024 to 0.0019 µg/mL. Fidaxomicin was dissolved in ethyl alcohol at a concentration of 10 µg/mL before making 2-fold dilutions ranging from 2 to 0.0019 µg/mL. Dilutions were incorporated in Brucella agar. After the preparation, *C. difficile* isolates were inoculated onto the plates and incubated at 37°C for 48 h under anaerobic conditions. *Clostridium difficile* (ATCC 700057), *Bacteroides thetaiotaomicron* (ATCC 29741), and *Bacteroides fragilis* (ATCC 25285) were used as the quality control strains for the MICs. The MIC breakpoint used to define in vitro resistance for rifaximin, metronidazole, and vancomycin was ≤32 µg/mL. The breakpoint for fidaxomicin was 1.0 µg/mL. The presence of antibiotic susceptibility of *C. difficile* strains at levels higher than breakpoints was studied. For agar dilution studies with

metronidazole and vancomycin, 216 strains were evaluated for MICs because one batch of 44 strains did not have control isolates that fell under the established ranges.

Results: MICs at which 90% of 260 *C. difficile* isolates were inhibited (MIC$_{90}$) for rifaximin and fidaxomicin was < 0.0019 and for metronidazole and vancomycin the MIC$_{90}$ was < 0.016. MICs above drug breakpoints, considered resistant strains, were seen in: 7 strains vs. rifaximin (2.7%); 5 strains vs. fidaxomicin (1.9%), 9 strains vs. metronidazole (4.2%) and 7 strains vs. vancomycin (3.2%). When a strain of *C. difficile* showed resistance to one drug, it often was resistant to other antibiotics. All strains tested against fidaxomicin were susceptible at 2 µg/ml. Four of 7 (57.1%) organisms resistant at 32 µg/ml were not resistant to rifaximin at 64 µg/ml and 5/7 (71.4%) originally resistant strains were susceptible to 256 µg/ml of rifaximin. For metronidazole and vancomycin, resistance at 32 µg/ml predicted resistance at higher concentrations.

Conclusion: Resistance levels of recently identified strains of *C. difficile* are generally low for the drugs used to treat CDI. The low levels of rifaximin resistance for *C. difficile* strains found were surprising to us. We previously published data that levels of resistance against rifaximin were rising between 2007 and 2011 in the study hospital used in this study that is known to heavily use the drug in patients with liver failure. The other important finding about rifaximin is that resistance to rifaximin largely disappeared as the organisms were exposed to higher concentrations of drug. This is important because our group has shown that rifaximin drug concentrations in the intestine after three days' therapy reach 8,000 µg/ml, although the biologically active concentration of this poorly soluble antibiotic in the aqueous colon is uncertain. This rising dose effect was not seen for metronidazole or vancomycin meaning that once resistant strains are identified, they are resistant to high levels of the drug. No moderate or high level resistance was seen for fidaxomicin. In summary, we found low levels of resistance for the drugs commonly used to treat CDI. We also found that rifaximin may be effective against strains normally considered resistant in vitro. The findings have implications for CDI therapy.

Appendix:

Table 1. Minimum inhibitory concentrations (MICs) for rifaximin, fidaxomicin, metronidazole and vancomycin against *Clostridium difficile* and percentage of resistant strains. MIC is measured in µg/mL.

Antibiotic	No. of isolates	No. of resistant strains	Percentage of isolates resistant	MIC$_{50}$	MIC$_{90}$	Range
Rifaximin	260	7	2.7%	<0.0019	<0.0019	<0.0019 to >1024
Fidaxomycin	260	5	1.9%	<0.0019	<0.0019	<0.0019 to 1
Metronidazole	216	9	4.1%	<0.016	0.125	<0.016 to >256
Vancomycin	216	7	3.2%	<0.016	2	<0.016 to >256

Table 2. Total number of resistant strains, percentage, and cross resistance shown for the four antibiotics.

Antibiotic	Total no. of resistant strains, n (%)	Total no. of strains also resistant to rifaximin, n (%)	Total no. of strains also resistant to fidaxomicin, n (%)	Total no. of strains also resistant to metronidazole, n (%)	Total no. of strains also resistant to vancomycin, n (%)
Rifaximin	7 (2.7)		4 (57.1)	4 (57.1)	4 (57.1)
Fidaxomicin	5 (1.9)	4 (80)		3 (60)	2 (40)
Metronidazole	9 (4.1)	4 (44.4)	3 (33.3)		6 (66.6)

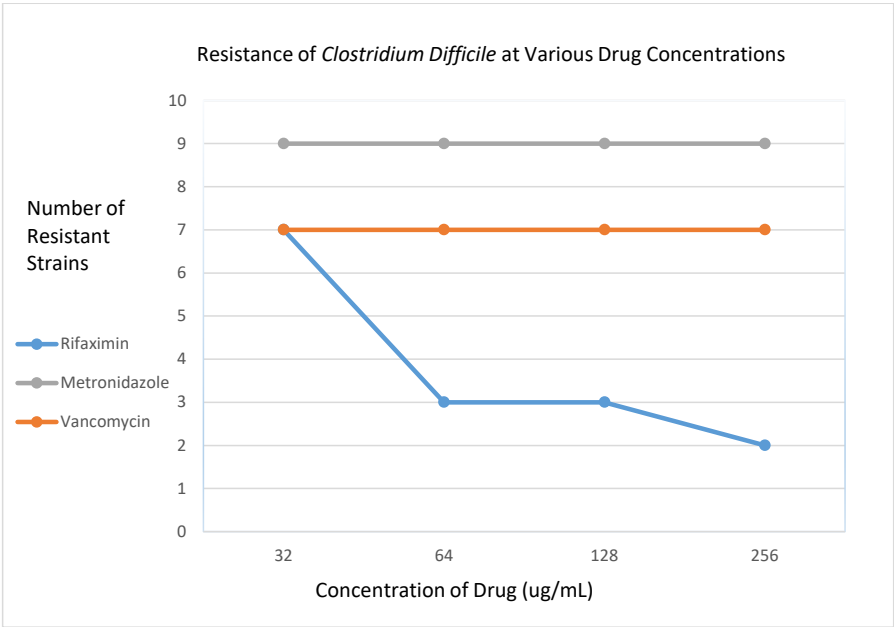| Vancomycin | 7 (3.2) | 4 (57.1) | 2 (28.6) | 6 (85.7) | |

Table 3. Distribution of total isolates, non-resistant and resistant, by MIC value, measured in µg/mL. Note that five fidaxomicin strains were found resistant at 1 µg/mL but none were found resistant at 2 µg/mL.

		No. of isolates with MIC value (µg/mL).							
Antibiotic	**Total no. of isolates**	**n≤0.016, (%)**	**<0.016<n≤0.25 (%)**	**0.25<n<1 (%)**	**=1 (%)**	**1<n<2 (%)**	**=2 (%)**	**2<n≤24 (%)**	**≥32 (%)**
Rifaximin	260	243 (93.5)	7 (2.7)	1 (0.38)	1 (0.38)	0 (0)	1 (0.38)	0 (0)	7 (2.7)
Fidaxomicin	260	240 (92.3)	14 (5.4)	1 (0.38)	5 (1.9)	0 (0)	0 (0)	N/A	N/A
Metronidazole	216	165 (76.4)	39 (18.1)	2 (0.9)	1 (0.5)	0 (0)	0 (0)	0 (0)	9 (4.2)
Vancomycin	216	143 (66.2)	16 (7.4)	18 (8.3)	10 (4.6)	6 (2.8)	6 (2.8)	10 (4.6)	7 (3.2)

Table 4. Susceptibility of strains found resistant to study drugs to higher drug concentrations. All metronidazole and vancomycin resistant strains were found resistant at over 256 µg/mL but the Etest strip only goes this far.

Drug	Resistant Strains	Concentration of Drug (µg/mL)			
		32 ug	**64**	**128**	**256**
Rifaximin	7	7/7(100%)	3/7(42.9%)	3/7(42.9%)	2/7(28.6%)
Metronidazole	9	9/9(100%)	9/9(100%)	9/9(100%)	9/9(100%)
Vancomycin	7	7/7(100%)	7/7(100%)	7/7(100%)	7/7(100%)

Figure 1. Plot of Resistant Strains for Rifaximin, Metronidazole, and Vancomycin at 32, 64, 128 and 256 µg/mL.

Resistance of *Clostridium Difficile* at Various Drug Concentrations

Number of
Resistant
Strains

- Rifaximin
- Metronidazole
- Vancomycin

Concentration of Drug (ug/mL)

ABSTRACT

The Hyperfibrinolytic Phenotype is the Most Lethal and Resource Intense Presentation of Fibrinolysis in Massive Transfusion Patients

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OBJECTIVE: Among bleeding patients, we hypothesized that the hyperfibrinolytic (HF) phenotype would be associated with the highest mortality, while shutdown (SD) patients would have the greatest complication burden.

METHODS: Severely injured patients predicted to receive a massive transfusion at 12 level-1 trauma centers were randomized to one of two transfusion ratios as described in the PROPPR trial. Fibrinolysis phenotypes were determined based on admission clot lysis at 30 minutes (LY30): SD ≤0.8%, physiologic (PHYS) 0.9-2.9% and HF ≥3%. Univariate and multivariate analysis was performed. Logistic regression was used to adjust for age, gender, arrival physiology, shock, injury severity, center-effect and treatment arm.

RESULTS: Among the 680 patients randomized, 547(80%) had admission TEG values available to determine fibrinolytic phenotypes. Compared to SD and PHYS, HF patients had higher ISS (25 vs. 25. vs. 34), greater base deficit (-8 vs. -6 vs. -12) and were more uniformly hypocoagulable on admission by PT, PTT and TEG values; all $p<0.001$. HF patients also received more RBC, plasma and platelets (at 3, 6 and 24 hours), had fewer ICU, ventilator and hospital-free days, and had higher 24-hr and 30-d mortality; all $p<0.001$. There were no differences in complications between the three phenotypes. Multivariate logistic regression demonstrated that HF on admission was associated with a 3-fold higher mortality (OR 3.06, 95% C.I. 1.57-5.95, $p=0.001$)

CONCLUSIONS: Previous data have shown that both the SD and HF phenotypes are associated with increased mortality and complications in the general trauma population. However, in a large cohort of bleeding patients, HF as confirmed to be a much more lethal and resource intense phenotype. These data suggest that further research into the understanding of SD and HF is warranted to improve outcomes in this patient population.

ABSTRACT

Evaluating the role of *Corynebacterium matruchotii* MdbA in oral biofilm formation

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Oxidative protein folding via disulfide bond formation is a well-characterized process in Gram-negative bacteria; however, this pathway has only recently been revealed in the two Gram-positive actinobacteria *Actinomyces oris* and *Corynebacterium diphtheriae*. In these organisms, post-translocational folding of the majority of proteins exported by the Sec translocon requires a thiol-disulfide oxidoreductase named MdbA. MdbA is essential for many cellular processes, as genetic disruption of *mdbA* causes aberrant cell morphology, grow arrest, and attenuation in bacterial virulence. The focus of my work is to characterize a putative MdbA homolog in *Corynebacterium matruchotii*, a newly found oral actinobacterium proposed to be a key organism in the development of oral biofilms. By molecular techniques, I generated a recombinant MdbA protein of *C. matruchotii* in *E. coli* and purified the protein in homogeneity for antibody production. In collaboration with Argonne National Laboratory, we have determined the crystal structure of *C. matruchotii* MdbA (1.7 Å resolution) that reveals two conserved features present in actinobacterial MdbA proteins: a thioredoxin-like domain and an extended α-helical domain. In addition, I found that *C. matruchotii* forms a monospecies biofilm as determined by the Congo Red assay and that *C. matruchotii* interacts with *Fusobacterium nucleatum*, a key pathogen involved in oral biofilm formation. Future experiments will focus on determining if genetic disruption of *C. matruchotii mdbA* affects cell growth, morphology, biofilm formation, and interaction with *F. nucleatum*. This study will contribute to our understanding of oxidative protein folding in actinobacteria and allow for research of MdbA inhibitors in actinobacterial pathogens including *A. oris*, *C. diphtheriae*, and *Mycobacterium tuberculosis*.

Undergraduate Student

ABSTRACT

Using Connectomics to Investigate Retinal Ganglion Cells

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Sponsored by: David W. Marshak, PhD, Department of Neurobiology and Anatomy
Supported by: University of Texas System Grant 363303 – Analysis of Neural Circuits by
                Electron Tomography
Key Words: Retinal ganglion cells, amacrine cells, connectomics, electron microscopy,
                motion detection

Background: Retinal ganglion cells integrate and transmit visual information from the retina to the brain. There are many distinct types of retinal ganglion cells whose functions vary along with their morphology. The focus of this study was on parasol ganglion cells, also known as M cells, which contribute to many aspects of vision and particularly to the perception of motion. Though parasol cells have long been studied by a variety of methods, retinal connectomics provides a novel way to study their synaptic connections. In particular, we propose that inputs from amacrine cells, inhibitory local circuit neurons of the inner retina, impart motion sensitivity to parasol cells. Previous work predicted these synaptic connections, and this project tested this hypothesis.

Methods: Serial block-face scanning electron microscopy was used to generate a series of images through the entire thickness of a piece of central macaque retina. The project utilized Viking software to navigate and annotate these sections. Ultimately, these annotations will be compiled to generate three-dimensional reconstructions of the cells and a high resolution map of their synaptic connections, also known as a connectome. Cells were classified based on their soma size and characteristics of their dendrites, including: field diameter, morphology and depth of stratification in the inner plexiform layer. Criteria for synaptic connections included pre-synaptic vesicle clouds and post-synaptic densities.

Results: We completed annotations of five ON parasol ganglion cells and one midget ganglion cell. Additionally, we partially annotated six other ganglion cells that are not yet classified. A subset of the somas in the ganglion cell layer appeared to be displaced amacrine cells. We investigated the morphology of twenty-four amacrine cells and have begun analysis of their synaptic connections with our completed parasol ganglion cells. We have observed inputs to ON parasol cells from both starburst and wiry morphological types of amacrine cells.

Discussion: Previous studies utilized light microscopy and electrophysiology to predict the existence of synapses from starburst and wiry amacrine cells to ON parasol ganglion cells. Our annotations provided direct, ultrastructural evidence for these synapses. Further analysis of the connectome will provide a more complete description of the retinal circuitry for motion detection.

ABSTRACT

S. cerevisiae model for assessing EXOSC2 mutations in the RNA exosome

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Sponsored by: Ambro van Hoof, PhD, Department of Microbiology and Molecular Genetics
Key Words: RNA exosome, EXOSC2, RNA degradation

Background: The RNA exosome is required for RNA degradation, processing, and surveillance. It is a ten-subunit complex that can be found in the cytoplasm as well as the nucleus of eukaryotic cells. Nine subunits compose the core and cap structure, while the subunit opposite the cap, Rrp44 in *S. cerevisiae*, serves the catalytic role. In humans, EXOSC3 and EXOSC2 both serve as cap proteins in the RNA exosome subunit model. Previously only mutations in EXOSC3 were implicated in human disease, specifically pontocerebellar hypoplasia type 1. Recently, mutations in the RNA exosome subunit EXOSC2 were identified as being linked to a novel Mendelian disorder with symptoms including retinitis pigmentosa, hearing loss, short stature, and mild intellectual disability. In a study conducted by Di Donato et. al. three patients exhibiting these symptoms were identified as having mutations to EXOSC2. All three patients had mutation Gly30Val. Patients I and II were homozygous for Gly30Val, while patient III was heterozygous for Gly30Val with an additional variant, Gly198Asp. While the novel condition produced by these variants is most likely caused by impaired RNA metabolism, the molecular basis for this condition is not yet understood. In order to understand the consequences of these mutations in cell growth, a yeast model of EXOSC2 (Rrp4 in *S. cerevisiae*) was made.
Methods: Site-directed mutagenesis was used to induce corresponding point mutations to Rrp4 of *S. cerevisiae*. In order to mirror the mutation in patients I and II, the mutation Gly58Val was induced in Rrp4. Patient III, who possessed the additional mutation Gly198Asp, was mirrored in yeast Rrp4 as mutation Gly226Asp. The product of site-directed mutagenesis was sent for sequencing. From this, the resulting plasmids with the correct point mutations were used to transform *E. coli*. The plasmid was used to transform a leu2 yeast strain containing a Rrp4 knock-out with an additional functional Rrp4 plasmid with URA3 selection. Transformed yeast was plated onto 5FOA plates in order to kick out the functional Rrp4. The positively transformed yeast was used to conduct a growth assay. Growth assay plates were incubated at 15°C, 30°C, 37°C, and room temperature.
Results: A growth assay revealed that yeast containing mutation Gly58Val may have a slight growth defect when compared to wild-type yeast with functional Rrp4. Yeast with mutation Gly226Asp are also viable mutants and do not appear to have a growth defect when compared to the wild-type yeast. In order to more accurately determine the growth effects of these mutations in the yeast model, a 24-hour liquid plate growth assay will be conducted. Further analysis will be conducted using a western blot to determine what effects the amount of mutant Rrp4 protein produced in our yeast cells might have on growth.
Conclusion: Results of the growth assay reveal that when Rrp4 point mutations correlating to human mutations are made in yeast, the yeast is still viable despite the mutations to Rrp4. Because Gly198Asp has only been found in compound heterozygous patients, it is not clear whether this mutation causes the disease, or is a null-mutation. Yeast with the equivalent Gly226Asp is viable, which indicates that this mutation may not be a lethal null mutation in humans.

ABSTRACT

Effects of Antihypertensive Medications
on the Success of Kidney Transplantation

Alan Vu   *University of Texas McGovern Medical School*   *Class of 2020*

Sponsored by: Donald A. Molony, MD, Department of Internal Medicine; Joshua A. Samuels, MD, MPH, Department of Pediatrics; Cynthia Bell, Department of Pediatrics

Supported by: Donald A. Molony, MD, Department of Internal Medicine; Joshua A. Samuels, MD, MPH, Department of Pediatrics; Cynthia Bell, Department of Pediatrics

Key Words: Antihypertensive, Kidney Transplantation, Allograft Failure

Introduction: Hypertension complicate the outcomes of kidney transplantation, leading to cardiovascular complications and decreased kidney graft survival. While the effects of hypertension on graft survival have been studied, the potential impact of antihypertensive treatment prior to transplantation on post-transplantation outcomes is less well understood. In this study, we intend to explore the effects of antihypertensive use prior to kidney transplantation on graft survival and mortality.

Methods: A national comprehensive data base of all transplant patients in the United States, the United Network for Organ Sharing (UNOS) Standard Transplant Analysis and Research Files from September 1987 to December 2013 was used. UNOS has given their permission to perform and publish this study. We analyzed (STATA 14.1) kidney graft survival time in patients stratified by their pre-transplantation antihypertensive medication use. Individuals were excluded from the analysis if they were retransplanted, had a simultaneous pancreas transplant or were transplanted after January 1, 2007. Analyses were performed using the Kaplan-Meier log rank test. For the analyses, three failure outcomes were explored: (1) allograft failure (excluding graft survival after death), (2) allograft failure or death, or (3) death. Data was stratified for ethnicity, gender, and diabetic status.

Results: A total of 165,889 patients were included in this study. The following median graft times (in days) show statistical significance (p-value<0.05) by displayed strata:

Event		General	Male	Female	White	Black	Hispanic	Non Diabetic
Allograft Failure Only	**Antihypertensives**	3694	3596	3858	3905	2843	4329	4153
	No Antihypertensives	4007	3996	4057	4179	3003	4293	4356
	P-value	0.0000	0.0000	0.0007	0.0000	0.0095	0.6394	0.0000
Allograft Failure or Death	**Antihypertensives**	5188	5047	5223	5724	3512	5652	5351
	No Antihypertensives	5088	5145	5021	5497	3566	5253	5219
	P-value	0.3427	0.0006	0.0144	0.0027	0.9357	0.0025	0.9719

Conclusion: A higher graft failure rate was seen in subjects on antihypertensive medication compared to those not exposure before transplant. Males, specifically non-diabetics and Blacks, showed particularly increased graft failure rates when exposed to antihypertensive medication before transplant. Conversely, females, specifically non-diabetic Hispanics and Pacific Islanders as well as diabetic Blacks, exhibited substantially reduced graft failure rates with pre-transplant antihypertensive exposure. The analysis will continue to account for comorbidities, the impact of the class of antihypertensive medication, and other factors which determine graft survival.

ABSTRACT

Studies of SSc-associated NOTCH4 in iPS cells

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Sponsored by: Xiaodong Zhou, MD, Department of Internal Medicine

Supported by: Xiaodong Zhou, MD, Department of Internal Medicine

Key Words: Systemic Sclerosis, Notch4 , iPS

Background: Systemic Sclerosis (SSc) is a rare chronic autoimmue rhematic disease. It is characterized by abnormal growth of the connective tissue and results in fibrosis of the skin and internal organs. There have been many genes from multiple studies identified to contribute to the susceptibility of SSc, such as STAT4, ITGAM, and IRF5, however, the etiology of this disease is still widely unknown. Recently, studies have shown that NOTCH4, a gene involved in the pathway of cell to cell communication, has been discovered to have multiple variants associated with susceptibility of SSc. The goal of part one of this study is to successfully perform an NOTCH4 iPS knockout in order to later analyze the effect of NOTCH4 variants on the susceptibility of SSc.

Methods: We first cultured the iPS cells and then used immunostaining to detect the presence of stem cell markers in iPS cells. The CRISPR/Cas 9 system, a targeted gene-editing tool, was used to permanently knockout the NOTCH4 genes in the iPS cells by transfection. After the transfection, the cells were cultured until there were enough colonies to split (about 10-14 days). The cells were split a total of 7 times. Using 2.5ug/ml of Blasticidin, the cells were screened starting from P8. In total this process took around 3 months. Specific PCR protocol was used to examine knockout clones, and the PCR product was sequenced to confirm the success of the knockout.

Results: We found no NOTCH4 sequence in iPS cells. The NOTCH4 gene was successfully knocked out.

Findings: In future studies, we will transfect and SSc-rick and non-risk NOTCH4 variants into iPS cell to compare their functions on cells such as proliferation and differentiation. This will ultimately help us observe the impact of NOTCH4 variants on SSc susceptibility.

![UTHealth Medical School - The University of Texas Health Science Center at Houston]

ABSTRACT

Regulation of proliferation and IGF-1R expression in mouse vascular smooth muscle cells by GTAP

Jeffrey Wang   *The University of Texas at Austin*   *Class of 2016*

Background and Aim: Galactosyltransferase 1-associating protein (GTAP) is a member of the E2 ubiquitin conjugating family. As an E2 enzyme, GTAP mediates the E3 ubiquitylation proteolytic pathway and plays a role in regulating functional protein levels in the cell. GTAP is especially upregulated in actively dividing cells, some cancers, and embryonic stem cells, indicating that GTAP may play an important role in controlling cell growth. Previous research has shown that when GTAP expression in mouse vascular smooth muscle cells (VSMC) is knocked down with miRNA, the expression of insulin-like growth factor-1 receptor (IGF-1R) is upregulated. IGF-1R is a growth related receptor protein, and its change in regulation may provide a potential link between GTAP and cell proliferation. Furthermore, the IGF-1/IGF-1R pathway plays a role in the pathogenesis of certain cardiovascular diseases and has cardiovascular protective effects. Our research aimed to define the link between GTAP and cell growth in vascular cells.

Methods and Results: We used a lentiviral vector to transduce murine VSMC cells with shRNA that knocked down GTAP expression (GTAP KD). Growth curves of GTAP KD and wild-type primary VSMC were determined by hemocytometer cell counting and MTT tetrazolium assay. A statistical assessment of differences in cell growth was conducted using a two-factor ANOVA test. Expression of GTAP and IGF-1R protein expression were measured by Western blot analysis. Densitometry of GTAP and IGF-1R protein bands was performed and the band density values were normalized with that of GAPDH, a housekeeping protein. We observed that GTAP KD cells grew significantly faster ($p < 0.05$) than wild-type control cells. Preliminary Western blot showed that the GTAP KD cells had lower levels of GTAP and IGF-1R than wild-type primary VSMC.

Conclusion and Discussion: The pilot study demonstrates that knocking down GTAP expression using shRNA increases growth and alters expression of IGF-1R in murine VSMC. These data suggest that GTAP regulates VSMC growth through IGF-1R. A potential limitation that confounds our conclusions is that low passage wild-type VSMC were used as controls for high passage VSMC with stable GTAP knockdown.

Future experiments will compare the GTAP KD cells with mock transduced cells. Nonetheless, our data indicates that GTAP plays an important role in controlling VSMC proliferation. Further tests using transduction controls, synchronized cells, and larger sample sizes will help reduce sample variance and better characterize GTAP's influence on growth. mRNA expression profiles will also be examined using RT-PCR.

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ABSTRACT

Identifying Receptors of Specialized Pro-resolving Mediators in Gingiva

Emily Wu   *Rice University*   *Class of 2017*

Sponsored by: Chun-Teh Lee, DDS, DMSc, MS, Periodontics and Dental Hygiene

Supported by: Chun-Teh Lee, DDS, DMSc, MS, Periodontics and Dental Hygiene; The University of Texas at Houston Dental School – Office of the Dean

Key Words: Periodontitis, Inflammation, Specialized Pro-resolving Mediators

Background and Objectives: Chronic periodontitis is a biofilm induced inflammatory disease characterized by a loss of alveolar bone around teeth that affects over 47% of adults over 30 in the USA. Traditional mechanical treatments have limited effects on susceptible patients. Specialized pro-resolving mediators (SPMs) including lipoxins, resolvins, protectins, and maresins are lipid mediators derived from essential fatty acids that have dual anti-inflammatory and pro-resolution actions. SPMs have been proven effective in treating experimental periodontitis in animal models by preventing bone loss and regenerating lost alveolar bone. The goal of this study was to profile the gene expression of SPM receptors at periodontally diseased sites and healthy sites of human gingiva to advance the application of SPMs in treating periodontitis.

Methods: Gingival tissues were collected from 3 healthy sites and 4 periodontally diseased sites in 5 subjects. Diseased sites were determined by having a probing depth ≥5 mm and clinical attachment loss≥3 mm. Healthy sites were determined by having a probing depth ≤3 mm and clinical attachment loss ≤2 mm. The average probing depth for the healthy sites was 2.93±0.12 mm and the average probing depth for diseased sites was 5.5±0.57 mm. RNA was extracted from gingival tissue and then reversely transcribed to cDNA. qRT-PCR with SYBR Green was performed to assess the expressions of SPM receptor genes (ALX, BLT1, ChemR23, CNR2, GPR32) and inflammation-related genes (IL1β, IL8, IL10, TNFα). Results were demonstrated as relative expression levels ($2^{-\Delta\Delta CT}$, Mean ± SEM). The Student's t-test was used to compare expression levels in the healthy and diseased groups.

Results: Several genes had higher relative expression levels (ALX: 4.14±2.50, ChemR23: 5.20±0.20, CNR2: 4.79±1.97, GPR32: 4.18±3.18, IL10: 2.93±0.93) in the diseased group than those in the healthy group (ALX: 0.99±0.05, ChemR23: 3.00±1.31, CNR2: 2.19±1.16, GPR32: 0.91±0.32, IL10: 1.36±0.37). However, there was no statistically significant difference of gene expressions between two groups.

Conclusion: There is a trend that the expression levels of SPM receptor genes are higher in diseased sites than the expression levels in healthy sites. Expression levels of SPM receptor genes appear to be related to the expression level of IL-10 gene. In the future, more samples will be collected to increase the statistical power.

ABSTRACT

Promotion of Osteogenesis

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Sponsored by: Johnny Huard, PhD, Department of Orthopaedic Surgery

Supported by: Krishna Sinha, PhD

Key Words: Tissue regeneration via stem cells

It was discovered that a unique population of slowly adhering myogenic progenitor cells are able to survive in the micro-environment of injured tissues after implantation. These cells were termed muscle-derived stem cells (MDSCs) due to properties such as increased survival and long-term proliferation, continued self-renewal, and multipotent differentiation ability. MDSCs have been used ever since in a variety of regenerative medicine approaches in an effort to improve the healing of articular cartilage, peripheral nerve, bone, ligaments, and the heart. More recently, many advances in research for MDSC therapy to delay the progression of aging and disease-related pathologies have been made. Ongoing clinical trials involve over 400 women with stress urinary incontinence and 12 patients suffering from myocardial infarction who have volunteered for stem cell therapy.

We are especially interested in epigenetic regulation of bone cell regeneration; the osteoblast-specific transcription factor Osterix (*Osx*) is pivotal for maintenance of bone homeostasis and formation of new bone after fracture. Even in bone marrow stromal cells, adipose-derived cells, and in satellite cells, forced expression of *Osx* induces the formation of osteoblasts. However, the mechanisms involved with the differentiation of MDSCs into bone-forming cells are poorly understood. Genetic modifications of MDSCs with *Osx* for bone formation has not been tested until now, although it was previously found that BMP4-expressing MDSCs have a tremendous capacity to enhance bone healing when implanted in a calvarial bone defect.

Through mass spectrometry analyses of *Osx* polypeptides isolated from BMP2-induced C2C12 cells, our data showed that *Osx* is most likely to be methylated on its lysine residues. *Osx* is either mono or di-methylated at lysine sites 45/46, 244, and 309, leading us to hypothesize that lysine methylation may have a significant role in the regulation of *Osx* function during osteoblastic differentiation. This was confirmed when we saw higher levels of methylated *Osx* at the 45/46, 244, and 309 sites in BMP-treated cells as compared to control cells, leading us to believe that *Osx* methylation is required for the activation of target gene expression. Furthermore, we are currently in the process of identifying which lysine methyltransferase (KMT) actually methylates *Osx*. Among the candidates is Suv39H1, whose role is not as clear. We are using Chaetocin, a specific inhibitor for Suv39H1, on *Osx*-expressing MDSCs to examine its effect on osteogenesis. Assessment involves studying the methylation of *Osx* with the Western blot using *Osx*-specific lysine antibodies in cellular extracts. Eventually, we hope to develop *Osx*-expressing MDSCs that can be reliably utilized to treat osteoporosis and/or other degenerative bone disease.

ABSTRACT

Small molecule inhibitor treatment and RNAi gene silencing of dynein heavy chain in bloodstream form *Trypanosoma brucei*

Weijie Yao   *University of Houston*   *Class of 2017*

Sponsored by: Ziyin Li, Ph.D. Department of Microbiology and Molecular Genetics
Supported by: NIH R01 AI101437
Key Words: Cell motility, dynein, flagellum, *Trypanosoma brucei*

The life cycle of *Trypanosoma brucei,* a parasitic protozoa causing African sleeping sickness, consists of two distinct morphological forms. The procyclic form exists in the tsetse fly, and the bloodstream form exists in the mammalian host. Previous work demonstrated that a calcium-binding protein, Centrin3, associates with an inner-arm dynein, IAD5-1, in the flagellar axoneme, and subsequent gene knockdown of either Centrin3 or dynein in the procyclic form results in defect in flagellar motility, but does not inhibit cell proliferation. Intriguingly, knockdown of Centrin3 in the bloodstream form of *T. brucei* causes rapid cell death, suggesting that Centrin3 is essential for cell viability of the mammalian infectious form of *T. brucei*. However, whether IAD5-1 is essential for cell viability in the bloodstream form is not known. Given that Centrin3 forms a complex with IAD5-1, I hypothesize that IAD5-1 is also essential for cell viability of the bloodstream form. Therefore, IAD5-1 is a potential drug target. Previous work suggested that EHNA (erythro-9-(2-hydroxy-3-nonyl)adenine) acts as a potent dynein inhibitor and impairs cell motility of *Leishmania*, a protozoan parasite closely related to *T. brucei*. In the current study, I investigate the function of IAD5-1 by genetic ablation in the bloodstream form, and evaluate the candidacy of IAD5-1 as a potential drug target using EHNA.

The genetic knockdown of IAD5-1 in the bloodstream form was performed using an inducible RNA interference (RNAi). After several attempts, a stable RNAi cell line was generated. Although western blotting is needed to confirm successful RNA silencing of IAD5-1, induction of RNAi by tetracycline showed an inhibition of cell growth. This result suggests that RNAi is likely working and that IAD5-1 is essential for cell viability. In addition to the genetic knockdown of IAD5-1, chemical inhibition of dynein by EHNA was also carried out. A range of EHNA concentrations and respective growth curves suggest that the lowest threshold for noticeable growth inhibition in bloodstream trypanosome fell around 100μM EHNA. Subsequent DAPI staining of 100μM EHNA treated cells following 48 hours indicates an increase in multi-nucleated and multi-kinetoplasts cells. The increase in binucleate cells indicates that the final stage of cytokinesis in the cell cycle of bloodstream *Trypanosoma brucei* may be impeded from completion due to the motility defect of the flagellum. Furthermore, western blotting of the RNAi cell line needs to be performed to verify the depletion of IAD5-1 protein upon RNAi induction. Similarly, DAPI staining of the RNAi cell line following 24 hours may be performed to quantify the increase in frequency of multi-nucleated and multi-kinetoplasts cells. Together, these results suggest the essential function of IAD5-1 in the bloodstream form of *T. brucei* and validate IAD5-1 as a good drug target.

ABSTRACT

Role of connexin36 (Cx36) in a model of photoreceptor degeneration

Sami Younes   *University of Houston*   *Class of 2018*

Background: Retinal dystrophies are a family of degenerating retinal diseases where photoreceptors progressively die. The mechanisms through which cell death signals propagate, in particular throughout photoreceptors of different types, remain obscure but the long-standing "bystander" hypothesis states that death signals may diffuse through transmembrane gap junction channels formed of connexins. Both rods and cones express connexin36 (Cx36). Mice that do not express Cx36 in cones or in rods have been recently created in the Ribelayga lab. Chronic exposure to room lights for several weeks triggers photoreceptor death. Here we set out to determine whether photoreceptor cell death under constant light conditions is dependent on Cx36.

Methods: Cone- (cone-Cx36-/-) or rod-specific Cx36 (rod-Cx36-/-) knockout mice and appropriate wild-type littermates (WT) were housed in constant light (app. 500 lux) or constant darkness for 2 weeks. Mice were euthanized by cervical dislocation under anesthesia and the eyeballs collected and fixed in paraformaldehyde. Retinal sections (20 μm) or whole-mounted retinas were reacted with antibodies against Cx36 and cone arrestin and the cell nuclei were stained with DAPI, and imaged by confocal microscopy (Zeiss 780 confocal microscope).

Results: We observed a 20% decrease in the thickness of the photoreceptor layer in the group of WT mice exposed to constant light compared to those exposed to constant dark. In this group, we also observed a decreased number of rod photoreceptor cells by 20% and of cone pedicles by 30-40%. No decrease in thickness or number of cells was observed in the mutant lines exposed to constant light compared to those kept in the dark.

Conclusion: The data suggest that the absence of gap junctions between photoreceptors has a protective effect against light-induced photoreceptor cell death. Therefore the data agree with the view that gap junctions represent a potential conduit for the diffusion of death signals in retinal dystrophies. Blocking gap junction channels may represent a therapeutic avenue to slow down the progression of retinal dystrophies.

ABSTRACT

Role of FAK and ROS Signaling Pathway in Apoptosis of Aortic SMC in *Myh11* R247C Mutants

Theodore Zhang   *Rice University*   *Class of 2018*

Sponsored by: Wang Shan Zhi, PhD, Internal Medicine; Diana Milewicz, PhD, MD, Internal Medicine

Supported by: Diana Milewicz, PhD, MD, Internal Medicine

Key Words: FAK, Myosin, ROS Signaling Pathway, Apoptosis, Aorta, Smooth Muscle Cells, *MYH11*

Introduction: *MYH11* R247C is a rare genetic variant present in cases of aortic aneurysms and dissections. In the *MYH11* R247C variation, the highly conserved arginine at the 247 position on the smooth muscle cell (SMC) Myosin HC is replaced by a cysteine. This significantly diminishes the contractile ability of the Myosin HC. The myosin head domain is comprised of the Heavy Chain (HC), the Regulatory Light Chain (RLC) and the Essential Light Chain. Myosin RLC Kinase phosphorylates the RLC to activate it, allowing the RLC to control and facilitate contractions by the HC. Focal Adhesion Kinase (FAK) is a non-receptor tyrosine kinase that is activated by integrin and acts as the upstream effector of Myosin RLC Kinase and phosphorylates RLC through the activity of Rho GTPase. FAK is also the upstream effector of NADPH Oxidase 4 (Nox4). Nox4 associates with 2 regulatory proteins, p22phox and poldip2, and oxidizes NADPH to $NADP^+$ by reducing molecular oxygen to reactive oxygen species (ROS). Accumulation of ROS can lead to stimulation of intrinsic apoptosis. Consequently, in order to compensate for the weaker contractions by the R247C HC, the myosin RLC will become more phosphorylated than usual. FAK activation can lead to increased Myosin RLC activation as well as increased activity of other downstream effectors.

Hypothesis: *In vitro* activation of FAK by exposing transgenic R247C mouse aortic SMC to fibronectin will increase the phosphorylation of myosin RLC as well as increase the activation of ROS signaling pathway. As a result, due to the buildup of ROS and increased expression of intrinsic apoptotic factors (Bid & Bax), aortic SMC will undergo apoptosis.

Methods: cDNA for samples were collected through reverse transcriptase PCR (rtPCR) using cytoplasmic mRNA from mouse aortic SMC. The expression levels of Nox1, Nox2, Nox4, p22phox, poldip2 were obtained through quantitative real-time PCR (qPCR). Protein levels of RLC, phosphorylated RLC, FAK, phosphorylated FAK, Bid and Bax were examined with Western Blot.

Results: Data collection is still in progress. Initial Western Blot shows marked increase in the protein levels of phosphorylated FAK in the R247C mutants when compared to the wild-type phosphorylated FAK levels. Phosphorylated RLC levels was also higher in R247C mutants compared to wild-type phosphorylated RLC levels. Furthermore, introduction of fibronectin

also increased phosphorylated FAK and phosphorylated RLC levels in both wild-type and mutants. Initial qPCR results show that levels of Nox2, Nox4, p22phox and poldip2 are all increased in mutant cells compared to wild-type cells while Nox1 expression was too low to be detected. Further experimental repeats are being done to confirm results and reach significance.

Conclusion: Based on these preliminary results, the increased activation of FAK inside the mutant mouse aortic SMC do in fact stimulate increased apoptosis through the activation of the both the ROS signaling pathway as well as the RLC activity. Increased phosphorylation of RLC requires increase ATP production which can result in mitochondrial degeneration and ultimately, apoptosis. This helps elucidates the specific pathway of action of FAK in the *MYH11* R247C variant and can allow for pharmaceutical intervention to prevent aortic aneurysms and dissections by interacting with the FAK and ROS signaling pathway intermediates.

ABSTRACT

Temporal and spatial distribution of lactoferrin expressing neutrophils in mouse brain after intracerebral hemorrhage (ICH)

Lucian Zhao   *Washington University in St. Louis*   *Class of 2020*

Background: Lactoferrin (LTF) is an iron-binding protein, abundant in the secondary granules of neutrophils, known for its immunomodulatory, anti-oxidative and anti-inflammatory capacities. After ICH, circulating blood neutrophils enter the ICH-afflicted brain where they release LTF into the brain tissue in a process known as degranulation. This degranulated LTF may bind to iron and hemoglobin (key components of hematoma) and assist in hematoma resolution. Neutrophil elastase (ELANE) is a cytosolic protein abundant in neutrophils. Using ELANE as a tracer for neutrophils, in the present study we measured both the temporal and spatial distribution of neutrophils containing LTF after ICH.

Methods: We injected 0.002U of collagenase to striatum to induce ICH in eight C57/BJ6 mice and analyzed brain at 1h, 6h, 1d, 2d, 3d, 5d, 7d, and 14d. For analyses, we intracardially perfused mice with saline (to remove blood from the brain vessels) and generated 10μm coronal cryosections of each brain for detection of ELANE and LTF by immunohistochemistry. Naïve mouse served as the negative control. Using rat anti-ELANE antibody and rabbit anti-LTF antibody to visualize LTF^+ neutrophils, we captured the digitized microscope images and counted the LTF^+-, $ELANE^+$-, and $LTF^+/ELANE^+$- cells in the brain sections from each time point using the cellSens software. The cell count was normalized for the hematoma surface area. Furthermore, using Western blot, we quantified the LTF protein amount in the ICH-affected brain tissues.

Results: After injecting collagenase into sub-cortical striatum, a visible hematoma starts to form within the first hour. Subsequently, the hematoma enlarges and reaches its maximum size by day two. The hematoma is then gradually resolved starting from day three and gets almost entirely absorbed by day 14.

The LTF or ELANE signals are non-detectable in Naïve mouse brain and in contralateral to ICH hemisphere throughout all time points. In the hematoma-affected hemispheres, the LTF^+-cell or $ELANE^+$- neutrophils appear as early as 1h, gradually increase from 6h to 24h, reach its peak at around 48h, and then gradually decrease from day 3. By day 14, there are almost no LTF^+ or $ELANE^+$-cells remaining in the brain. The LTF Western blot staining profile is consistent with the above temporal changes of LTF^+-cell numbers.

From the entire population of neutrophils ($ELANE^+$-cells), a majority of neutrophils (>50%) are LTF^+-cells. However, the number of exclusively LTF^+- cells is greater than that of exclusively $ELANE^+$-neutrophils, suggesting that not only neutrophils express LTF. The ratio of LTF^+-cells over $ELANE^+$-cells is 2.45±0.50 (n=6). Morphologically, $ELANE^+$-neutrophils have segmented,

lobular nuclei, suggesting that ELANE may primarily label mature neutrophils; whereas LTF+-cells show the diverse nuclear morphology, which may stand for different stages of maturity among neutrophils or indicate contribution of other than neutrophil cell type.

Conclusion: After ICH, neutrophils quickly enter the hematoma area. The temporal profile of their infiltration and disappearance is associated with the hematoma expansion and then resolution, respectively. Both LTF and ELANE can be used as markers of neutrophils to measure neutrophils in the brain after ICH. However, disparity in context of numbers of LTF- and ELANE- immune-labeled cells may suggest that using one single marker (such as ELANE) may underestimate the true number of neutrophils present in the brain.

Future Direction: The questions if LTF is also expressed in other brain cell types besides neutrophils, or if the neutrophils-released LTF may be encapsulated by other phagocytes, still require further confirmation.

International Medical Students

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ABSTRACT

Compare two different incubation time and temperature in Micro BCA

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Sponsored by: Claudio Soto, PHD, Professor, Department of Neurology,
                    The University of Texas Health Science Center at Houston

Keywords: Micro BCA; incubation time; concentration; protocol; measurement

Background: Micro BCA protein assay is a protein concentration measurement method was developed by P.K. Smith in 1985. The assay is based on the principle that proteins can reduce Cu^{2+} to Cu^{1+} in alkaline condition. BCA detects Cu^{1+} and forms a stable purple-colored water soluble complex. The purple-colored complex strongly shows an absorbance at 562nm. This absorbance exhibits a linear relationship with protein concentration. The protein, peptide bonds and the amino acids (cysteine, cystine, tryptophan and tyrosine) are reported to be responsible for color formation in BCA assay. Since the development of the purple- colored end product depends on the reaction time, we hypothesized that higher reaction time is necessary to detect low concentration of proteins using this assay.

Method: Different concentrations of Bovine Serum Albumin, ranging from 200 µg/mL to 3.125 µg/mL, in duplicate, were incubated 100 µL developing reagent prepared as per the instruction of Micro BCA kit. The assay was performed in 96 well microplate. We have tested two different conditions. 1, 30 minutes incubation at 37℃ and 2, 30 minutes incubation at 37℃ followed by overnight incubation at room temperature.

Results and conclusion: Our results indicate that longer incubation of sample with developing reagent enables us to detect lower concentration range of proteins. We were able to detect 4µg/mL of BSA by overnight incubation of the sample with developing reagent. This was not possible by 30 minute detection at 37 C.

ABSTRACT

Effects of shear stress on mesenchymal stromal cell function

Rui Omichi   *Tokushima University*   *Class of 2019*

Sponsored by: Pamela L. Wenzel, PhD, Pediatric Surgery

Keywords: Biomechanical force, Shear Stress, Mesenchymal stem cells, Inflammation

Background

The mesenchymal stromal cell (MSC) is a somatic stem cell originating from mesodermal tissue which holds exceptional promise for use in regenerative medicine because of its ability to differentiate into multiple cell types. However, this cell also has proven functions in anti-inflammatory signaling. In unpublished work from the Wenzel Lab, physical force caused by fluid flow typical within arterial vasculature was found to change expression of genes associated with MSC immunomodulatory function. We have chosen to more closely examine MSCs from human bone marrow to examine their response to various biomechanical forces from molecular and functional perspectives.

Experiments

We examined handling techniques that would expose cells either to minimal mechanical force (static) or high levels of fricitional force (wall shear stress, WSS). WSS treatment was applied by vigorously agitating the cells for 1 min in a tube by micropipette. The first set of experiments were designed to quantify the level of an inflammatory cytokine (TNF-a) produced by activated immune cells. We co-cultured MSCs with splenocytes that produce extremely large amounts of TNF-a in response to bacterial lipopolysaccharide (LPS). Following activation by LPS and overnight co-culture, we collected medium from co-cultures and measured secreted TNF-a by ELISA. Secondly, to test whether high densities of MSCs suppress TNF-a more strongly than low densities, we co-cultured MSCs of four different concentrations with splenocytes and analyzed TNF-a, as above. Thirdly, we measured gene expression by RNA extraction and quantitative-PCR to evaluate the effects of WSS on *PTGS2*, *HMOX1*, *TNFAIP6*, and *IL1RN*, four genes implicated in mediating MSC immunomodulatory function.

Results and discussion

The ELISA showed that splenocytes cultured with MSCs exposed WSS produced less TNF-a than those cultured with MSCs exposed to minimal force. Agitation by pipetting is simple, quick, and produces highly variable WSS. Nevertheless, our data suggest that vigorous pipetting is enough to endow MSCs greater potency than non-agitated MSCs in TNF-a suppression. In our second experiments, we found that lower concentrations of MSCs permit higher TNF-a production by splenocytes. However, even the lowest concentration of the four tested, 100K MSCs/ml, still produce less TNF-a than without MSCs. Lastly, we confirmed that WSS by vigorous pipetting upregulates expression of *COX2*, *HMOX1*, and *IL1RN*. Together these results strongly suggest that MSCs, if treated by WSS, can be effective therapeutically at lower cell doses.

Conclusion

Agitation by pipetting can enhance the ability of MSCs to suppress inflammation. In ongoing studies, we are exposing MSCs to well-defined physical forces. For example, we are using microfluidics to control hydrostatic pressure and WSS intensity. We are now testing the effects of cyclic strain (stretching). By elucidating the mechanism employed by MSCs to influence immune cells, our research promises to lead to effective clinical applications in the near future.

ABSTRACT

The functional role of Net1 in breast cancer cells and macrophages

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Sponsored by: Jeffrey A. Frost, Ph.D, Department of Integrative Biology and Pharmacology

Keywords: Net1, Breast Cancer, Macrophage, Cell Migration.

Background: Net1 is a RhoA-specific guanine nucleotide exchange factor that is overexpressed in breast cancer and contributes to cancer initiation and progression. It has two isoforms, Net1 and Net1A. Net1 plays crucial roles in mediating cancer cell proliferation and mitosis, while Net1A is important for cancer cell migration and invasion. In Dr. Frost's lab we have generated mice lacking the Net1 gene. By breeding with mice which express the Polyoma middle T antigen oncogene in the mammary gland, we found that Net1 deficiency can significantly inhibit breast cancer initiation and metastasis. Tumor initiation and progression is a complicated process which depends on the communication and coordination between tumor cells and micro-environment, especially tumor-associated M2-like macrophages. Macrophages promote tumor progression by stimulating angiogenesis and creating an inflammatory environment that enhances cancer cell migration and invasion. To further investigate the distinct roles of Net1 in tumor cells and macrophages, I studied (1) how Net1 mediates the polarization of mouse bone marrow macrophages (BMM) into M1 and M2 phenotypes, and how the different cytokines secreted by WT and Net1 KO macrophages affect breast cancer cell (MDA-MB-231) migration; and (2) defined the role of Net1 in regulating the PI3K signaling pathway in various human basal-like breast cancer cell lines.

Methods: (1) BMM precursors from WT and Net1 KO mice were cultured in M-CSF supplemented DMEM/F12 complete medium for 7 days. M1 and M2 macrophages were polarized by exposure to IFNγ and LPS (M1) or only IL4 or IL10 (M2) for 24 hours. Cultured medium from WT and Net1 KO BMMs, M1 and M2 macrophages were collected for MDA-MB-231 cell migration. After serum starvation overnight, MDA-MB-231 cells were seed in the 8.0 μm cell insert chambers and allowed to migrate towards cultured various mediums for 24h. After staining with 1μg/mL DAPI, migrated cells were photographed and analyzed under microscope.

(2) 5 basal breast cell lines (MDA-MB-231, SUM-149, SUM-159, Hs578T, BT549) were transfected with control siRNA, or siRNAs targeting both Net1 isoforms and allowed to grow for 72h. Cells were lysed in 2% SDS lysis buffer and Western blot was performed to detect the expression of Net1, pAKT and pERK.

Results: Preliminary data indicates that MDA-MB-231 cells migrated significantly less in cultured medium from Net1 KO BM macrophages and M1 macrophages compared with WT, which suggests there are different cytokine profiles between WT and Net1 KO macrophages. We observed that expression of pAKT was reduced in some basal cell line such as SUM-149, SUM-159 and Hs578T after Net1 knockdown. No effects on pERK were observed.

Conclusions: *In vitro* BM polarization experiments indicate that Net1 deficiency may affect cytokine secretions from macrophages, and in turn inhibit their ability to stimulate tumor cell migration. In addition, we have found that loss of Net1 expression in basal breast cancer cell lines impairs AKT activation, similar to that observed in Net1 KO PyMT mammary gland tumors. This suggests that the ability of Net1 to regulate Akt activation, which is necessary for tumor cell survival and metastasis, may a a generalizable effect.

ABSTRACT

Role of Heat Shock Protein During Inflammatory Response

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Lipopolysaccharides (LPS) are recognized as a key factor in inducing inflammation and oxidative stress. Chronic inflammation is thought to be a cause of stress and hypertension. Corticosterone is a hormone which is released in order to combat increasing stress levels. Heat-Shock Proteins (HSPs) are thought to play a role in the inflammatory response, specifically through the role of cytokines.

We hypothesize that stress levels over time in rats will decrease after initial inflammation induced by LPS. Fifteen male Sprague-Dawley rats were anesthetized using oxygen and isoflurane in order to insert a catheter in the femoral vein, and were given three days to recover. After recovery, a blood sample was taken for each rat prior to being given LPS (20 mg/kg IV). Group 1 (n=5) was sacrificed at one month after LPS administration, and the brain (intact), lung samples, and CSF (Central Spinal Fluid) were taken for further analysis. Group 2 (n=5) and Group 3 (n=5) will be sacrificed at two months and three months after LPS administration respectively, with the same samples being taken as Group 1. Blood samples were taken once a week per rat, and were centrifuged to collect serum. The serum from Groups 1 and 2 for weeks one through four as well as the baseline sample taken prior to LPS administration were used to complete a corticosterone assay to measure stress levels per week after initial inflammation. The intact brains will be used for immunostaining and a HSP 70 assay to measure the presence of HSPs due to inflammation. The lung and CSF will be analyzed in the future to measure HSPs and their possible role in inflammation throughout the body.

The results of the corticosterone assay on the weeks one-four and baseline serum have not been analyzed yet. In the near future we will be able to determine if stress levels generally increase or decrease over the course of one month after initial inflammation (LPS). The HSP assay will be completed in the near future, as more brains are needed for accurate results. Therefore, the role of HSPs in chronic inflammation cannot be demonstrated at this point in time.

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ABSTRACT

VEGF-A promotes lipolysis through beta-3 adrenergic pathway

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Sponsored by: Dr. Kai Sun

Keywords: Adipose tissue remodeling, VEGF-A, Lipolysis

During the progression of obesity, local hypoxia is developed in the adipose tissue (AT) due to limited oxygen diffusion[1]. Hypoxia inducible factor-1α (HIF-1α) is induced due to local tissue hypoxia. Vascular endothelial growth factor-A (VEGF-A) is one of the classic downstream molecules regulated by HIF-1α[2]. HIF-1α specifically induces a pro-fibrotic state in AT instead of upregulation of VEGF-A[3]. However, physiological activities, such as cold exposure and exercise, may dramatically stimulate VEGF-A overexpression in AT. Previously we reported that overexpression in AT can enhance a brown adipose phenotype in white adipose tissue (WAT) by increasing the level of PGC-1α and UCP-1[2]. But the direct effect of VEGF-A on lipolysis remains unknown.

Here, we hypothesize that VEGF-A enhances lipolysis in WAT by increasing phosphorylation of hormone sensitive lipase (HSL) through beta-3 adrenergic pathway to improve adipose tissue function.

To test the hypothesis, a doxycycline-inducible mouse model which over-expresses VEGF-A was used. After one week induction of VEGF-A in local AT by doxycycline, the mice were sacrificed and AT was collected. Adrenergic beta-3 receptor and phosphorylation level HSL in epididymal and subcutaneous AT were analyzed.

Real time PCR shows that the adrenergic β_3 receptor in the WAT of VEGF-A transgenic group was significantly upregulated. Western-blotting further reveals that the phosphorylation level of HSL were dramatically elevated.

Our preliminary results suggest that over-expression of VEGF-A can induce lipolysis by upregulating the adrenergic β_3 receptor. Further studies are required to confirm the observations. Our studies demonstrate that VEGF-A is a critical factors that regulate metabolic homeostasis in the obese AT.

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ABSTRACT

GRKS Suppresses the Expression of Androgen Receptor Gene And Regulates positively correlates with NE marker(ENO2)   in NEPC cells

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Neuroendocrine prostate cancer (NEPC) is an aggressive subtype of prostate cancer that commonly arises through neuroendocrine differentiation (NED) of prostate adenocarcinoma (PAC) after therapy, such as radiation therapy and androgen deprivation treatment (ADT). Neuroendocrine prostate cancer (NEPC) is characterized by loss of androgen receptor (AR) expression, resistance to hormonal therapies, and elevated levels of NE-related proteins. Recently, we have shown that G-protein coupled receptor kinase (GRK-3) is a new critical activator of prostate cancer progression. However, its molecular mechanisms remain poorly understood. We aim to investigate the GRK3 expression in different PAC cells and its influence to AR expression as well as NE-related proteins.

Results:

To investigate the signaling pathways and molecular mechanisms of neuroendocrine prostate cancer cells, we compared the level of AR and NE marker between AR-positive adenocarcinoma (PAC) LNCaP.C4-2 cells with NEPC cells.NEPC cells(NE1.3, LN3,LNCap-AI) have low levels of AR and high levels of NE marker ENO2. This is consistent with the literature that long term ADT induces NED in PAC cells, mouse models and patients. We hypothesized that GRK3 promotes NEPC development. We found that GRK3 was significantly up-regulated in NEPC cells NE1.3 ,LN3 at both mRNA and protein levels as compared with the PAC cells LNCap and C4-2. Cells. G-protein coupled receptor kinase 3 (GRK3) is a critical activator of prostate cancer progression . Not only is it necessary for the survival and proliferation of metastatic cancer cells in vitro and in vivo, but it is also sufficient to promote primary tumor growth in the prostate and metastases in soft tissues (lungs and lymph nodes). We found different levels of AR and NE marker between NEPC cells and non-NEPC cells. Moreover, we determined that GRK-3 expression positively correlates with NE marker(ENO2).

Conclusion:

We examined the levels of androgen receptor(AR) and NE marker(ENO2) expression in PAC cells. And we found AR expressions were low in NEPC cells which always with higher expression of ENO2.Moreover, levels of GRK3 were positively correlate with ENO2 in NEPC cells.

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ABSTRACT

Direct conversion of astrocytes into neurons for spinal cord injury repair

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Sponsored by: Qilin Cao, M.D.; Department of Neurosurgery

Keywords: Reprogramming, Astrocyte, Neuron, Spinal cord injury

The traumatic spinal cord injury (SCI) causes neuronal death which contributes to the dysfunction after the injury. One of the critical issues for SCI repair is how to replace the lost neurons. Transplantation of variety of stem or progenitor cells has been extensively studied for neuronal replacement after SCI. However, there are many issues associated with cell transplantation approach: the safety of cell transplantation, the risk of tumor formation, the limited cell resources, the need for immunosuppression, and the ethnic controversy, et al. In this study, we have tested a novel approach to directly reprogram astrocytes into neurons without transplantation. Astrocytes become activated and form astroglial scar after SCI. Glial scar represent the major physical and chemical barriers for axonal regeneration after SCI. We hypothesize that direct conversion of astrocytes into neurons will promote functional recovery after SCI by two synergistic mechanisms: 1) reducing the inhibition of glial scar to promote axonal recovery and 2) to replace the lost neurons. We have cloned several transcription factors related to neuronal development into lentiviral vector and produced lentiviruses expressing these transcription factors. Astrocytes purified from the injured rat spinal cord are divided into seven groups to overexpress 1) mcherry, 2) sox2-mcherry, 3) mash1-mcherry, 4) neurogenin2-mcherry, 5) sox2-mash1-mcherry, 6) sox2-neurogenin2-mcherry or 7) mash1-neurogenin2-mcherry, using lentiviral infection. The infected astrocytes are induced to differentiate for 2 and 6 weeks with or without the small molecular. The converted efficiency is evaluated by immunohistochemistry. Our initial results show that over-expression of sox2 and mash1 or sox2 and neurogenin 2 can reprogram astrocytes into neurons at 2 weeks after differentiation and small molecular enhance the reprogramming efficiency. The quantification analyses for reprogramming efficiency and phenotype differentiation are undergoing. The in vivo animal experiments are also undergoing. The results from both in vitro and in vivo will help us to determine the effects of neuronal reprogramming of astrocytes in SCI repair. Completion of these studies could help us to develop a novel effective therapeutic approach to promote functional recovery after SCI.