

OUHSC 2015

SUMMER
UNDERGRADUATE
RESEARCH
PROGRAMS

Student
Research
Abstracts

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2015

I_{DeA}
NETWORK of
BIOMEDICAL
RESEARCH
EXCELLENCE

FOREWARD

The INBRE Summer Research Program, formerly known as BRIN, is celebrating its fifteenth year. Over 400 students have participated in the program since its inception. The program has succeeded in encouraging students to pursue careers in the STEM disciplines. Twenty-six percent of former students have gone on to health related professions, sixteen percent to medical schools, and seventeen percent to biomedical graduate schools.

This summer, there are thirty-seven students from ten Oklahoma undergraduate institutions participating in the program. The students were selected based on their applications by an independent panel, and each student was paired with a mentor conducting research in a shared area of interest. The goal of the program is to expose undergraduate students to the world of biomedical research and inspire them to pursue careers in the fields of science and technology.

The program is funded through a grant by the National Institutes of Health's Institutional Development Award (IDeA) Program and through the Oklahoma State Regents for Higher Education. Oklahoma INBRE (IDeA Network of Biomedical Research Excellence) is a five year grant, and it is currently in its third renewal. The grant brings a total dollar amount of \$18 million to the state. The IDeA Program was designed to broaden the geographic distribution of NIH funding for health related research. Federal-state partnerships were formed to enhance the science research and technology capabilities of states that receive smaller amounts of federal research and development dollars. The Oklahoma INBRE grant provides research funding to fourteen higher education institutions throughout the state. Oklahoma's INBRE assists these institutions' efforts in conducting biomedical research by supporting promising new faculty, recruiting students into biomedical research careers, and sustaining vital core facilities. For additional information about the grant, please visit the INBRE website at oumedicine.com/mi/ok-inbre.

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Mentor: Dr. Phillip Coburn

Institution: University of Oklahoma Health Sciences Center

Student Home Institution: University of Central Oklahoma

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Mentor: Dr. Jicheng Fu

Institution: University of Central Oklahoma

Student Home Institution: University of Central Oklahoma

INBRE ABSTRACTS
INBRE Students' Abstracts

Abstract #1

EFFECTS OF BETA-FNA ON TAK-1 PHOSPHORYLATION IN NORMAL HUMAN ASTROCYTES

Eric Bates, K. McCracken, D.J. Buck and R.L. Davis

Department of Pharmacology and Physiology, Oklahoma State University Center for Health Sciences

Introduction: Neuroinflammation is a component of many neurological disorders including CNS infections, neurodegenerative diseases, depression and other mental health disorders. β -funaltrexamine (β -FNA) shows promise as an effective means to combat neuroinflammation. More specifically, β -FNA inhibits interleukin-1 β (IL-1 β)-induced chemokine expression in normal human astrocytes (NHA). β -FNA is best characterized as a mu-opioid receptor (MOR) antagonist but, the anti-inflammatory actions identified thus far are MOR-independent. Our objective was to better understand the mechanism by which β -FNA inhibits inflammatory signaling by assessing its effects on the activation (phosphorylation) of transforming growth factor beta-activated kinase 1 (TAK-1).

Methods: NHA were maintained in cell culture with media replenished every 48-72 h until 80% confluence was reached. Cells were exposed to IL-1 β (3 ng/ml), β -FNA (10 μ M), IL-1 β + β -FNA or untreated for 30-120 minutes. Western Blot analysis was used to measure phosphorylated TAK-1 (p-TAK-1), TAK-1 and β -tubulin expression in whole cell lysates.

Results: A trend toward increased p-TAK-1 in IL-1 β treated cells was observed, but did not reach the level of statistical significance. Similarly, β -FNA tended to decrease IL-1 β -induced TAK-1 phosphorylation, but again not to the level of significance.

Conclusion: β -FNA may decrease TAK-1 phosphorylation, but further experiments are required, likely with modified experimental conditions (e.g., increased IL-1 β concentration and earlier time points).

Funding: Funding: This project was supported in part by the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20GM103447 and OCAST HR14-007 (RLD).

Abstract #2

WETTABILITY AND ANTIBACTERIAL EFFICACY OF DENTAL ADHESIVE CONTAINING DOPED TiO₂ NANOPARTICLES

Matthew Benton¹, *F. Florez*¹, *R. Hiers*¹, *A. Rondinone*², *S. Khajotia*¹

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Introduction: The development of antibacterial adhesive resins is one approach to reducing secondary dental caries. The objective of this study was to assess the wettability and antibacterial efficacy of dental adhesive containing Nitrogen-doped TiO₂ nanoparticles (N-TiO₂).

Methods: Experimental adhesives were synthesized by adding N-TiO₂ (Oak Ridge National Laboratory) to OptiBond Solo Plus adhesive resin in concentrations of 0, 50, 67 and 80% (v/v). Thin-films (t \approx 15 μ m, d=12mm) were fabricated to test wettability (n=4/concentration) and antibacterial efficacy (n=10/concentration). Wettability of ultrapure water (2 μ L) was tested at four locations per specimen in an OCA15-Plus contact angle goniometer. Profiles of axisymmetric sessile drops (25frames/s, 37°C) were analyzed to determine contact angle values at time=0s (θ INITIAL) and time=59s (θ FINAL). Antibacterial efficacy was tested by growing *Streptococcus mutans* biofilms (UA159-ldh, 1:500 dilution, 3 hours, 37°C, anaerobic) on the surfaces of thin-films with or without continuous visible light irradiation (410nm, 39J/cm²). Then, thin-films with biofilms were sonicated (20kHz, 4min, \approx 78KJ) to remove the adherent biomass. Counting of viable cells was performed using the CFU/mL method. Data was analyzed by General Linear Models and Student-Newman-Keuls post-hoc tests (α =0.05).

Results: Significant differences were observed among the groups for both wettability and antibacterial efficacy (p<0.0001). Mean θ INITIAL values ranged from 86° (control) to 95° (80%) and for θ FINAL from 44° (control) to 48° (67%). CFU/mL mean values ranged from 5.66E+05 (control) to 6.98E+04 (80%) without light irradiation, and from 5.30E+04 (control) to 1.58E+04 (80%) with continuous irradiation.

Conclusion: Adhesives with higher N-TiO₂ were less wettable and more antibacterial than the control group, as hypothesized.

Funding: This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20GM103447. A portion of this research was conducted at the Center for Nanophase Materials Sciences, which is a DOE Office of Science User Facility.

Abstract #3

ISOLATION AND PURIFICATION OF BACTERIOPHAGES: AN ALTERNATIVE METHOD OF KILLING BACTERIA

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Introduction: Bacteriophages are viruses which utilize bacteria as hosts. With antibiotic discovery, bacteriophage studies were largely abandoned by western medicine. Increasing drug resistances among bacteria make bacteriophages interesting to the scientific community now because their host choice is highly selective and viral reproduction lyses host bacteria. Our goal was to isolate, purify and characterize virulent bacteriophages from fecal material which possess lytic capability against *Escherichia coli*.

Methods: Bacteriophages present in mammalian feces were amplified by mixing with bacterial cultures and overnight incubation. Enriched cultures were sterilized and spot-tests were employed to identify bacteriophage-positive lysates. Once identified, bacteriophage lysates were titrated onto agar plates to count plaque forming units (pfu) and isolate individual bacteriophages. Consecutive plaque isolates were used for bacteriophage purification. After three successive purifications, bacteriophage genomic DNA was isolated, and underwent restriction enzyme digests and electrophoresis to verify phage presence and approximate genome size.

Results: Horse and goat sources yielded bacteriophage-positive lysates against an *E. coli* strain. An average of 2.28×10^9 pfu/ml and 1.36×10^9 pfu/ml were obtained from the goat and horse samples, respectively. Restriction enzyme digests indicated double stranded DNA and an approximate genome size of 48 Kb.

Conclusion: Utilizing mammalian fecal material as a source, it is possible to isolate and purify bacteriophages based on host selection. Sequencing genomic DNA of novel phages will allow for further characterization of isolated bacteriophages. The bacteriophage-host systems will be utilized in model systems to study bacteriophages as alternatives to antibiotics.

Funding: Funding source: This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health through Grant Numbers 8P20GM103447 and GM110593. Additional support was provided by the Department of Biochemistry and Microbiology, OSU Center for Health Sciences.

Abstract #4

LRRTM4 IS SPECIFICALLY LOCALIZED TO PHOTORECEPTOR-ON BIPOLAR CELL SYNAPSES IN THE MOUSE RETINA.

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¹INBRE Summer Undergraduate Research Program; ²Department of Cell Biology; ³Oklahoma Center for Neuroscience; ⁴Department of Pharmaceutical Sciences, University of Oklahoma Health Science Center

INTRODUCTION: A key stumbling block to cell-based therapies to restore sight in degenerative retinal diseases is that grafted photoreceptors or stem cells form synapses with the host retina very poorly. Synaptic adhesion molecules are excellent candidates to regulate synapse formation between photoreceptors and their appropriate synaptic targets. However, very little is known about the synaptic adhesion molecules at photoreceptor synapses. The Leucine Rich Repeat Transmembrane (LRRTM) family of synaptic adhesion proteins comprise four members that are known to regulate synapse formation in the brain. We tested whether the LRRTMs might be positioned to regulate specific synapses interactions between photoreceptors and their targets.

METHODS: LRRTM expression and localization at photoreceptor synapses was assessed using immunolabeling of frozen sections of mouse retina together with fluorescence and confocal microscopy.

RESULTS: All four LRRTMs were expressed in the retina and showed distinct distributions. In particular, LRRTM4 was specifically associated with photoreceptor terminals. Double and triple immunolabeling for LRRTM4 together with cell- and synapse-specific markers showed that LRRTM4 specifically localized to the synapses between rod and cone photoreceptors and ON-type bipolar cells at ribbon synapses. In addition, LRRTM4 also appeared to be associated with the ribbon apparatus of the photoreceptor terminal itself.

CONCLUSION: All four LRRTM isoforms are expressed in the retina in distinct patterns. LRRTM4 in particular is strategically positioned to regulate synapse formation and function at the ribbon synapses between rod and cone photoreceptors and their ON bipolar cell targets.

Funding: This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20GM103447, and the Presbyterian Health Foundation. Mohammed Mazhar M Bombaywala was supported by the INBRE Summer Undergraduate Research Program.

Abstract #5

OPTIMIZING PROTEIN-PROTEIN CROSSLINKING: A TOOL FOR PROTEOMICS

James Brown, and S. Das-Bradoo

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Introduction: Minichromosome maintenance protein 10 (Mcm10) is a vital replication factor in eukaryotes and has been observed to exhibit abnormal expression in many forms of cancer. To better understand Mcm10's role in healthy and cancerous cells our lab aims to analyze its binding partners at a molecular level. To this end, we have cross-linked proteins by formaldehyde with the intent of purifying Mcm10 by Nickel column and analyzing interactions by mass spectrometry.

Method: *S. cerevisiae* strains yb025 (Myc tagged genomic Mcm10) and DB030 (protease deficient strain transformed with a vector containing a 6x His tagged Mcm10) were incubated in the presence of 1% formaldehyde to produce reversible protein-protein linkages. Cell lysates were prepared utilizing trichloroacetic acid (TCA) precipitation and whole cell extract (WCE) methods. The lysates were then analyzed by western blot to determine the yield of cross-linked complexes.

Results: Protein precipitation by TCA was found to be incompatible with formaldehyde cross-linking. Cell lysis with glass beads in the presence of protease inhibitors and urea was successful in producing lysates containing Mcm10-protein complexes.

Conclusion: Successful cross-linking of Mcm10 and its interaction partners was achieved. In conjunction with our laboratory's recent success with purification of Mcm10 using nickel columns, we are confident of identifying novel binding partners of Mcm10 via proteomic analysis.

Funding: This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20GM103447.

Abstract #6

E. COLI EXPRESSION OF A MEDICAGO TRUNCATULA CDNA-ENCODED ANTHOCYANIN REDUCTASE (ANR)

Ludmila Chandler, and N. Paiva

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Introduction: Condensed tannins benefit human health by serving as natural antioxidants, reportedly reducing cancer rates and heart disease. Anthocyanin reductase (ANR) controls condensed tannin biosynthesis in plants, but few biochemical characterizations of ANR are published. Our main emphasis was to improve expression levels of ANR protein in an *E.coli* pMAL expression system using an available *Medicago truncatula* ANR cDNA clone. We also initiated isolation of a new ANR cDNA clone from condensed tannin-rich *Cercis canadensis* seeds.

Methods: Plasmids constructed to express the MtANR cDNA ORF fused to *E.coli* maltose binding protein (MBP) with and without periplasmic targeting signal (pMAL-P5x and pMAL-C5x derivatives) were transformed into hosts JM109 and NEB-Express. Levels of ANR-MBP protein were visualized using immunoblots and SDS-PAGE gels. Immature *Cercis* seeds were harvested by dissecting green pods and freezing immediately in liquid nitrogen. RNA was extracted using TriReagent.

Results: NEB-Express cells yielded more ANR-MBP protein (1.80 mg) than JM109 cells (1.05 mg). Maltose-agarose affinity chromatography worked effectively in purifying ANR-MBP fusion protein. Cytoplasmic constructs yielded more ANR than periplasmic constructs. High yields of *Cercis* RNA were obtained but samples contained starch contamination. Vanillin/HCl staining confirmed high levels of condensed tannins in *Cercis* seed coats.

Conclusion: There was an improvement in expression levels and yield of ANR-MBP protein using *Medicago truncatula* ANR when using NEB-Express competent cells and cytoplasmic expression, although degradation was still evident. Hosts with fewer proteases could further improve yields. RNA was successfully obtained from *Cercis* seeds accumulating condensed tannins, for future ANR cloning efforts.

Funding: This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20GM103447.

Abstract #7

INDUCTION OF AUTOPHAGY PARTIALLY RESCUES LOCOMOTION DEFECTS IN C. ELEGANS NEMATODES

Lewis Clark, and A. Holgado

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Introduction: In humans, CRMPs (Collapsin Response Mediator Proteins) are involved in axon development and elongation. Mutations in UNC-33, the *Caenorhabditis elegans* CRMP homolog, result in improper neuronal development and impaired mobility in worms. Recent work from the SWOSU neuroscience laboratory has shown that the brain metabolite LK (Lanthionine Ketimine) partially rescues *unc-33* mutant neuronal defects. Moreover, preliminary work from Hensley and collaborators uncovered a probable link between LK and activation of the cellular recycling process of autophagy. We therefore present data towards testing the hypothesis that enhancement of autophagy is sufficient to partially rescue the mobility defects in *unc-33* mutants.

Methods: Nematodes harboring the *unc-33(e204)* mutation were synchronized at the egg stage and allowed to develop into larva for a 48-hour period. After growing for the specified time, mutant rescue was indirectly measured via locomotion assays in liquid and on agar media. The effects of autophagy was tested by inducing dauer formation, with starvation, or by introducing the *daf-2(e1370)* mutation.

Results: Liquid medium locomotion assays showed a 32.6% increase in thrashing rates among dauers versus their non-dauer counterparts, while *daf-2* mutant strains had a 61.3% collective increase in thrashing rates. Further assays showed a 124.6% increase in coordinated sinusoidal movements in dauers versus non-dauers, and a 100.0% increase among *daf-2* mutants compared to control. Assay of locomotion on agar also showed significant differences between dauers and non-dauers.

Conclusions: Preliminary evaluation of mobility rescue shows that *C. elegans unc-33* mutants attain significantly improved locomotion when developing under conditions of enhanced autophagy.

Funding: This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20GM103447.

Abstract #8

IMMUNOHISTOCHEMICAL IDENTIFICATION AND CHARACTERIZATION OF NEURONAL PRIMARY CILIA IN RATS

Jordan Cox, G. Cook¹, N. Wilson¹

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Introduction: Primary cilia have been implicated in many important developmental processes, ranging from normal physical growth to neurologic function. The objective of this research was to optimize an immunohistochemical method where several types of microscopy (i.e. fluorescence, confocal) can be employed to effectively image neuronal primary cilia for qualitative as well as quantitative analysis.

Methods: Using modified versions of two commonly used protocols (floating sections and freeze-thaw method), rat brain slices were prepared and mounted on slides for analysis. Labeling with a fluorophore-conjugated adenylate cyclase III (ACIII) antibody to identify primary cilia followed by labeling with 4',6-diamidino-2-phenylindole (DAPI) to identify nuclear DNA was used to determine which method, if any, would be suitable for identification and observation of neuronal primary cilia in vivo.

Results: Tissue slices prepared using the floating sections method stained successfully but contained excessive background noise, making identification of individual organelles challenging. Also, the free-floating method prevented us from observing actual tissue morphology as it appears in vivo. Tissue slices prepared using the freeze-thaw method also stained successfully, but with considerably less background noise, making the images much clearer and positive identification of individual organelles easier. Applying the sections directly to gel-coated slides and allowing them to dry also made preservation of actual tissue morphology more consistent.

Conclusions: Based on comparative results, we determined that the freeze-thaw method of tissue preparation is most suitable for our current research applications

Funding: This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20GM103447.

Abstract #9

STRATEGY TO REMOVE UNNECESSARY GENES FROM GENETICALLY MODIFIED PLANTS USING RECOMBINASES

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Introduction: Transgenic plants are a valuable asset to the global economy and addressing consumer safety concern is a priority. Antibiotic resistance genes found in many of the commercially available transgenic plants unnecessarily remain in the genome after initial screenings. These genes, are necessary in screening processes to obtain stable transgenic lines, however, removing these genes prior to market is ideal. Our goal is to produce marker-free transgenic plants utilizing recombinase technology.

Methods: Expression vector pCambia2300-Phas1470-Nos was used to introduce four independent binary vectors, both codon-optimized and original gene fragments for ParA and CinH recombinase cassettes, into *Nicotiana tabacum* SR1 by *Agrobacterium* mediated transformation. Recombinase expression is driven by phas, a seed-specific promoter. Selective kanamycin (100mg/mL) medium and GUS staining assay was used to screen putative transformants. T1 seedlings were analyzed on kanamycin selection medium and by GUS staining. Genomic DNA was extracted from T1 seedlings, and PCR using primers against the selectable marker NptII, GUS, and the full cassette is underway.

Results: Kanamycin inhibited T1 seedlings from ParA- and CinH-original transformants. These seedlings demonstrated chlorosis, small size, and lack of true leaves. T1 Seedlings from these transgenic lines were GUS positive in the staining assay. Kanamycin did not inhibit seedlings from transformants expressing the optimized gene fragment.

Conclusions: Preliminary results suggest removal of antibiotic resistance genes flanked by recombinase recognition sites can be mediated by controlled expression of ParA and CinH recombinases under phas. DNA analysis by PCR and southern blot will confirm effectiveness of the marker removal system.

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Abstract #10

CHARACTERIZATION OF A MODEL OF GLUTAMATE EXCITO-TOXICITY IN NEURONAL AND MICROGLIAL CELLS

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Introduction: Traumatic brain injury (TBI) is the leading cause of death and disability in Americans younger than 40. Apoptosis and neuronal degeneration are the main causes of long-term deficits experienced in patients with TBI and neurodegenerative disorders (e.g. Alzheimer's disease). Excessive glutamate release and excitotoxicity is one of the triggers for activation of apoptotic pathways leading to brain damage. Our aim is to establish and characterize an in vitro model of glutamate excitotoxicity in neuronal and microglial cells that simulates apoptosis seen in TBI and neurodegenerative diseases.

Methods: Both neuronal (SH-SY5Y) and microglial (BV2) cells were treated with glutamate (L-glutamic acid) at varying concentrations (5 to 50 mM) for 24 hours. Cell viability was measured using the MTT assay. Activation of two major apoptotic proteins; caspase 3 and poly ADP-ribose polymerase (PARP) were quantified using immunoblotting analysis.

Results: SH-SY5Y cells were more sensitive to glutamate excitotoxicity than BV2 cells with significantly different inhibitory IC₅₀ values of 18.4 and 32.0 mM respectively ($P < 0.05$, $n = 4-6$). Maximal glutamate-induced excitotoxicity/cell death was detected in both cell lines at 50 mM concentrations. Cleaved caspase 3 was significantly increased in SH-SY5Y cells after treatment with 10 mM glutamate for 24 hrs ($P < 0.05$, $n = 2$). Cleaved PARP and BV2 experiments are currently ongoing.

Conclusions: This in vitro model is useful to study glutamate-induced apoptosis using SH-SY5Y cell lines after treatment with 10 mM glutamate for 24 hours, as detected by the significant increase in cleaved caspase 3 levels. Neuronal cells have higher sensitivity to glutamate excitotoxicity than microglial cells.

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Abstract #11

ESTROGEN AND ESTROGEN RECEPTOR ANTAGONIST EFFECT ON CD55 EXPRESSION AND E. COLI INFECTION IN BLADDER

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Introduction: About 50% of women contract a urinary tract infection (UTI) by E. coli at least once in their lifetime. Lack of estrogen in menopausal women increases UTI susceptibility. Estrogen replacement along with antibiotics used as therapeutics show conflicting results and needs further investigation. Our studies have shown that Dr adhesin bearing E. coli cause UTI by binding to cells via CD55 regulated by hormones. In this study, we investigated estrogen regulation of CD55 and Dr E. coli invasion in T24 bladder cells and in bladder tissues of hormone treated C3H/HeJ mice, with and without UTI.

Methods: T24 bladder epithelial cells were treated with various doses of β -estradiol (E2) and estrogen receptor (ER) agonists and antagonists for 24 hours before infection. Bacterial invasion was determined using gentamicin protection assay. CD55 expression on T24 cells was determined using flow cytometry and in mice bladder tissues by immunohistochemistry.

Results: In T24 bladder cells, physiological levels of E2 appears to lower CD55 expression and bacterial invasion, although not significantly. In uninfected mice, E2 treatment significantly increased CD55 in muscularis of bladder tissues compared to the untreated control group. Vehicle-treated, infected mice induced a significant increase of CD55 expression, as compared to uninfected mice. In contrast, ER antagonist-treated, infected mice had reduced CD55 with increased bacterial colonization when compared to vehicle-treated, infected mice.

Conclusions: Bladder cell lines other than T24 may be required for optimal testing of estrogen effects on CD55 and bacterial infection. Estrogen via ERs regulates CD55 in uninfected and infected mice.

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Abstract #12

LIPID NANOPARTICLES IN THE TREATMENT OF DIABETES

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Background: Pancreatic beta-cell dysfunction and insulin resistance are the two important factors contributing to the pathogenesis of diabetes. Our goal is to develop an ideal pancreatic cell-specific gene delivery system with low antigenic potential, high capacity to accommodate genetic material, high transduction efficiency, controlled and targeted transgene expression, and reasonable expense and safety for both the patients and the environment. The application of viruses as carriers to deliver genes to pancreatic tissue was successful, although unsafe. A safer, non-viral, biocompatible lipid-based nanoparticle has never been tested in the treatment of diabetes. In this study, we developed a non-viral gene delivery system to pancreatic cells for therapeutic applications.

Methods: We have cloned a cDNA-encoding neon green fluorescent protein under the control of a CAG promoter. The neon green plasmid DNA was complexed with lipid nanoparticles (LPD) and injected through the mouse tail vein. One week later, pancreatic tissues were cryosectioned and examined for fluorescence under microscopy. We have also established beta cell cultures from mouse pancreas, and transfected neon green by LPD. Immunocytochemistry was used to confirm pancreatic specific expression of insulin.

Results: Our data suggest that tail vein injection of LPD is able to deliver genes into the pancreas. We also observed the expression of neon green in our pancreatic beta cells which were positive for insulin, a marker for beta cell islets.

Conclusions: We successfully constructed a non-viral gene delivery system to pancreatic cells. This system could be used to deliver therapeutic genes to alleviate pancreatic β -cell failure in diabetes.

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Abstract #13

ESTROGEN INCREASES VOLUNTARY EXERCISE BY OVARIECTOMIZED RATS

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Introduction: The benefits of exercise on physical and psychological health are well-known. Exercise can be influenced by different physiological factors including body fluid status. Estrogen is known to affect body fluid balance as well as locomotor activity. However, little is known about the effects of estrogen on voluntary exercise, and even less about how interactions between estrogen and body fluid challenges affect voluntary exercise. Previous studies in the lab suggest that water deprivation influences forced running. The objective of the current study was to investigate the effects of estrogen and body sodium challenge on voluntary running.

Methods: 16 female Sprague-Dawley rats were bilaterally ovariectomized and allowed to recover for a week. They then were administered estradiol benzoate (EB) or oil on two consecutive days, repeated at weekly intervals for 3 weeks. Four oil-treated rats and four EB-treated rats were housed in cages in which running wheels were attached and were allowed to run voluntarily; four oil-treated rats and four EB-treated rats remained sedentary throughout the study. During week 1 and 3, running distance was recorded while rats were maintained on a regular diet (baseline 1 and 2). During week 2, running distance was recorded while rats were maintained on a sodium deficient diet (NaD).

Results: EB treatment increased distance run compared to oil treatment. Effects of NaD were modest; however, experience increased distance run, particularly in EB-treated rats.

Conclusion: These results suggest that body fluid challenges affect voluntary running, but estrogen has a larger impact, especially with increasing exercise experience.

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Abstract #14

CANCER GENE COOPERATION MODELED IN DROSOPHILA.

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Introduction: Studies have shown that certain oncogenes are commonly mutated in many different types of cancers. Many of these cancers have mutations in multiple oncogenes. Whereas activation of one oncogene may not be sufficient to cause malignancy, some evidence shows that a combination of mutations in different signaling pathways can cooperate to cause a drastic effect. Our purpose was to determine which cancer pathways can cooperate to cause malignancy in *Drosophila melanogaster*.

Methods: In this experiment, mutations in select oncogenes or tumor suppressors were expressed in *Drosophila*, either alone or in combination. GAL4 was used to express these cancer genes in the developing eye tissue.

Results: The Notch pathway showed cooperation with the Receptor Tyrosine Kinase and PI3 Kinase signaling pathways. For example, activation of either Notch or AKT alone gives a normally-patterned eye. However, a malignant phenotype results when these two are combined.

Conclusions: We used *Drosophila* homologues of human genes to model interactions between cancer pathways. Because these signaling pathways are conserved across species; a *Drosophila* model can be studied to understand how these oncogenes interact in humans. A growing understanding of how these oncogene pathways interact could lead to a more targeted treatment of cancer.

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Abstract #15

IDENTIFYING NOVEL RAG2-ASSOCIATED PROTEINS IN DNA DAMAGE-ASSOCIATED CELL CYCLE CONTROL

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Introduction: The RAG1 and RAG2 proteins initiate V(D)J recombination in developing lymphocytes. The V(D)J reaction involves the formation of DNA double strand breaks (DSBs). Inefficient repair of DNA DSBs generated during V(D)J recombination drive the formation of some types of neoplasms. Recent findings from our laboratory show RAG2 undergoes export from the nucleus to the cytoplasm following the formation of DNA DSBs in pro-B-lymphocytes. Furthermore, the cytoplasmic pool of RAG2 exhibits enrichment at the centrosome. We hypothesize that the centrosome-associated RAG2 participates in the regulation of the cell cycle by signaling ongoing V(D)J recombination and preventing duplication of the centrosome and entry into S-phase, until DNA repair is complete.

Methods: To identify proteins that may function with RAG2 in cell cycle control at the centrosome, we are using GFP-antibody coated magnetic beads to pull-down a GFP-labeled bait following DNA damage. Novel candidates will be identified by mass spectrometry following separation by SDS-PAGE.

Results: Using our described method, GFP-labeled RAG2 has been successfully pulled down. We have initiated optimization of methods to pull down RAG2 associated proteins following DNA damage.

Conclusions: We have concluded that the use of GFP-antibody coated magnetic beads to pull-down a GFP-labeled bait is an efficient method for isolating proteins that may function with RAG2 at the centrosome. Further studies will determine the identity and RAG2-associated function of these proteins.

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Abstract #16

EFFECTS OF CDKN1B PROMOTOR SNP, RS34330, ON LUPUS SUSCEPTIBILITY

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Introduction Systemic Lupus Erythematosus (SLE or lupus) is a clinically heterogeneous autoimmune disease with strong genetic and environmental components. A promoter variant (rs34330) in CDKN1B gene is identified as an SLE susceptibility in Asian population. The objective of this study was to replicate the association of this genetic variant (rs34330) in Korean population for SLE susceptibility. We also studied the effect of this variant (rs34330) in CDKN1b gene expression and its neighboring genes (APLOD, DDX47).

Methods To assess the genetic association in SLE, We genotyped the CDKN1b SNP (rs34330) in the Korean population (1947 SLE cases and 1617 controls), by TaqMan genotyping assay. We also measured the relative expression of CDKN1b and its neighboring genes using the quantitative real time PCR.

Results The study comprised of 1947 SLE cases and 1617 controls and we found that the genetic variant (rs34330) was shown to be associated with SLE (odds ratio [OR] 0.84, 95% confidence interval [95% CI] 0.76-0.93, $p = 0.0006$). Furthermore, the individual's with "CC" genotype shows higher expression of CDKN1b gene than individuals with "TT" genotypes. Present data suggest that this SNP (rs34330) is a regulatory variant, modulating the CDKN1b gene expression and is highly associated with SLE susceptibility.

Conclusion Our association and gene expression data suggest that the functionality of rs34330 is a functional variant associated with lupus.

Funding: INBRE Students Only: The following statement must be put in the Funding Source section, "This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20GM103447." It is also found on the website under the example tab.

Abstract #17

EFFECTS OF TREATMENT DURATION ON ANTI-TUMOR RESPONSE INDUCED BY NON- INVASIVE LASER IMMUNOTHERAPY

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Introduction: Laser immunotherapy is a promising therapy for metastatic cancers by combining laser irradiation with immunostimulation. Previous studies showed that non-invasive laser immunotherapy, using immunologically-modified carbon nanotubes, is an effective treatment method for metastatic cancers. This study focused on determining the optimal treatment parameters for non-invasive laser immunotherapy.

Methods: Single-walled carbon nanotubes (SWNTs) were dissolved in glycated chitosan (GC), a novel immunostimulant. For animal studies, 1.0×10^5 DMBA-4 cells were injected into the flanks of female Wistar Furth rats. Rats were split into 5 treatment groups: control, laser, laser+SWNT+NaC, and laser+SWNT+GC. Two hours before irradiation, 0.2 ml of SWNT-GC or SWNT-NaC was injected into the primary tumor. Rats were irradiated non-invasively with 980-nm laser light at a power density of 1.0 W/cm^2 for 5 or 10 minutes when tumor size was approximately 0.2 to 0.5 cm^3 .

Results: The 10-minute laser+SWNT-GC treatment induced complete primary tumor regression in 7 of 10 rats (70 %) with a final survival rate of 50%. In comparison, the 5-minute Laser+SWNT-GC treatment was capable of inducing complete primary tumor regression in only 1 of 8 rats, with no long-term survivors. Additionally, the 10-minute laser+SWNT-GC treatment induced regression of metastatic tumors at remote sites in the long-term surviving rats.

Conclusion: We demonstrated that laser+SWNT-GC treatment with 10-minute irradiation, opposed to 5 minutes, at 980-nm wavelength with a power density of 1.0 W/cm^2 , was capable of inducing regressions of both primary and metastatic tumors. These findings will guide future studies for improving laser immunotherapy as a treatment modality for cancers.

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Abstract #18

CREATION OF A DNA CONSTRUCT FOR THE STUDY OF VAMP TRANSCRIPTIONAL REGULATION

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Introduction: The occurrence of aging associated neurological disorders is increasing due to the general population's ability to live longer (among other factors) thanks to modern medicine. To address the issue of dementia in the elderly, we are looking specifically at how changes in levels of vesical associated membrane protein 2 (Vamp-2) and vesical associated membrane protein 1 (Vamp-1) overtime, affects neurotransmitter release. The goal of our experiment is to look at how nerve growth factors and transcription factors in general effect Vamp-1 and Vamp-2 expression.

Methods: To accomplish this goal, the promotor regions of Vamp-1 and Vamp-2 genes were amplified using PCR and cut with restriction enzymes for latter incorporation into a luciferase reporter vector. Furthermore, a dual luciferase reporter assay will be used to measure the level of expression.

Results: We used the readily available database of the mouse genome to identify the promoter regions of Vamp1 and 2 genes. We then designed constructs to amplify fragments of the promoter and downstream sections. Also, we have prepared full genome DNA extracts from adult mice and used it as a PCR template. Next, we digested and subcloned the inserts into the Promega luciferase vector.

Conclusion: This experiment resulted in the creation of the Vamp-1 insert, and incorporation of the Vamp-1 insert into a luciferase reporter vector. In turn, this experiment, along with subsequent experiments, should help us understanding how Vamp-1 is regulated and allow us to develop better treatment options for patients suffering from age related cognitive decline.

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Abstract #19

C-FUNCTIONALIZED TRANSITION METAL COMPLEX CXCR4 ANTAGONISTS

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Introduction: Chemokine receptors, together with their specific natural ligands, play a role in a number of disease states. We propose to systematically synthesize and evaluate potential C-functionalized CXCR4 antagonists based on our published potent N-functionalized transition metal complex CXCR4 antagonists. Upon synthesis and chemical characterization, and with the help of collaborators, we will evaluate the antagonism of CXCR4 in cell lines previously developed for such studies—with the results of these screens feeding back into the iterative re-design of additional C-functionalized complexes.

Methods: Synthetic routes were developed extending side- and cross-bridged ligand syntheses to include reactive primary amine groups attached to C-atom positions of the macrocycle ring to impart the ability to conjugate these CXCR4-targeting compounds to useful imaging compounds. Cu²⁺, Ni²⁺, Co²⁺, and Zn²⁺, complexes were synthesized. Electrospray mass spectra, UV-Visible spectra, cyclic voltammograms, and ¹H and ¹³C NMR spectra were collected to characterize the complexes.

Results: The ligand synthesis of the C-functionalized ligands is more synthetically challenging than our typical ethyl cross-bridged ligands. However, single-macrocycle and bis-macrocycle ligands have been made. Complexation with the desired metal ions proceeded as expected. Characterization of the metal complexes is ongoing.

Conclusion: CXCR4 antagonist tetraazamacrocycles C-functionalized for conjugation to other components are challenging to produce. Once synthesized, metal ion complexation proceeds smoothly following known procedures. The resulting complexes will inform our understanding of the requirements for producing efficient conjugatable CXCR4 antagonists.

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Abstract #20

CD82 STRENGTHENS THE MAINTENANCE OF ENDOTHELIAL CABLE NETWORKS ON 3D MATRICES.

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Introduction: CD82 is a member of the tetraspanin superfamily and has been identified as an important tumor metastasis suppressor. CD82 is widely expressed on various endothelial cells but, its vascular function remains unknown until very recently. The goal of this study is to investigate the function of CD82 in Human Microvascular Endothelial Cells (HMECs) using in vitro endothelial cable network formation and maintenance assay. We hypothesized that CD82 will accelerate the regression of endothelial of endothelial cable networks by reducing endothelial cell-cell adhesiveness

Materials and Method: We examined in vitro endothelial cable network formation and maintenance in HMECs transfectants of CD82 knockdown (KD) and control siRNA by culturing 15, 000 endothelial cells from each group on top of matrigel in a 96-well plate. The absence of CD82 was confirmed by flow cytometry. We imaged the endothelial cable networks at 6, 16, 20, and 24 hours. The pictures were analyzed by counting the branches of the formed cables using the ImageJ software.

Results: At 6hours, there is no significant difference between the two groups. However, at 16, 20, and 24hours, the CD82KD group regressed significantly faster compared to the siRNA control group.

Conclusion: Our observation did not support our hypothesis that CD82 accelerates the regression of endothelial cable networks. However, our results suggests that CD82 stabilize the endothelial cable networks. Also, this result suggests that CD82 is not required for the formation of endothelial cable networks on 3D matrices. Furthermore, the result suggests that CD82 strengthens the maintenance of newly formed endothelial cable networks. At this stage we could not exclude the off-target effect of our CD82-silencing RNA.

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Abstract #21

PROFILING MIRNA EXPRESSION PATTERNS FROM EXOSOMES ISOLATED FROM URINE OF ENDOMETRIAL CANCER PATIENTS

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Introduction: Exosomes are small extracellular vesicles of 30-100 nm in size, produced by all cells and are present in bodily fluids. Exosomes contain DNA, RNA, micro (mi) RNA, and proteins in their lumen. It has been postulated that the contents of exosomes isolated from cancer cells are unique and can serve as biomarkers. Hence, we hypothesized that exosomes present in the urine of endometrial cancer patients would present a unique miRNA profile that will be useful in cancer diagnosis, prognosis and predicted response to treatment.

Methods: Exosomes isolated from the urine of 11 endometrial cancer patients and 5 normal individuals were characterized for shape and size by transmission electron microscope (TEM). Subsequently, miRNAs were isolated from the exosomes and subjected to miRNA expression profiling using commercially available quantitative polymerase chain reaction (qPCR)-based 84 miRNA array.

Results: TEM showed exosomes were 20-100 nm in size and spherical. miRNA analysis showed that the expression level of all 84 miRNAs was markedly increased in the exosomes isolated from the cancer patients compared to miRNA levels in the exosomes of normal individuals. Hierarchical ranking showed hsa-miR-23b-3p and hsa-miR-200c-3p was increased by 86.8 and 83.1 fold over control.

Conclusion: We have demonstrated that the miRNA profile in exosomes of cancer patients differ from that of normal individuals. Further, miR23b-3p and miR-200c-3p identified in the present study have previously been reported to be deregulated in endometrial cancer. Our study results, though preliminary, warrant further testing prior to clinical application.

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Abstract #22

CERIUM OXIDE NANOPARTICLES PREVENT BLOOD-RETINAL BARRIER LOSS IN AMD MICE

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Purpose: Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly in developed countries. The very low density lipoprotein receptor knockout (vldlr^{-/-}) mouse is a model for wet AMD, which is characterized by dysfunction of the retinal pigment epithelium (RPE), breakdown of the blood-retinal barrier (BRB), increased vascular permeability, and neovascularization. In this study, cerium oxide nanoparticles, which catalytically destroy toxic molecules called reactive oxygen species (ROS), are evaluated for their ability to restore normal function to the BRB.

Methods: Albino vldlr^{-/-} mice were intravitreally injected with 1 µl of 1 mM nanoceria in saline at P28d. Eyes were analyzed at P35d and P7m with BalbC wild type (wt), uninjected and saline injected vldlr^{-/-} mice as controls. Expression of RPE65 and junctional proteins was analyzed with immunofluorescence staining of flat mounts, cryosections, and western blots. Vascular leakage was assessed by fluorescein angiography.

Results: vldlr^{-/-} mice have damaged RPE cells and BRB structure, and severe vascular leakage. Levels of RPE65, ZO-1, Cadherin, and Occludin in vldlr^{-/-} mice were decreased compared to wt. Nanoceria injection up regulated these proteins and alleviated vascular leakage. These effects were pronounced in P35d mice. Preliminary data shows an increase in the expression of Occludin and Cadherin proteins by nanoceria treatment at P7m. Experiments analyzing the expression of RPE65 and ZO-1 at P7m are ongoing.

Conclusion: Nanoceria are effective in protecting RPE integrity, BRB loss and prevent increased vascular permeability. Nanoceria may be an effective treatment strategy for AMD and other vascular diseases.

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Abstract #23

PMA INDUCES GROWTH INHIBITION AND MORPHOLOGICAL CHANGES IN HT-1080 CELLS

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Introduction: Ras oncogene activations are present in approximately 30% of human malignancies including colon, pancreas, thyroid and hematopoietic cancers. Our earlier studies reveal that oncogenic K-Ras-transformed cells are highly sensitive to inhibition by phorbol 12-myristate 13-acetate (PMA). In this study, we utilized a human fibrosarcoma cell line (HT-1080) with a mutated N-Ras allele to investigate further the effects of PMA on Ras-transformed cells.

Methods: The entire coding region of N-Ras was amplified from HT-1080 cell cDNA by PCR and sequenced. HT-1080 cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum at 37°C, 5% CO₂, and humidified conditions in the presence or absence of PMA. Cell counts were obtained on a hemocytometer and phase contrast microscope. Cell density and morphology were observed with Wright-Giemsa and immunofluorescence staining. Activation of Erk1/2 was assessed using Western blot analysis and immunofluorescence staining.

Results: Heterozygous N-RasQ61K mutation was found in HT-1080 cells. Cultures treated with a high dose of PMA (10uM) consistently showed a significant ($p < 0.05$) decrease in cell number compared to the respective control culture. Results for HT-1080 cell cultures treated with a low dose of PMA (0.02uM) were less consistent and the decrease was not always significant ($p > 0.05$). PMA-treated cells have a stretched appearance with prominent actin reorganization and appear differentiated.

Conclusions: PMA induces extensive cell growth inhibition and morphology changes in HT-1080 fibrosarcoma cells. Our study further verified the sensitivity of Ras-transformed cells to PMA, which may have implications for development of anti-cancer drugs targeting oncogenic RAS.

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Abstract #24

TARGETING AGGRESSIVE CANCERS THROUGH P27 REGULATED METABOLISM

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Introduction: The tumor suppressor p27 controls a metabolic switch allowing cells to use glutamine or glucose as a nutrient. Early stage tumors lack a blood supply and thus might downregulate p27 to use glutamine. Vascularized tumors could upregulate p27 to use glucose. Reducing glucose levels in advanced tumors with deregulated p27 might force a switch back to glutamine, whereupon cancer cells can be selectively killed by inhibiting the glutamine pathway.

Methods: Transformed h293 kidney epithelial cells have deregulated p27 and were used as a model system. Cells were grown in media containing different amounts of glucose and glutamine, then treated with inhibitors targeting glycolysis (2-Deoxy-D-Glucose) or glutamine metabolism (rotenone). Effects were evaluated by measuring the amount of cellular adenosine triphosphate (ATP) using an ATP dependent luciferase reaction and a luminometer.

Results: With high glucose, h293 cells produced ATP mainly through glycolysis. Decreasing glucose in the media promoted the switch to glutamine, as indicated by rotenone sensitivity. The switch could also be activated using 2-Deoxy-D-Glucose, which acts as a competitive inhibitor to mimic lower glucose levels. Cells pre-treated with 2-Deoxy-D-Glucose and then rotenone inhibited ATP ~90%. Cells did not recover ATP production with addition of glucose after rotenone inhibition.

Conclusions: Decreasing glucose levels will cause cells with deregulated p27 to switch from glycolysis to glutaminolysis. This switch can be activated by 2-Deoxy-D-Glucose, a glycolysis inhibitor currently in clinical trials. Thus, it may be possible to combine 2-Deoxy-D-Glucose with inhibitors of glutaminolysis to kill cancer cells with deregulated p27.

Funding: This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20GM103447.

Abstract #25

AICAR, METFORMIN, AND PHENFORMIN INHIBIT TGF-B INDUCED DUPUYTREN'S CONTRACTURE CELLS

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Introduction: Dupuytren's contracture is a proliferative connective tissue disease that causes the fingers of the hand to contract towards the palm. Myofibroblasts are the effector cells of Dupuytren's disease. Myofibroblasts in other diseases were inhibited by AMPK activators. Our goal was to test whether AMPK activators AICAR, Metformin, and Phenformin reduce TGF-b induced proliferation and myofibroblast differentiation of Dupuytren's cells. The ability to inhibit proliferatization in myofibroblasts and fibroblasts can be beneficial for those suffering of Dupuytren's contracture.

Methods: Serum-starved Dupuytren's cells were plated on coverslips in the presence of AICAR (500mM), Metformin (10mM), and Phenformin (2mM) 30 minutes prior to TGF-b 1ng/ml. A nucleotide probe was added to coverslips for 16 hours to monitor proliferation. After 48 hours, the cells were MeOH fixed then immunostained to observe proliferation and differentiation.

Results: When observing the results from the stain the control group had an average of 0.625 proliferative fibroblasts (pf) with a standard deviation of .744. The control average for proliferative myofibroblasts (pmf) was (Avg:0 with a SD:0). The TGF-b treated group average pf was (Avg: 2.375 and SD: 2.83) and pmf (Avg of 2.625 and SD of 2). AICAR pf was (Avg: 1.375 and SD 1.4) and pmf (Avg: .375 and SD: .518). Metformin pf was (Avg: 0 SD:0)and the for the pmf. Phenformin had the same results for pf and pmf as the Metformin. Myofibroblast differentiation was similarly affected.

Conclusions: Metformin, Phenformin, and to a lesser extent, AICAR, reduced proliferation and differentiation of both fibroblasts and myofibroblasts.

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Abstract #26

SLED DOG TRANSCRIPTOMICS DATA VALIDATION

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Introduction: Sled dogs are remarkable athletes with incredible endurance. Microarrays have been used to interrogate the transcriptional profiles of sled dog muscle tissue during training and racing. Biological replicates existed for eight dogs at time points before conditioning, after two months of conditioning, at the end of conditioning, and after a four day, four-hundred mile exercise challenge. Our goal this summer was to perform microarray technical replicates on RNA samples from a single dog at all time points.

Methods: RNA (1.0µg) from a single dog at all four time points were reverse-transcribed into double-stranded cDNA, then transcribed into amino-allyl RNA before being labeled with Alexa Fluor fluorescent dyes 488, 555, and 647. Samples were mixed and hybridized to Agilent 4X44k Canine v2 microarrays to assess any experimental bias due to the fluors themselves. Each of the four arrays had three time points resulting in two technical replicates of all time points. Statistical analyses using MEV4 (reference) determined the levels and relative amounts of differential gene expression. Differentially expressed genes were submitted to KEGG (reference here) for pathway analyses.

Results: Technical variability was lower (0.65) than biological variability (0.88). At p=0.05, technical replicates showed between 11% and 17% of differentially expressed genes compared to 21% in the pooled biological data. The individual dog differential expression pattern correlated (>30%) with pooled biological data. A threshold for biological variability on the larger dataset was indicated.

Conclusions: Technical replicates showed lower variability and fewer differentially expressed genes than pooled data. Pathways were subsequently validated.

Funding: This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20GM103447.

Abstract #27

EFFECTS OF MUTATED PROTEIN PHOSPHATE 5 (PP5) OVEREXPRESSION ON BREAST CANCER (MCF-7) CELLS

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Introduction: Serine/threonine Phosphatase 5 (PP5) is an enzyme that has a role in cell signaling. It has been shown that overexpression of PP5 increases the growth and survival of breast cancer cells (MCF-7s). Higher levels of PP5 in neuronal cells seem to reduce the severity of Alzheimer's disease yet result in cell death in other healthy cells. To better understand whether PP5's effect depends on general overexpression (physical effect) versus increased levels of phosphatase activity, we compared catalytic PP5 mutant to fully functional PP5 overexpression in MCF-7s.

Methods: We used lipofectamine-based transfection with PLUS reagent (Life Technologies) for PP5-EGFP constructs. We exposed cells to low oxygen using the BD Gas Pack EZ: Anaerobe gas generation pouch. We used SYTOX Orange dye to observe survival between mutated and non-mutated PP5-EGFP constructs. At various intervals, we observed cells using fluorescent microscopy under transmittance and fluorescent channels GFP and RFP to detect EGFP expression and cell death respectively.

Results: Based on published research overexpression of PP5 in breast cancer MCF-7 cells inhibits apoptosis. MCF-7 cells seem to thrive in presence of overexpressed PP5 especially when under stress. We used overexpression of PP5 with mutated catalytic domains from transfected constructs in MCF-7 cells under low oxygen stress. Preliminary data suggests that the overexpression of PP5 with a mutated catalytic domain does not provide cells with protection.

Conclusions: Our results indicate that the proper catalytic function of PP5 is responsible for providing MCF-7 cells with protection from stress.

Funding: This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20GM103447

Abstract #28

EFFECT OF AGE AND ESTROGEN DEPLETION ON HYPOTHALAMIC RESPONSES TO THE BETA - ADRENERGIC AGONIST, ISOPROTERENOL

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Introduction: Women have increased rates of cardiovascular disease after menopause; however, it is not clear whether this increase is due to age or to postmenopausal loss of estrogen. Our objective was to address this issue in a rat model. We used young and aged ovariectomized (OVX) female rats and focused on the hypothalamic Supraoptic Nucleus (SON), which contributes to blood pressure regulation and body fluid homeostasis via secretion of the hormones vasopressin and oxytocin.

Methods: Rats: Young (5 months old) and aged (24 months old) adult female OVX rats were used. Isoproterenol Treatment: Isoproterenol (ISOP; 30 µg/kg; young-n=5, aged-n=4) or vehicle (0.15 M NaCl; 750 µl/kg; young-n=3, aged-n=3) was injected subcutaneously. Immunohistochemistry: 90 minutes after ISOP or vehicle, rats were deeply anesthetized and then perfused with paraformaldehyde. Brains were removed, sectioned at 40µm, and processed for the protein FOS (Santa Cruz; 1:50,000), a marker of neuronal activation, and the neuropeptide Oxytocin (Chemicon; 1:25,000). Immunolabeling was quantified in the SON.

Results: Total FOS immunolabeling in the SON was increased by ISOP in both young and aged OVX rats. The increase in neural activation was not selective, as FOS and Oxytocin double-labeling and labeling for FOS only increased after ISOP in both groups.

Conclusion: These experiments revealed no effect of age on neural activation in the SON. Thus, changes in blood pressure regulation after menopause may be due to loss of estrogen. Alternatively different central areas may play a larger role in this effect.

Funding: This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20GM103447

Abstract #29

EVALUATING A TARGETED AND EFFICIENT IN VIVO BIOTINYLATION METHOD FOR PARE TOXINS

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Introduction: The preparation of labeled proteins for in vitro measurements of direct interactions are laborious and non-specific. The project objective is to directly label ParE toxin proteins using an in vivo method for biotinylation. This should be more efficient than chemical biotinylation methods due to biotinylation site specificity and will allow direct capture of biotinylated ParE proteins from cell lysate.

Methods: ParE sequences were amplified from existing vectors and placed in expression vectors encoding a biotinylation signal at either the N- or C-terminals. These sequences are recognized and biotinylated in appropriate E. coli strains. ParE sequences were amplified by PCR and the products digested with restriction enzymes for directional ligation. The resulting transformants were tested by colony PCR and by direct analysis of plasmids. Plasmids with inserts of appropriate size were sent for DNA sequencing.

Conclusions: More colonies were obtained for vectors encoding C-terminal biotinylation tags than N-terminal tags. C-terminal tags may quell the toxicity of ParE sequences, unlike N-terminal tags. DNA sequencing results indicated a frame shift in all C-terminal samples arising from the primer design. The procedure is being repeated with new primers. Overall efficiency of sub-cloning was low despite successful PCR and based on previous observations it may be due to leaky expression of the toxic ParE proteins. Targeted biotinylation should allow efficient production of protein tagged in a known location. Biotinylated protein binding assays will be used to determine how tightly ParE sequences bind to DNA gyrase and if sequence differences affect this interaction.

Funding: This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20GM103447 and by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103640.

Abstract #30

PERCEIVED AND TESTED WORK CAPACITY IN EMPLOYED AND UNEMPLOYED MEN WITH OSTEOMYOPLASTIC TRANSTIBIAL AMPUTATION

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Introduction: Traumatic amputation requires lengthy rehabilitation, which may not ensure ability to return to previous employment. Osteomyoplastic transtibial amputation (OTA) provides a more robust residuum that allows near-normal mobility. Specific work-related risks for injury are previously unexamined in the work-eligible with OTA.

Methods: 15 otherwise healthy men were classified as: [non-amputees] n=5 (C); employed OTA n=5 (E); or unemployed OTA n=5 (U). Data on floor-to-knuckle lift, two-minute self-paced and brisk walk tests and psychosocial factors from the Prosthetics Evaluation Questionnaire (PEQ), and Locomotive Capacity Index-5(LCI-5) were retrospectively examined. Work demand levels were based by federal standards.

Results: Controls lifted less weight (C-49lbs.3 vs. U-86.1 & E-90.6) yet reported higher perceived exertion RPE/20 (C-14.3 vs. U-10.7 & E-8.8). Unemployed OTA participants walked shorter distances in both walking tasks than either employed OTAs or controls. Unemployed OTAs scored lower in perceived mobility scores than employed OTAs (E-54.8 vs. U-47.4). Between OTA groups, three areas of disparity on PEQ were Ambulation (E-92.1vs.U-55.6), Appearance (E-94.4vs.U-76.4), and Utility (E -87.3vs.U-69.4). All OTAs met job demand level 40% of the time; controls met demand 60% of the time.

Conclusions: Disparity between physical capability of men with OTA and job demand level may place them at risk for work-related biomechanical injury. Small sample size provides implications, requiring expanded study to substantiate generalized need for additional, job-specific rehabilitation and/or vocational education before return to work.

Funding: This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20GM103447

Abstract #31

SEX DIFFERENCES IN BLOOD PRESSURE AND RENAL SODIUM TRANSPORT IN MICE ON HIGH FRUCTOSE AND SALT DIET

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Introduction: High fructose and salt consumption causes obesity and hypertension. Females are often protected from these conditions compared to males. Our goal was to investigate sex differences in mice consuming high levels of fructose and salt (F+S).

Methods: Female and male mice (n=6/group) in metabolic cages consumed normal diet and water for 4 days followed by 30 days of F+S diet consisting of 4% salt chow with a drinking solution of 20% fructose and 1% salt. Measurements included blood pressure via the tail-cuff method and urinary sodium excretion. Separate mice were used for molecular analysis of the renal sodium transporters via real-time PCR.

Results: Mean blood pressure (MBP, mmHg) was not different between females and males in the control period (72.3 ± 2.6 and 73.4 ± 1.3 , respectively). In the second week of F+S diet, MBP increased in females to 86.4 ± 2.2 ($p < 0.05$ from control) whereas males had a non-significant increase to 77.2 ± 1.0 . In the 4th week of F+S diet MBP was significantly higher in females and males compared to control (90.1 ± 3.2 and 89.4 ± 2.6 , respectively, $p < 0.01$). Females had lower sodium excretion-to-sodium intake ratio (%) than males (62.0 ± 4.4 vs 74.8 ± 3.7 , respectively, $p < 0.03$) and female expressed higher levels of renal sodium transporters.

Conclusion: Consuming the F+S diet increased blood pressure in both female and male mice. Interestingly, females were not protected. We propose that the higher expression of sodium transporters and enhanced sodium retention explains this lack of protection in females.

Funding: This project was supported by the National Institute of General Medical Sciences of National Institutes of Health through Grant Number 8P20GM103447.

Abstract #32

HUR IS PRESENT IN THE CYTOPLASM OF TH2 CELLS AFTER INHIBITION OF IRE1A

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Introduction: While TH2 cells promote immune responses against external pathogens by producing cytokine IL-4, over production of IL-4 can lead to diseases such as asthma. IL-4 production is promoted by IRE1 α , a molecule important for activating the unfolded protein response in cells. Inhibiting IRE1 α in mouse cells reduces IL-4 cytokine expression, mRNA stability, and map kinase p38 activation. HuR binds to IL-4 mRNA and mediates its stability, and it is translocated from the nucleus to the cytoplasm by phospho-p38. We seek to determine if reduced IL-4 production in cells treated with 4u8c is due to reduced HuR translocation from the nucleus to the cytoplasm.

Methods: D10 cells, a TH2 mouse cell line, were tested under various conditions to determine the best way to stimulate the cells and inhibit IRE1 α activity. After determining the appropriate conditions, D10 cells were stimulated in the presence of the inhibitor or DMSO for 48 hrs. Western blot was performed in order to measure HuR expression in the nucleus and the cytoplasm, ELISA was used to test the amount of IL-4 being produced, and qRT-PCR was used to measure the level of spliced Xbp-1 and total Xbp-1.

Results: While IL-4 protein expression is reduced, HuR translocates from the nucleus to the cytoplasm in the presence of the inhibitor.

Conclusions: HuR is translocating to the cytoplasm in the presence of the inhibitor. Further experiments will have to be performed to determine if HuR is binding to IL-4 in the cytoplasm.

Funding: This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20GM103447

Abstract #33

SYNTHESIS OF A HUMAN UROGUANYLIN ANALOG USING SEQUENTIAL FORMATION OF REGIOSELECTIVE DISULFIDE BONDS

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Introduction: Colorectal cancer is the second leading cause of cancer-related deaths in the United States. Guanylyl cyclase C (GC-C) receptor is the target for the endogenous intestinal paracrine hormones uroguanylin and guanylin as well as the bacterial heat-stable enterotoxins. It is selectively expressed in brush-border membranes of intestinal epithelial cells. It mediates guanosine triphosphate to cyclic guanosine monophosphate conversion and maintains intestinal homeostasis. Loss of paracrine hormone expression has shown to dysregulate GC-C and its downstream signaling that suppress tumor initiation and progression. In this regard, Waldman and coworkers (Clin Pharmacol Ther. 2007, 82(4):441-7) has recently proposed the paracrine hormone hypothesis of colorectal cancer and the potential use of oral hormone replacement therapy with uroguanylin to prevent and treat primary colorectal cancers. The main objective of this project was to synthesize the human uroguanylin analog, SP304, and examine the possible chemoprevention effect on the development of colon polyps and colorectal tumors in a mouse model in the future.

Methods: Initially, the linear SP304 peptide containing two Acn-protected cysteine residues was manually synthesized using solid phase peptide synthesis method. Then two disulfide bonds were formed by successive reactions using 2-PDS and iodine. The peptide was purified by reversed-phase high performance liquid chromatography and characterized by high resolution electrospray mass spectrometry.

Results: The SP304 peptide was obtained in an overall yield of <2%.

Conclusion: The SP304 peptide synthesis was challenging due to presence of two disulfide bonds that led to a low yield.

Funding: Funding Sources: Funding was provided by NIH Grant 8P20GM103447.

Abstract #34

FMRI AND PHYSIOLOGICAL EVIDENCE OF EMOTIONAL HYPO-REACTIVITY IN THE OFFSPRING OF ALCOHOL ABUSERS

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Introduction: Family history of alcohol use disorder (AUD) is a genetic liability expressed as a neurobehavioral-disinhibition phenotype exhibiting cognitive, behavioral, and emotional dysregulation. The hypothesis assumes lower emotional reactivity in participants with positive (FHP) than with negative (FHN) family history of AUD. Testing entails comparing FHP to FHN samples in terms of task-related or resting-state fMRI brain-activation patterns, emotion-laden probe-task performance and emotion dysregulation indices (EDI) by reviewing existing studies.

Methods: Twenty relevant studies were reviewed for differences between FHN and FHP participants in four criteria: probe-task performance, demographics and matching variables, task-related fMRI brain-activation patterns/regions, and EDI scores. Against this evidence, the following predictions were tested: a) lower probe-task performance in FHP samples; b) significant fMRI activation-pattern differences between FHP and FHN samples, in emotion-processing or behavioral-control brain regions; c) significant EDI differences; and d) significant correlation between the three predicted differences.

Results: Nine different probe tasks and six different EDIs were used with demographically-matched FHP and FHN teenagers/young adults. The existing evidence showed: a) five probe tasks produced significant between-sample performance differences; b) three studies reported between-sample activation differences in emotion-processing regions; five studies showed sample differences in behavioral-control regions; and c) fMRI activation patterns correlated closely with EDIs and physiological probe-task responses but not with behavioral probe-task performance.

Conclusion: Currently, there is conflicting and insufficient evidence demonstrating that FHP individuals exhibit emotional hypo-reactivity. Studies employing physiological emotion indices provide stronger support to the hypothesis than those employing behavioral responses.

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Abstract #35

THE SYNTHESIS AND FREEZE CASTING OF HYDROXYAPATITE NANOROD SCAFFOLDS

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Introduction: Hydroxyapatite Nanorod (HAP NR) scaffolds have been successful in bone regeneration. Bone regeneration is important for normal bone fractures to major bone degeneration. HAP NRs have a good biocompatibility with tissue, skin, and muscles which make them better for orthopedic services.

Methods: During synthesis there are hydrophilic and a hydrophobic nanorods that are produced. The hydrophilic nanorods have a polyacrylic acid coating which makes them bad candidates for bone regeneration. The non-coated hydrophobic HAP NRs are mixed by a 1:1 ratio with collagen to replicate the porosity and morphology of the natural bone. The slurry of HAP NRs and collagen are freeze casted and then freeze dried to construct a rod like structure.

Results: The individual properties of collagen scaffolds and hydrophobic HAP NR scaffolds explain why they need to be mixed. The collagen is too sponge like and the HAP NRs are fragile and break too easily for bone regeneration. After the correct ratio of collagen and HAP NR scaffolds are created, further procedures are taken to test the scaffolds on bones. The mechanical properties are not very strong so the most common place for the nanorods to be placed is none weight bearing bones such as the jaw.

Conclusion: HAP NRs are very useful modern bioengineering products used in the orthopedic fields. It's important to keep excelling with this new technology to create more reliable HAP NRs that can be used for weight bearing bones as well.

Funding: This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20GM103447.

Abstract #36

TLR4-DEPENDENT RETINAL GENE EXPRESSION IN BACILLUS CEREUS ENDOPHTHALMITIS

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Introduction: *Bacillus cereus* is leading cause of endophthalmitis, a frequently blinding intraocular infection. Toll-like receptors (TLR) recognize *B. cereus* cellular components and initiate the inflammatory response in the eye. We previously observed significantly less inflammation and retinal damage in TLR4^{-/-} mice than wildtype mice after intraocular infection. In the current study, we evaluated the retinal transcriptional response early during infection to identify TLR4-dependent genes.

Methods: The right eyes of male C57BL/6J or TLR4^{-/-} mice were intravitreally injected with 100 CFU of *B. cereus* ATCC 14579. Left eyes served as uninfected controls. After 4 hours, retinas were harvested, total RNA isolated, and quantitative PCR was performed using primers specific to mouse genes previously shown by microarray to be significantly upregulated in C57BL/6J retinas after infection.

Results: Microarray analysis previously identified a set of genes related to the acute inflammatory response that were significantly upregulated in C57BL/6J, but remained unchanged in TLR4^{-/-} retinas 4 hours post-infection. In the current study, we used qPCR to verify these results. Genes significantly upregulated in C57BL/6J but not in TLR4^{-/-} retinas after infection were CXCL1, CXCL2, CXCL10, CCL2, CCL3, IL6, ICAM-1, SOCS3, LIF, PTGS2/COX-2, and PTX3.

Conclusions: These studies demonstrate that TLR4 regulates cytokine and chemokine genes important for the acute inflammatory response, as well as genes related to photoreceptor survival and pathogen recognition and clearance. Future studies will evaluate the retinal and global ocular inflammatory responses over the course of *B. cereus* endophthalmitis to identify pathway-based anti-inflammatory targets, specifically, those that are regulated by TLR4.

Funding: The study was supported by R01EY024140, R01EY012985, and P30EY12191 and an unrestricted grant from Research to Prevent Blindness. This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health through Grant Number 8P20GM103447.

Abstract #37

A NOVEL MOBILE-CLOUD SYSTEM FOR IMPROVING MANUAL WHEELCHAIR TILT/RECLINE USAGE

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Introduction: People with spinal cord injury (SCI) who rarely tilt or recline (TR) their wheelchairs are at higher risk for developing pressure ulcers (PUs). One of the major reasons for the ineffective TR usage is that most wheelchairs nowadays do not provide the mechanism of measuring TR angles. Wheelchair users have to adjust TR angles by their perceptions and preference. Besides, existing research lacks of a practical way to keep track of the longitudinal data of wheelchair TR usage. Thus, our objective is to address these issues by using a mobile and cloud computing-based system and artificial intelligence techniques.

Methods: We implemented a novel algorithm for all kinds of Windows 8.1 devices that have a built-in accelerometer. The algorithm utilizes advanced math and physics methods. No matter how the device is positioned, it measures angles accurately. The records of wheelchair TR usage are transmitted to the cloud subsystem for monitoring users' activities. Statistic graphs are generated based on the records as feedbacks for wheelchair users. Novel text-to-speech and voice recognition techniques are employed to achieve hand-free operations of the mobile subsystem.

Results: We installed our application on a Lumia 930 Windows phone, and attached it to a manual wheelchair with a commercial goniometer to cross-validate the results. As expected, the angle readings from the smartphone and goniometer were consistent.

Conclusion: Our system is promising to improve the effectiveness of wheelchair TR usage because it enriches wheelchair mechanism, logs every adjustment, and guides users to adjust wheelchair TR effectively.

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FOREWORD

The Native American Research Centers for Health (NARCH) Student Development Program, funded through a grant from the National Institute of General Medical Sciences (NIGMS, a program within the National Institutes of Health or NIH) in collaboration with the Indian Health Service (IHS), has the purpose of encouraging and facilitating American Indian undergraduate students to apply to, enter, progress through, and graduate from colleges and universities and choose to enter medical research careers. The NARCH summer undergraduate research opportunities focus on health research issues relevant to Native Americans including diabetes-related research and cancer, and emphasizes why doing research is so important by relating it back to the health of their own tribal communities. The ultimate objectives of the NARCH Student Development Program are to increase the representation of Native American students in health related research professions where they can serve as role models for future generations.

At the conclusion of the program, each participant submits a scientific abstract and presents his or her findings at a poster session. Native American participants are encouraged to continue interactions with their sponsoring laboratories after successful completion of the program. The following collection of abstracts showcases the research activities of our 2015 participants.

We are grateful to NIH and the Indian Health Service for their support (NARCH VI: U26 IHS300412; P.I. Gloria Grim, Cherokee Nation of Oklahoma).

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NARCH ABSTRACTS
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Abstract #38

GOLD NANOPARTICLES DISRUPT CROSSTALK BETWEEN PANCREATIC CANCER CELLS AND PANCREATIC STELLATE CELLS

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Introduction: Pancreatic ductal adenocarcinoma (PDAC) is the fourth most common cause of death by cancer and presents one of the most pressing and challenging problems for therapeutic management. Altered tumor microenvironment (TME) arising from a bidirectional crosstalk between the pancreatic cancer cells (PCCs) and the pancreatic stellate cells (PSCs) is implicated in this dismal prognosis. PSCs, besides supporting PCC growth, also secrete growth factors (GFs) to support the cancer stem cell niche implicated in drug resistance. We hypothesized that unmodified gold nanoparticles (AuNPs), known to bind and denature heparin-binding GFs, could be potentially utilized to disrupt this crosstalk primarily driven by secreted GFs.

Methods: To better replicate the tumor microenvironment in PDAC, we first optimized the relative ratios of PSCs and PCCs in indirect coculture, 2D coculture and 3D coculture on uncoated, matrigel and poly-hema coated plates. 20 nm gold nanoparticles alone or in combination with gemcitabine were then tested to further observe the effects of disrupting crosstalk on the phenotype and organization of PSCs and PCCs in 2D and 3D culture models.

Results: Indirect coculture of PCC and PSCs resulted in mutual induction of cell proliferation which was inhibited by gold nanoparticle treatment. Also a significant increase in number of colonies formed by PCCs was observed when cultured with PSCs than alone.

Conclusion: The results support our hypothesis that PCC-PSC crosstalk is mutually beneficial in promoting pancreatic tumor growth and utilizing gold nanoparticles to disrupt this crosstalk is highly promising to improve the prognosis in pancreatic cancer.

Funding: This project was supported by grants from the National Institutes of Health (NIH), National Institute of General Medical Sciences (NIGMS) in collaboration with the Indian Health Service (IHS).

Abstract #39

IDENTIFICATION OF BREAST CANCER EXOSOME MEMBRANE PROTEINS

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Introduction: Exosomes are nanometer-sized (50-100nm) vesicles secreted by all cells, often carrying information that can be used in cell-to-cell signaling. Exosomes contain cytosolic proteins, lipids, nucleic acids, as well as, a lipid bilayer membrane with functional membrane bound proteins. The membrane bound exosome proteins vary by their cell of origin and may potentially be used as a source to identify plasma exosomes that have been secreted specifically from breast cancer cells. Identification of breast cancer exosome membrane proteins may provide potential biomarkers for breast cancer detection, even in the earliest stages of breast cancer.

Methods: Exosomes were isolated from various breast cancer cell lines and verified by western blotting for the known exosome markers, CD9 and CD63. An antibody array was performed to identify breast cancer exosome membrane proteins. Membrane proteins identified in the antibody array were validated by western blotting.

Results: Protein expression of CD9 and CD63 was evident in all exosomes isolated. Seven membrane proteins that are specifically expressed in breast cancer exosomes were identified by the antibody array. Among them, CD40LG was verified by western blotting showing its selective enrichment in exosomes derived from cancer cell lines (MCF7, SKBR3, and MDA-MB 231), but absent in exosomes derived from normal mammary cells (MCF10A).

Conclusions: Seven membrane proteins were identified to be selectively expressed in breast cancer exosomes. Among those identified, CD40LG is further confirmed by western blotting and maybe utilized to isolate breast cancer exosomes from patient plasma.

Funding: Funding Source: This project was supported by the Oklahoma Center for the Advancement of Science and Technology (OCAST) and the Oklahoma Shared Clinical and Translational Resources (OSCTR).

Abstract #40

THE CHARACTERIZATION OF APOLIPOPROTEIN A1: A NOVEL RETINOIC ACID BINDING PROTEIN

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Evidence suggests that retinoic acid (RA) has implications in the development of near-sightedness, or myopia. Previous experiments suggest that RA is bound by a 27kD protein present in ocular tissue, identified as Apolipoprotein-A1 (Apo-A1), suggesting Apo-A1 could have implications in the regulation of ocular growth. The goal of the study was to generate purified mature Apo-A1 protein to further study its binding interaction with RA. White leg horn chickens were euthanized via isoflurane overdose and cervical dislocation. Trunk blood was collected and spun at 12,000xg to isolate serum. Apo-A1 was isolated from serum using a NaBr density gradient, ethanol/ether washes, and centrifugation. The protein concentration, after reconstitution in PBS, was determined via Bradford Assay and spectrophotometry. The samples' purities were checked by staining with Coomassie Blue following SDS-PAGE. Western blotting was performed using anti-chicken Apo-A1 antibody. Purified protein was concentrated and reconstituted in 1.2M NaCHO for crystallization. Protein was tested with photo-affinity binding assays using 3H-RA, SDS-PAGE and fluorography. Highly purified Apo-A1 was extracted from the yellow organic layer following density gradient ultracentrifugation. Western blot confirmed Apo-A1 at 27kD. Preliminary results from photo-affinity binding assays suggest that Apo-A1 in serum binds RA with higher affinity than delipidated native Apo-A1 or recombinant mature Apo-A1. Using chicken blood serum, Apolipoprotein-A1 protein was successfully isolated. Isolated protein confirmed that Apo-A1 binds RA, and suggests the binding interaction requires the presence of lipids. The structural basis for the RA-Apo-A1 binding interaction is being tested to help determine RA's role in the development of myopia.

Funding: This project was funded by the grant: "Regulation of Scleral Growth and Remodeling in Myopia." Grant Number: EY09391 (NEI/NIH).

Abstract #41

STREPTOCOCCUS PYOGENES PHAGE A25: ASSESSING ITS POTENTIAL FOR PHAGE THERAPY IN A GALLERIA MELLONELLA MODEL

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Introduction: A25 is a lytic bacteriophage of *Streptococcus pyogenes* (Group A streptococcus (GAS)), a Gram-positive bacterium that causes a wide range of disease. Our objective was to sequence the A25 genome and develop a phage therapy model using A25 in a waxworm model (*Galleria mellonella*).

Methods: GAS susceptibility to A25 was assessed by measuring waxworm health and survival over 5 days post infection (107 cfu/ml; n=10/strain). Hemolymph from A25 inoculated waxworms was extracted, and circulating phage was measured by plaque assay. Waxworms (n=15) infected with GAS received immediate, delayed, or no phage treatment. Health and survival rates were measured. A25 sequencing was performed at the OUHSC Laboratory for Genomics and Bioinformatics.

Results: Waxworms infected with MGAS5005, SF370, ATCC12204, CEM1delta4, and K56 had 5 day mean health indices of 1.7, 1.4, 2.8, 4.1, and 7.2 out of 10 and survival rates of 20%, 25%, 30%, 45%, and 77.5% respectively. A25 remained stable within the waxworm 6 hours post injection, decreasing over time. A significant difference (p=0.04) by t-test was observed in 24-hour mean health of the immediate (5.8±0.4) versus non-treated group (2.7±0.5). Overall 24-hour survival rates were 80%, 60%, and 40% for immediate, delayed, and non-treatment groups respectively. A25 has a 33,900 bp virome with homology to streptococcal lysogens from several species.

Conclusion: Overall, waxworms that received immediate treatment fared better in health and overall survival compared to delayed or no phage treatment suggesting the potential use of A25 for selective clearance of infection. The genome sequence suggests that A25 escaped lysogeny.

Funding: This work was made possible by NIH Grant Number R15A1072718 to WMM.

Abstract #42

DOK3 NEGATIVELY REGULATES OSTEOCLASTOGENESIS

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Introduction: Bone homeostasis is maintained by the balance of osteoblasts (OB) mediated bone formation and osteoclasts (OC) mediated bone resorption. RANKL promotes OC differentiation but requires costimulation of immunoreceptor tyrosine-based activation motif (ITAM)-adapters such as DAP12. Downstream of tyrosine kinases (DOK) are cytosolic adaptors which assemble multi-molecular signaling complexes that function to limit tyrosine kinase receptor mediated signaling. Recently, we found that DOK3 negatively regulates DAP12 signaling and inflammatory cytokine production in response to toll-like receptor (TLR) stimulation of macrophages. We hypothesize that DOK3 negatively regulates osteoclastogenesis.

Methods: To test this hypothesis, bone micro-architecture, histomorphometry, and histology of sex matched 16 week old wild type (WT) and DOK3-deficient (DOK3 KO) mice were evaluated. In vitro derived OC from DOK3 KO and WT bone marrow were analyzed for morphology and function. Intracellular signaling in response to MCSF and RANKL was investigated.

Results: Our results show that DOK3 KO mice have significantly reduced trabecular bone mass at the tibia and femur whereas cortical bone is unchanged. In vivo, OC numbers were increased between WT and DOK3 KO. In vitro derived OC assays revealed that DOK3 pre-OC had increased proliferation in response to MCSF and were more sensitive to RANKL with increased OC differentiation and resorptive capacity. Mechanistically, DOK3 regulates osteoclastogenesis by inhibiting Syk and Erk activation.

Conclusion: In conclusion, our data supports an important role for DOK3 in the regulation of osteoclastogenesis in vivo and ex vivo.

Funding: This project was supported by Native American Research Centers for Health (NARCH), Veterans Affairs (MBH) and NIH RO1 AR064211 (MBH).



2015

Other
Summer
Students

FOREWORD

Undergraduate and high school students sometimes have individual arrangements with OUHSC laboratories to conduct mentored biomedical research projects outside of the purview of SURP. Space permitting, we welcome these students to come to the Poster Day Celebration at the end of the summer to present their work and learn about the projects from other laboratories. Abstracts from their projects are included within this section.

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Other Summer Students ABSTRACTS

Abstract #43

GROWING LARGER CRYSTALS BY OPTIMIZING THE PHYSICAL PARAMETERS OF HANGING DROP EXPERIMENTS

Akila Venkataramany¹, *B. Venkataramany*¹, *C. Kanyumbu*¹, *S. Gulati*¹, *A. Shakir*¹, *K. Rice*¹, *T. McKay*¹, *V. Mooers*¹, *J. Sherpa*¹, *A. Singh*¹, and *B. Mooers*^{1,2}

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Introduction: Large crystals (> 200 microns long) of biological macromolecules give stronger diffraction of X-rays and thus better data leading to high quality structural models suitable for structure-based drug design. Large crystals are traditionally found after a series of full factorial experiments varying the crystallization solution's chemical composition and maybe temperature. We hypothesize that optimizing only the physical parameters of the crystallization experiment will also give larger crystals.

Methods: Using RNA in hanging drop experiments, we considered reservoir volume (5 levels), RNA concentration (3 levels), drop volume (9 levels), and the reservoir solution volume:RNA solution volume in the crystallization drop (6 levels). Instead of a full factorial experiment with 810 drops, we used a response surface method: the Hardin-Sloane J-optimal spherical experimental design with 20 drops. Our response variable was the length of the drop's longest crystal.

Results: We used two-dimensional contour plots of the four-dimensional response surface to evaluate pairs of physical factors. The reservoir solution volume:RNA solution volume in the drop gave the largest response. No optimal ratio was found because gravity limited the drop volume to about 14-15 μL , so further optimization of crystal length would require the use of sitting drops. Nonetheless, the longest crystal was 740 μm long in comparison to about 175 μm , the mean length of the longest crystals in 73 conventional hanging drops.

Conclusions: The optimization of the crystallization drop's physical parameters lead to longer crystals. In addition, the response surface methods experimental design was an efficient way of improving crystal size.

Funding: This work was supported in part by the National Institutes of Health grants R01 AI088011 (PI: Mooers) and P20 GM103504 (PI: Ann West).

Abstract #44

PRACTICAL DESIGN AND ANALYSIS OF CRYSTAL OPTIMIZATION EXPERIMENTS USING RESPONSE SURFACE METHODS

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Introduction: After finding a crystallization lead in high throughput screens, biological crystallographers do a series of inefficient and expensive, “vary one factor at a time” experiments to improve the quality and size of the crystals for molecular structure determination. In contrast, response surface method experiments (RSMs) find the optimal crystal size by varying several factors together and thereby consume less valuable sample (Carter 1997; Carter and Yin, 1994; Carter & Ries-Kautt, 2007). The lack of free software for quickly designing RSM crystallization experiments has prevented the wide adoption of RSM by crystallographers. We hypothesize that simple spreadsheet templates will ease the adoption of RSMs by crystallographers.

Methods: We made spreadsheets for the popular central composite designs and for the efficient J-optimal designs for all available crystallization tray sizes. The user selects the worksheet with the desired number of factors and number of crystallization tray size and then enters the name and low and high values of each factor. The worksheet computes the recipe for each crystallization drop and thereby saves hours of tedious calculations. We also provide R scripts to fit the RSM polynomial model to the data, to evaluate that fit, and to display the response surface as a contour plot and as a 3-D response surface.

Results: The completion of the worksheet takes less than 5 minutes. This convenience is critical for the use of RSMs in daily work.

Conclusions: Our result suggests that biological crystallographers will find that our worksheets and scripts ease the adoption of RSM.

Funding: This work was supported in part by the National Institutes of Health grants R01 AI088011 (PI: Mooers) and P20 GM103504 (PI: Ann West).



2015

STEPHENSON

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UNDERGRADUATE

PROGRAM

FOREWORD

The Stephenson Cancer Center Summer Research Scholars Program was developed in 2010. The program enables outstanding undergraduate students to work under the mentorship of senior cancer scientists and encourages their interest in and preparation for careers in cancer research and medicine.

In 2013, the Stephenson program joined the Summer Undergraduate Research Program (SURP), which consists of three other student groups: SURE, INBRE, and NARCH. As part of the SURP program, the Stephenson scholars have the opportunity to network with other areas of biomedical research and research methodology.

During the course of the program, Stephenson students receive intensive hands-on research training in the laboratories of a select group of OUHSC biomedical faculty. At the conclusion of the nine-week program, the scholars participate in a poster presentation to gain experience in organizing, interpreting, and discussing their research results. The following is a collection of abstracts showcasing the research activities of the participants in the 2015 Stephenson Summer Research Scholars Program.

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SCUP ABSTRACTS
SCUP Students' Abstracts

Abstract #45

GENOMIC SEQUENCE VARIATION INDICATE POSSIBLE REGULATORY DIFFERENCES IN MAST CELL PROTEASE EXPRESSION

Garret Boren¹ and **Zhizhuang Joe Zhao**².

¹Stephenson Cancer Center Summer Research Program, ²Department of Pathology, University of Oklahoma Health Sciences Center

Introduction: Mast cells (MC) are implicated as causal factors in allergies, heart disease, and certain cancer types. DBA/2 strain mouse MC overexpresses certain proteases (MCP2 and MCP4) without known cause. We hypothesized that the genomic sequences of MCP2 and MCP4 and the sequence between the two adjacent genes may contribute to protease expression differences in different mouse strains.

Methods: DNA sequence comparison was performed using the BLAST program with data of several mouse strains from the Wellcome Trust Sanger Institute genome database. The regions with insertion, deletion, and significant sequence variation on chromosome 14 in between MCP2 and MCP4 genes were PCR amplified and sequenced to determine if the differences were read errors.

Results: Two deletions, an insertion, and an unmapped region were indicated in the DBA/2 sequence when compared to normal mice (C57BL/6 strain). Our sequencing results from DBA/2 indicated an insertion of 19 base pairs at the first indicated area, a deletion of 1,053bp at the second area, and a deletion of 25bp at the third area when compared to published C57BL/6 data and C57BL/6 sequencing data. Sequencing data in the unmapped fourth area revealed that this sequence is highly homologous to the correspondent region in the C57BL/6 genome.

Conclusion: These results support predictions of insertions and deletions, indicate large genomic differences between the two mouse strains, and identify targets of future study to understand MCP2 and MCP4 overexpression. This increased understanding will assist in developing treatments of heart disease and cancers associated with MC and their proteases.

Funding: This project was funded by the Stephenson Cancer Center, the Broken Arrow Lady Elks Auxiliary, and the Oklahoma Tobacco Settlement Endowment Trust.

Abstract #46

ONCOGENIC LNCRNA UCA1 ROLE IN OVARIAN CANCER: REGULATION OF GLUCOSE TRANSPORTER, GLUT1

Jiali Dong, Rangasudhagar Radhakrishnan, Ji Hee Ha, Muralidharan Jayaraman, and Danny N. Dhanasekaran
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Introduction: Ovarian cancer is a highly aggressive cancer with only 46% 5-yr survival rate. The major reason for poor prognosis is due to lack of early diagnosis and therapy options. In an attempt to aid in early detection, the lab has identified a long non-coding RNA (lncRNA) namely Urothelial Carcinoma One (UCA1), which is aberrantly expressed in ovarian cancer. Since UCA1 regulates many genes, also acts on many signaling pathways, it may serve as a biomarker for early detection of ovarian cancer. The objective of this study is to explore the functionality of UCA1 and its therapeutic potential.

Method: RNA was isolated from OVCAR8, ovarian cancer cells transfected with control siRNA (siControl) and siRNA specific against UCA1 (siUCA1). A RT2 profiler PCR array was used to analyze the expression of an array of signal transduction pathway genes affected by UCA1 knockdown. Quantitative Polymerase Chain Reaction (qPCR) was performed to validate the genes affected by UCA1 knockdown.

Results: Based on the array results, the two highly upregulated proteins were Patched 1 and chemokine ligand 5 while the two most downregulated proteins were Inhibitor of DNA binding 1 and Solute carrier family 2 member 1 (GLUT1). Since GLUT1 is a glucose transporter and a major regulator of glycolysis in cancer cells, it was further studied. Validation of GLUT1 mRNA expression in UCA1 knockdown cells showed 4-fold decrease compared to control cells.

Conclusion: In ovarian cancer cells, UCA1 regulates GLUT1 mRNA expression, further investigations on its protein expression levels and the UCA1 involvement in glucose transport are being pursued.

Funding: We thank the Stephenson Cancer Center and Oklahoma Tobacco Settlement Endowment Trust. We also thank the Broken Arrow Lady Elks Auxiliary.

Abstract #47

SMNDC1 AS A POTENTIAL THERAPEUTIC DRUG TARGET FOR THE TREATMENT OF EPITHELIAL OVARIAN CANCER

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Introduction: According to the American Cancer Society 21,290 women are expected to be diagnosed with epithelial ovarian cancer (EOC) in 2015 with a high rate of mortality. EOC is a particularly difficult cancer to treat because of multiple drug resistance and reoccurrence with the existing treatments. To combat EOC new drug therapies need to be established.

Methods: Unique proteins involved in EOC were identified using a nanoproteomic approach. Proteins on the nanoparticle's surface were analyzed using algorithms to detect protein connectivity, and a protein known as SMNDC1 emerged with a high connectivity. The distinctive protein, SMNDC1, is known to be constituent of the spliceosome and may have anti-apoptotic function. The role of SMNDC1 in cancer is yet unknown, which makes it a target for exploration to determine its role in EOC cell phenotypes and signaling pathways. We used a gene silencing approach with siRNA technology to knockout the SMNDC1 gene in EOC cell line, OV90 and studied various phenotypes employing routing biochemical techniques.

Results: SMNDC1 was found to be strongly expressed in cisplatin resistant patient tissue samples and also the oncomine database suggested higher SMNDC1 expression correlates with poor survivability. We found SMNDC1 silencing abrogated cellular viability, induced S-phase cell cycle arrest, reduced cellular motility and diminished colony-forming abilities of OV90 cells.

Conclusions: In depth study of this protein and its role in EOC could lead to drugs targeting SMNDC1 in order to treat EOC cancer patients more effectively and overcoming drug resistance, reoccurrence, and mortality.

Funding: Thank you to the Broken Arrow Lady Elks Auxiliary, Oklahoma Tobacco Settlement Endowment Trust, and Stephenson Cancer Center for supporting the Summer Research Scholars Program. This work was supported by National Institutes of Health Grant NHLBI CA135011 and CA136494 (to P.M.).

Abstract #48

LONG NONCODING RNAS PLAY A KEY ROLE IN THE REGULATION OF HSA-LET-7A IN OVARIAN CANCER

Cheyenne Hornback, Rangasudhagar Radhakrishnan, Jihee Ha, Muralidharan Jayaraman, and Danny N. Dhanasekaran
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Introduction: Long non-coding RNAs (lncRNAs) are drivers of cancer through regulatory functions on tumor suppressive and oncogenic genes in cancer cells. UCA1 is a lncRNA involved in the progression of bladder, breast, prostate, and colorectal cancers; however, its role in ovarian cancer remains largely unknown. One of several mechanisms by which lncRNAs function is through binding and modulating miRNA bioavailability. The objective of this study is to identify miRNAs regulated by UCA1 in ovarian cancer cells.

Methods: An *in silico* approach revealed several putative hsa-let-7 binding sites in UCA1. To determine the role of UCA1 in regulating hsa-let-7 family, RT-qPCR analysis was conducted in UCA1 silenced OVCAR8 cells.

Results: Results from our study on UCA1 silencing in OVCAR8 cells show an increased expression of about five miRNAs, decreased expression of two miRNAs, and eleven miRNAs with no change, among the 18 let-7 family miRNAs screened. One of the hsa-let-7a family members, hsa-let-7a-5p showed an increased expression of approximately 668%. Validation indicated a consistent upregulation of hsa-let-7a-5p in siUCA1 OVCAR8 cells. Analysis of the targets for hsa-let-7a-5p identified interleukin-6 (IL-6), a cytokine involved in cancer cell proliferation, as a candidate. Since IL-6 plays a critical role in the pathogenesis of ovarian cancers, the role of UCA1 in regulating IL-6 was analyzed. Results indicate that silencing UCA1 abrogates IL-6 expression.

Conclusion: The current study identifies for the first time hsa-let-7a-5p as a node through which UCA1 could regulate IL-6. Further studies are underway to delineate the UCA1 mediated regulation of IL-6 through hsa-let-7a-5p.

Funding: We thank the Stephenson Cancer Center and the Oklahoma Tobacco Settlement Endowment Trust. We also thank the Broken Arrow Lady Elks Auxiliary.

Abstract #49

TGF-BETA, A POTENTIAL CLINICAL TARGET IN UTERINE CARCINOSARCOMA

*Samantha Horton*², *Shailendra Kumar Dhar Dwivedi*^{1,2}, *Resham Bhattacharya*^{1,2}.

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Introduction: Uterine carcinosarcomas (UCS) are aggressive, rare, biphasic (contain both epithelial and mesenchymal components) uterine tumors accounting for 3-4% of gynecologic malignancies while displaying a disproportionately high mortality (16.4%) among all uterine malignancies. Lack of targeted therapy and a report demonstrating amplification of the TGF- β locus at 19q13.1 prompted us to investigate the role of TGF- β signaling in UCS.

Methods: The UCS cell line, CS-99, was cultured in DMEM-1. Cells were treated with or without TGF- β 1, the Smad3 inhibitor (SIS3), a TGF- β -I receptor I kinase inhibitor (LY2157299), and a JNK pathway inhibitor (SP600125). MTT cell viability assay was performed to evaluate the effect of these inhibitors in presence or absence of TGF- β 1. Western blot and scratch migration assay were performed to probe the effect of these inhibitors on TGF- β 1 induced EMT and migration responses in CS-99.

Results: Here we demonstrated that the components of the TGF- β pathway are expressed and functional in UCS. TGF- β -I induced significant Smad2/3 phosphorylation, JNK phosphorylation, migration and EMT responses in the CS-99 cell line which could be attenuated by the TGF- β receptor I (TGF β R-I) inhibitor developed by Eli Lilly. The inhibition of Smad 3 led to a dose-dependent decrease in phosphorylation of the JNK pathway which reveals the nature of the JNK pathway as Smad-dependent. The various EMT markers reveal that the Smad and JNK pathways of TGF- β signaling are responsible for EMT in UCS.

Conclusion: The Smad and JNK pathways were identified as efficacious target for therapeutic treatment of EMT and migration in UCS.

Funding: Work supported by the Stephenson Cancer Center as well as the National Cancer Institute grant RO1 CA157481 awarded to Dr. Resham Bhattacharya. We thank the Oklahoma Tobacco Settlement Endowment Trust along with the Broken Arrow Lady Elks Auxiliary.

Abstract #50

GUANYLATE CYCLASE SIGNALING AND THE DEVELOPMENT OF HIGH-RISK METASTATIC NEUROBLASTOMA

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Introduction: Our recent studies recognized the transcriptional/translational loss of Retinal degeneration Protein 3 (RD3) in high-risk metastatic neuroblastoma (HR-NB) and defined its novel metastasis suppressor function. It has been shown that RD3 competitively binds to guanylate cyclases (GCs) and maintains GC expression, stability, as well as restores GC localization. Accordingly, we investigated the role of GCs, if any, in the evolution of HR-NB.

Methods: Utilizing a vivo mouse model of spontaneous HR-NB coupled with ex vivo approaches using metastatic site derived aggressive cells (MSDACs), we examined the transcriptional (whole genome microarray) and translational (immunoblotting) expression of GCs and validated with high-content confocal immunofluorescence. Further, utilizing a cGMP competitive enzyme immunoassay, we investigated both intracellular and soluble GC activity.

Results: Whole genome microarray analysis revealed a differential activation of GC signaling transcriptional machinery in three different clones of MSDACs (compared to SH-SY5Y cells) ex vivo. Immunoblotting identified a consistent translation of GCs (GC-1 and GC-2) in a manifold of metastatic tumors (compared to non-metastatic primary xenografts) in vivo and in MSDACs. Confocal immunofluorescence analysis validated the near-identical expression of GC1 and GC2 both in SH-SY5Y cells and MSDACs. cGMP immunoassay recognized indistinguishable intracellular and soluble GC activity in clones of differentiated/undifferentiated MSDACs as opposed to the clones of SH-SY5Y.

Conclusion: For the first time, the results of this study define the transcription and translation blueprint of GCs in HR-NB and, further imply that the RD3-loss may dictate GCs-signaling independent alternate molecular flow-through in the evolution of HR-NB.

Funding: Stephenson Cancer Center, NIH COBRE 1P20GM103639-01, Broken Arrow Lady Elks Auxiliary, and Oklahoma Tobacco Settlement Endowment Trust

Abstract #51

EVALUATION OF APELIN AND ITS RECEPTOR APJ IN PROMOTING ANGIOGENIC EFFECTS IN VITRO

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¹Department of Pharmaceutical Sciences, College of Pharmacy, University of Oklahoma Health Sciences Center, ²Stephenson Cancer Center, University of Oklahoma Health Sciences Center

Introduction: Angiogenesis is required for tumor progression and metastasis and a valid target for anticancer therapy. However, the rapid development of resistance to antiangiogenic therapies is a major challenge. Our previous study has evaluated resistance gene signature to anti-VEGF treatment using preclinical ovarian cancer models and identified significant upregulation of microvascular proliferation markers, apelin receptor (Apj) and its ligand (Apln), in sorafenib-resistant tumors compared to sensitive tumors ($P < 0.0005$). Our overall objective is to elucidate the effects of Apelin-APJ pathway on antiangiogenic resistance. In this study, we characterized the angiogenic effects of Apelin-APJ signaling in tumor and endothelial cells using various in vitro systems.

Methods: We transfected apelin-GFP into SKOV-3 human ovarian cancer cells (SKOV-3+APLN) and APJ into human umbilical vein endothelial cells (HUVEC+APJ). Tube formation assay was performed using HUVEC and HUVEC+APJ under normoxic and hypoxic (1% O₂) conditions in the presence of Apelin (100 ng/mL), VEGF (50 ng/mL), and/or sorafenib. Cell growth inhibition was determined on SKOV-3 and SKOV-3+APLN using sorafenib. A transwell co-culture migration assay was performed using combinations of HUVEC/HUVEC+APJ and SKOV-3/SKOV-3+APLN to assess the HUVEC migration.

Results: HUVEC+APJ formed 47 % more tube length compared to untransfected HUVEC. VEGF treatment further increased the tube length by 30 % in HUVEC+APJ, but not in control HUVEC.

Conclusions: Preliminary results indicate that the cells overexpressing Apelin or APJ showed more angiogenic potential compared to their respective controls. Further in vivo studies are needed to fully characterize antiangiogenic resistance effects of apelin-APJ pathway.

Funding: We thank the Stephenson Cancer Center, Broken Arrow Lady Elks Auxiliary, and Oklahoma Tobacco Settlement Endowment Trust.

Abstract #52

THE P66SHC ADAPTOR PROTEIN INHIBITS THE CASPASE CASCADE

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Introduction: Inhibition of apoptotic processes is one of the ways malignant tumors survive and proliferate. p66Shc is a protein known to modulate cell response to stress and external stimuli, though the precise mechanisms are not well defined. Our previous work demonstrated that purified p66Shc binds cytochrome c (cyt c). Thus, we hypothesized that p66Shc serves as a caspase cascade regulator via disruption of apoptosome formation in response to cyt c release from mitochondria.

Methods: WI-38, PCS-201 fibroblasts (20,000 cells), and purified human caspase 3 were separately seeded into a 96-well, white, flat-bottom plate and treated with purified p66Shc, BSA, or control buffer (PBS). The Caspase-Glo 9 Assay and the Caspase-Glo 3/7 Assay (Promega) were used to measure caspase 9 activity and caspase 3/7 activity, respectively. The Caspase-Glo 3/7 Assay (Promega) was used to measure purified caspase 3 activity. Statistical significance was determined using t-test calculations.

Results: The caspase 3/7 and caspase 9 activities of WI-38 and PCS-201 fibroblasts were significantly inhibited by p66Shc. BSA did not affect caspase activity in the cell lines. The addition of p66Shc to purified caspase 3 presented no change in caspase 3 activity relative to control or BSA.

Conclusion: p66Shc significantly inhibits caspase 3/7 and caspase 9 activities using in vitro assays. Co-incubation with purified p66Shc with purified caspase 3 does not result in attenuation of caspase 3 activity. Thus, p66Shc functions as a caspase-independent inhibitor of the caspase cascade.

Funding: This work was supported by the Stephenson Cancer Center, Broken Arrow Lady Elks Auxiliary, the Oklahoma Tobacco Settlement Endowment Trust, and an Institutional Development Award from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103639

Abstract #53

UBAP2 IS A NEW TARGET FOR PANCREATIC CANCER RESEARCH

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Introduction: Pancreatic Ductal Adenocarcinoma (PDAC) is one of the most mortal cancers worldwide. Our group has identified ubiquitin binding associated protein 2 (UBAP2) as a new regulator of pancreatic cancer progression, but the full function of UBAP2 is far from being clear.

Method: Localization of UBAP2 was detected from different subcellular fractions by western blotting and visualized by immunofluorescence. To test the critical role of Ubiquitin-binding Associated (UBA) domain, we overexpressed full length or UBA-truncated UBAP2 in BxPC3 cells respectively, and detected the KRAS activation via pulldown based assay. Furthermore, the function of UBAP2 in vivo was also evaluated by immunohistochemistry staining for α -smooth muscle actin (α -SMA) and PCNA in mice xenograft tumors.

Results: UBAP2 widely expressed in the nucleus, cytosol, low density microsomal membrane, and plasma membrane. In AsPC1 cells, UBAP2 targets to WAVE2-containing cell membrane ruffles. Most interestingly, UBAP2 co-localized with USP10, a ubiquitin specific peptidase, at the cell leading edge. We also found that full length UBAP2 but not UBA domain truncation upregulated activated KRAS. Moreover, UBAP2-depletion significantly decreased both α -SMA and PCNA positive cell populations in mice xenograft tumors

Summary: UBAP2 is a new regulator of pancreatic cancer growth under multi-mechanisms via promoting micropinocytosis, cell proliferation, fibroblast activation as well as KRAS stabilization. Additionally, UBAP2 might deubiquitinate KRAS by functional associated with USP10 and UBA domain is critical for its ubiquitin-dependent function. Further exploitation of the molecular function of UBAP2 will open a new insight into PDAC therapies and even beyond.

Funding: This work was supported by Stephenson Cancer Center, National Institutes of Health Grant CA135011 and CA136494, Broken Arrow Lady Elks Auxiliary, and Oklahoma Tobacco Settlement Endowment Trust.

Abstract #54

ROLE OF HDACS IN THE EVOLUTION OF HIGH-RISK NEUROBLASTOMA

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Introduction: Determining the ongoing acquisition of genetic/epigenetic alterations and consequent functional molecular signal transduction that drives favorable neuroblastoma to high-risk metastatic disease (HR-NB) has momentous importance in neuroblastoma cure. Herein we investigated the role of histone deacetylases (HDACs) in the evolution of HR-NB.

Methods: Utilizing *in vivo* mouse model of spontaneous HR-NB coupled with *ex vivo* approaches using metastatic site derived aggressive cells (MSDACs), we examined the transcriptional (QPCR) and translational (immunoblotting) expression of HDACs/SIRT. Exploiting a novel second-generation HDAC inhibitor with concentration dependent HDACs inhibitory potency, we investigated the role of HDACs in tumorsphere formation capacity. Individual gene expression systems for HDACs 1 through 8 were utilized to define the role of select HDAC or HDACs in tumor progression.

Results: QPCR analysis revealed a significant activation of HDAC (HDACs 1 through 11 and SIRTs 1 through 3) transcriptional machinery in a manifold of metastatic tumors (compared to non-metastatic primary xenografts) *in vivo* and in MSDACs (compared to SH-SY5Y cells) *ex vivo*. Immunoblotting identified functional translation of HDACs 5, 7 and 11 in metastatic tumors and in MSDACs. Complete inhibition of tumorsphere formation above 1 μ M concentration of Quisinostat selectively defined the role of HDAC7 in stemness maintenance and tumorsphere formation.

Conclusion: For the first time, the results of this study define the transcription and translation blueprint of HDACs in the evolution of neuroblastoma. Further these results imply that HDAC7 may serve as the key player in the stemness maintenance and could dictate the switch from favorable disease to HR-NB.

Funding: Stephenson Cancer Center, NIH COBRE 1P20GM103639-01, Broken Arrow Lady Elks Auxiliary, and Oklahoma Tobacco Settlement Endowment Trust



2015

SUMMER
UNDERGRADUATE
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FOREWORD

The Summer Undergraduate Research Experience (SURE) Program was developed in 1989 with the goal of introducing outstanding undergraduate students to the rigors of academic preparation required for successful biomedical research careers. The program also encourages students to select the University of Oklahoma Health Sciences Center for their graduate education.

During the course of the program, SURE participants receive intensive hands-on research training in the laboratories of a select group of OUHSC biomedical faculty members and mentors. We hope that they will keep contact with their host laboratories for continuing guidance, and to keep track of the science that will emerge from their summer activities. The following is a collection of abstracts showcasing the research activities of the participants in the 2015 SURE Program.

We are grateful to the Provost and the Graduate School of the University of Oklahoma Health Sciences Center for their generous support.

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A NOVEL THERAPEUTIC STRATEGY IN FRIEDREICH ATAXIA*

Mentor: Dr. Sanjay Bidichandani

Institution: The University of Oklahoma Health Sciences Center

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SURE ABSTRACTS
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Abstract #55

ISOLATION AND SUCCESSFUL UPTAKE OF VERY LONG-CHAIN SATURATED FATTY ACIDS FROM WAX ESTERS IN BEESWAX

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Introduction: Very Long-Chain Fatty Acids (VLC-FAs, $\geq C28$) may play unique roles in membrane structure and contribute to overall human health. The elongase ELOVL4 is necessary for the synthesis of VLC-FAs. Saturated VLC-FAs (VLC-SFAs) are found in the skin and brain. Mutations in ELOVL4 lead to compromised skin permeability barrier function, retinal degenerations, and improper brain development and function. We propose that a lack of VLC-FAs causes these detrimental phenotypes. Our goal was to isolate VLC-SFAs from beeswax and incorporate them into mouse feed to determine if they can be absorbed.

Methods: Beeswax lipids were saponified and VLC-SFAs were enriched. The VLC-SFA's composition was verified by Gas Chromatography-Mass Spectrometry (GC-MS). The VLC-SFAs were mixed with safflower oil and incorporated into mouse feed. The mice were euthanized after feeding for 4 days and plasma was collected. Upon lipid extraction of the plasma and methyl ester derivatization, fatty acid methyl esters were analyzed by GC-MS to determine the presence and amounts of VLC-SFAs. Results were compared to a control group given chow with safflower oil only.

Results: GC-MS analysis showed VLC-SFAs were isolated from beeswax with an average 13.6% yield. GC-MS revealed a significant increase of VLC-SFAs in the plasma of mice given VLC-SFA feed (5.20 pmoles/100 μ L) compared to controls (1.33 pmoles/100 μ L).

Conclusion: VLC-SFAs can be absorbed by the intestine and transported by the blood. If these fatty acids are incorporated into target tissues (brain, skin, retina, and testes), it may be possible to treat diseases caused by ELOVL4 mutations by oral supplementation.

Funding: This project was supported by the National Institutes of Health through Grants Number 1R21NS090117-01/02 and R01 EY04149-33/36.

Abstract #56

ELUCIDATING MECHANISMS UNDERLYING NON-AUDIOVISUAL SYMPTOMS OF USHER SYNDROME USING AN USH2A MODEL

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Introduction: Usher syndrome (USH) is the leading cause of blindness/deafness. In addition, schizophrenic-like symptoms and painful ovulation in USH females have been reported; however it is unclear what other systems are affected by USH. We generated USH2A knockin (KI) mice with a common human mutation, c.2299delG, and inserted in the same locus GFP to assess usherin expression in other tissues to elucidate potential pathophysiological abnormalities outside the eye/cochlea to further our understanding of non-canonical USH phenotypes.

Methods: Tissues were collected, embedded, sectioned from wild-type (WT) and KI mice at postnatal day 30 (P30) and P360 and assessed for any potential morphological changes. Immunofluorescence using GFP antibody was used to determine usherin expression.

Results: We observed GFP expression in the reproductive and respiratory systems of P30 KI mice. Interestingly, most positively identified tissues lost GFP expression by P360. Morphological changes were identified in the ovary. This abnormality is consistent with symptoms in female USH patients and with our observation that litter size is significantly smaller in KI vs. WT. GFP was also identified in a small subset of cells in the P30 brain, a tissue previously reported to not express usherin.

Conclusions: This work confirms that USH2A is found outside the audiovisual systems and identifies novel tissues not previously known to express usherin. Our data will help explain mechanisms underlying the complex USH patient phenotypes and facilitate a better understanding of the role of usherin protein.

Funding: This project was supported by Foundation Fighting blindness, and NEI (EY10609, EY18656, EY022778).

Abstract #57

INNATE IMMUNITY DYSREGULATION IN HIGH FAT DIET CONTRIBUTING TO OSTEOARTHRITIS

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Introduction: Obesity increases osteoarthritis (OA) in weight-bearing and non-weight-bearing joints due to inflammation. Saturated fatty acids in high fat (HF) diets leads to activation of Toll-Like Receptor 4 (TLR4), promoting a pro-inflammatory state. TREM2 receptor, associated with DAP12, negatively regulates TLR4 driven inflammatory responses. We hypothesize that HF diet-induced OA is mediated by abnormal TLR4 driven cytokine production, leading to inflammation, and DAP12 negatively regulates these responses.

Methods: To test our hypothesis, three groups of female mice (WT, DAP12 KO and TLR4 KO) were placed on a HF or control diet (Con) for 12 weeks. At 12 weeks, glucose tolerance testing (GTT) was performed. Gonadal fat tissue was stained with F4/80 antibody for quantification of Crown-Like Structures (CLS) as a measure of inflammation. Osteophytes presence in the knee was quantified by microCT.

Results: Analysis revealed that HF-diet induced significant glucose intolerance in WT and TLR4 KO mice, in contrast to DAP12 KO mice that were minimally affected. HF-diet induced significantly more CLS compared to Con diet in WT and DAP12 KO; whereas no difference was observed in TLR4 KO mice between diets. HF-diet induced significant increases in OA osteophyte grades in DAP12 KO mice compared to the other strains.

Conclusion: Analysis of knee joint histology is underway to evaluate additional features of osteoarthritis. Our data suggests that TLR4 contributes to fat induced inflammation and OA while DAP12 has a protective regulatory role HF diet- induced osteoarthritis in aged mice. Furthermore, a new role of DAP12 in glucose intolerance was discovered.

Funding: Funding Source: Children's Hospital Foundation Fellows Research Award

Abstract #58

EARLY ENDOSOMAL RAB GTPASES IN AUTOPHAGOSOME BIOGENESIS

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Introduction: Autophagy is a basic and important process in all eukaryotic cells in which damaged or unnecessary intracellular components are destroyed and recycled in response to cell stress. Recent work has demonstrated that the small molecular weight GTPase, Rab5, is a necessary contributor of autophagosome biogenesis and function. Within the Rab5 sub-family, Rab17 and Rab22 are closely related to Rab5 in both structure and function, but their contributions to autophagy are largely unknown. Experiments performed in our lab have shown Rab5 and Rab17 as positive regulators of autophagy and Rab22 as a negative regulator of autophagy, though it is unclear what their exact contributions might be. Our hypothesis is that Rab5, Rab17, and Rab22 facilitate fusion of early endosomes to autophagosomes.

Methods: We produced expression vectors containing a known autophagosome marker, LC3B, as well as wild-type and dominant negative mutants of our proteins of interest fused to fluorescent proteins. This dual expression vector allows for visualization of both LC3B and a Rab GTPase simultaneously under autophagic conditions.

Results: Analysis by confocal microscopy of PC12 cells transfected with our novel fusion vectors revealed that Rab5, Rab17, and Rab22 do not co-localize with LC3B during autophagy. Therefore, Rab5, Rab17, and Rab22 do not directly facilitate fusion of early endosomes to autophagosomes during autophagy.

Conclusions: This investigation questions the exact role of early endosomes during autophagic progression. Future experiments must be conducted to further understand the involvement of early endosomal Rab GTPases in the structure and function of autophagosome biogenesis and maturation.

Funding: NIH R01 GM074692

Abstract #59

EFFECTS OF MUNC18-1 HAPLOINSUFFICIENCY ON NEUROTRANSMISSION

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Introduction: Early infantile epileptic encephalopathy with suppression-burst (EIEE), or Ohtahara syndrome, is one of the most severe forms of epilepsy, characterized by the onset of tonic spasms within the first 3 months of life and often leads to psychomotor impairment and death. A mutation in the syntaxin binding protein 1, MUNC18-1, has been linked to the encephalopathy. MUNC18-1 is a brain-specific protein known to have a role in neurotransmitter release and is essential in vesicle priming. The point mutations investigated in this project are assumed to cause the degradation of MUNC18 protein, leading to haploinsufficiency (heterozygous expression). The objective of this project was to explore the effect of a MUNC18-1 mutation on synaptic activity. We hypothesized that the mutation would affect neurotransmission and lead to an increase in synaptic release rates.

Methods: We used FM staining to image a heterozygous neuronal model in order to mimic haploinsufficiency and measure synaptic activity and created LentiViral constructs to mimic specific point mutations.

Results: FM imaging revealed that synaptic release in MUNC18-1 heterozygotes was surprisingly reduced compared to neurons from wild-type littermate controls.

Conclusions: Our results show that reduction of MUNC18-1 leads to reduced vesicle release rates. Mutant MUNC18-1 proteins may have dominant negative or novel functions and epilepsy may result from such effects. Furthermore, it is possible that inhibitory and excitatory synapses react differently to MUNC18-1 mutations. We think that further research is warranted to explore these possibilities and future directions aim to assess the point mutations using the same study design.

Funding: This project was supported by the Summer Undergraduate Research Experience, sponsored by the Graduate Program in Biomedical Sciences at the University of Oklahoma Health Science Center.

Abstract #60

INTERLEUKIN-6 REGULATION OF MURINE MACROPHAGE POLARIZATION

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Introduction: Interleukin-6 is a highly pleiotropic cytokine and its involvement in macrophage polarization may partially explain its multiple activities. Previous studies offer contradictory evidence about the role of IL-6 in macrophage polarization. Macrophages are a heterogeneous population of cells that can be divided into three phenotypes: M ϕ (non-activated monocytes), M1 (pro-inflammatory), and M2 (anti-inflammatory). Therefore, the goal of this project was to study the function of this cytokine in the differentiation of macrophage-like cells.

Methods: RAW 264.74 mouse monocyte cell cultures were pre-treated with varying concentrations of IL-4, IL-6 or IFN- γ for 30 minutes then with LPS for 2 hours (RNA) or overnight (flow cytometry). Total RNA was isolated and mRNA expression of IL-4, IL-6, CD86 (all macrophage), CD206 (M2 macrophage), and TGF- β was assessed using qPCR. For flow cytometric analysis, treated cells were also stained with anti-F4/80 (macrophage), and CD206 (M2).

Results: Flow cytometric analysis showed that IL-6 and IL-4 increased the expression CD206 while IFN- γ tended to decrease it. Interestingly, three populations were observable in the forward scatter vs. side scatter plot of IFN- γ treated samples, suggesting physical differences among M1, M2, and M ϕ macrophages. qPCR revealed that treatment with IFN- γ upregulated CD86 and CD206 but downregulated IL-4, IL-6, and TGF- β mRNA expression. Treatment with IL-4 and IL-6 downregulated mRNA expression of each gene analyzed.

Conclusions: The data presented suggest that IL-6 favors polarization toward the M2 macrophage phenotype and suggest physical differences among M1, M2, and M ϕ macrophages.

Funding: This research was funded by CDC/NIOSH grant R01-OH0 010241.

Abstract #61

NEURONAL EXPRESSION OF N/OFQ IN A RAT MODEL OF PTSD

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Introduction: Post-Traumatic Stress Disorder (PTSD) produces anxiety, hyperarousal and disruption of learning, memory and recall; all involve hippocampal neurons and circuitry. Rat models of PTSD mimic many symptoms and pathology noted in humans. Our lab utilizes the Single-Prolonged Stress (SPS) model of PTSD. SPS alters expression of Nociceptin/orphanin FQ (N/OFQ), an endogenous 17-amino acid opioid neuropeptide that modulates anxiety, stress, cytokine expression, learning and memory. N/OFQ can be synthesized in neurons or glia, but little N/OFQ was found in glial cells in SPS. We hypothesized that increased N/OFQ is primarily localized to neurons. Our goals were to optimize N/OFQ immunolabeling conditions and identify specific N/OFQ-expressing neurons in the hippocampus that might be affected by PTSD to aid development of targeted therapies.

Methods: Male Sprague-Dawley rats were subjected to sham or SPS procedure (sequential exposure to restraint, forced swim, anesthesia and isolation) in the presence or absence of the N/OFQ peptide receptor antagonist, JTC-801 (6 mg/kg/day). Rats were euthanized after 21-28 days; and hippocampal sections were fluorescently immunolabeled for N/OFQ and synaptic markers.

Results: N/OFQ was expressed in neurons of CA1 through CA3 (with CA2 showing the highest levels), and in the Dentate Gyrus, particularly in interneurons. N/OFQ labeling in CA1-CA3 was similar to controls after SPS, but labeling in Dentate Gyrus was substantially reduced. JTC-801 did not affect N/OFQ labeling patterns in any group.

Conclusions: N/OFQ is expressed primarily by neurons in both SPS and control animals. However, N/OFQ levels in the Dentate Gyrus were reduced following SPS.

Funding: This study was supported by the Department of the Army DMRDP W81XWH-11-2-0077 and the Presbyterian Health Foundation; animal protocols were approved by the University of Oklahoma Health Sciences Center IACUC committee and U.S. Army Research and Material Command ACURO office.

Abstract #62

SYNCAM3 IS POSITIONED TO MODULATE CONE PHOTORECEPTOR SYNAPSES.

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Introduction: Failure of grafted stem cells or photoreceptors to regenerate synapses is a major difficulty in cell-based therapies to restore sight in degenerative retinal disease. However, the mechanisms that control photoreceptor synapse formation are poorly understood. The Synaptic Cell Adhesion Molecule (SynCAM) family of synaptic adhesion factors comprises four members that mediate trans-synaptic adhesion between pre- and post-synaptic partners in the brain via hetero- and homotypic transynaptic binding, and are excellent candidates to mediate synaptic adhesion in the retina as well. We tested the hypothesis that SynCAMs might be positioned to mediate cell-specific adhesion between photoreceptors and their proper synaptic partners.

Methods: The cell-specific expression of the SynCAMs in the retina was determined by immunofluorescent labeling of frozen sections of mouse retina and fluorescence and confocal microscopy.

Results: Each SynCAM showed a unique expression pattern in the retina. SynCAM3 in particular appeared to be positioned to mediate adhesion at cone photoreceptor synapses. Cones and a subset of cells with the characteristics of cone bipolar cells in the inner retina showed high levels of SynCAM3 labeling. Double and triple immunolabeling identified Type 3b and Type4 OFF-cone bipolar cells as SynCAM3-expressing cells. In contrast, rods and rod bipolar cells did not show SynCAM3 labeling.

Conclusions: These results indicate that SynCAM3 is positioned specifically to mediate synaptic adhesion in cone photoreceptor circuits and may be particularly important for cell-cell recognition and formation of synapses between cones and Type3b and Type 4 OFF cone bipolar cells.

Funding: This study was supported by the OUHSC Summer Undergraduate Research Experience Program and the Presbyterian Health Foundation.

Abstract #63

MALARIA ASSOCIATED REGULATORY B CELLS SUPPRESS T CELL PROLIFERATION VIA PD-1 INDEPENDENT PATHWAYS

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Introduction: The adaptive immune system is critical for controlling malaria, but long-lasting, sterilizing immunity against Plasmodium parasites does not occur naturally. Our overarching hypothesis is that Plasmodium infection triggers the expansion of “regulatory/suppressive” immune cells that limit T cell responses and parasite clearance. In support of this, our laboratory has identified that Plasmodium infection expands a unique population of regulatory B cells (Bregs) expressing the adhesion molecule CD138. Moreover, Bregs can suppress effector immune responses both in vitro and in vivo. However, mechanisms by which Bregs down-regulate immunity are unknown. We hypothesized that two additional Breg cell surface molecules, FasL and PDL1, are essential for their suppressive function.

Methods: Flow cytometry was used to evaluate surface expression of FasL and PDL1 on Bregs. Cell sorting was used to purify Bregs for in vitro suppression assays. To test the biological relevance of PDL1, a suppression assay was performed with an antibody that targets and blocks the PDL1 receptor on T cells.

Results: Compared to other B cells, expression of PDL1 and FasL was 2-3-fold higher on Bregs. Addition of the α -PD-1 antibody to the suppression assay resulted in no significant differences in T cell proliferation, compared to cultures receiving an isotype control antibody.

Conclusion: Our data suggest that Bregs utilize PD1-independent pathways to suppress effector T cell activity. Studies examining the role of FasL are ongoing. Defining the mechanisms by which Bregs limit anti-malarial immunity will identify novel immune-based interventions to improve immunity against malaria, ultimately aiding in malaria control.

Funding: This work was supported by grants from the NIH (1K22AI099070 to N.S.B.), the American Heart Association (13BGIA17140002 to N.S.B.), and the Presbyterian Health Foundation (PHF Seed Grant to N.S.B.). N.S.B. is also an OK-INBRE scholar supported by a grant from the NIH/NIGMS (8P20GM103447).

Abstract #64

STRUCTURAL STUDIES OF TRYPANOSOMAL RNA EDITING FACTOR H2F2

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Introduction: African trypanosomiasis (i.e., African sleeping sickness) threatens the health of hundreds of million people in sub-Saharan Africa. This fatal disease is caused by infection with *Trypanosoma brucei*, a parasitic protozoan. One candidate drug target is the unique RNA editing system in the trypanosome mitochondrion. The protein H2F2 is part of a complex associated with RNA editing. Our sequence analysis and homology modeling suggests that H2F2 has four zinc fingers. We hypothesize that the zinc fingers bind double-stranded RNA.

Methods: We are using recombinant protein and X-ray crystallography to determine the structure of the protein. We made a homology model to plan our protein expression and purification experiments and to think about the protein's biological function. We have subcloned the H2F2 gene into a pET15b plasmid and are doing protein overexpression trials. The purified protein will be used in crystallization trials without and with dsRNA.

Results: Our bioinformatics analysis indicates 99.5 % sequence identity between the amino acid sequence of our gene of interest in *T.b. brucei* and the same protein in the pathogenic *T.b. gambiense*. In addition, there was a 72% sequence identity with H2F2 from four strains of *T. cruzi*, the causative agent of Chagas disease in the Americas.

Conclusion: Knowledge of the structure and function of this new protein that is used to design drugs to inhibit the function of H2F2 should be transferable to other diseases caused by trypanosomes because of the high sequence similarities between H2F2 from different species of trypanosomes.

Funding: This work was supported in part by the National Institutes of Health grants R01 AI088011 (PI: Mooers) and P20 GM103504 (PI: Ann West).

Abstract #65

IMPROVING MRNA STABILITY & TRANSLATION EFFICIENCY: A NOVEL THERAPEUTIC STRATEGY IN FRIEDREICH ATAXIA

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Introduction: Friedreich Ataxia (FRDA) is an autosomal recessive inherited disorder caused by a GAA triplet-repeat expansion in intron 1 of the FXN gene. FRDA patients have abnormally low levels of frataxin protein secondary to deficient FXN transcription, which results in degeneration of the nervous system mainly affecting the spinal cord and heart. Since the genetic mutation is in a noncoding region of the FXN gene, the protein coding sequence is intact. Therefore increasing the transcriptional output, improving transcript stability and / or translational efficiency are attractive therapeutic strategies for FRDA. Hypothesis: The 3' untranslated region (UTR) of a transcript often plays an important role in mediating mRNA stability through cis- and trans-acting regulatory elements. We hypothesized that overexpression of the 3'UTR of the FXN transcript could potentially act as a sponge and deplete negative FXN regulatory elements and thereby improve FXN mRNA stability and / or translational efficiency.

Methods: Both major 3'UTRs of the FXN gene were cloned downstream of the tdTomato fluorescent marker and over-expressed in HEK cells. 3'RACE was performed to identify the 3'end of the tdTomato-linked 3'UTRs. Endogenous FXN mRNA levels were measured by qRT-PCR on RNA extracted from transiently transfected HEK cells.

Results: By sequencing we showed that FXN 3'UTRs are expressed downstream of tdTomato mRNA. qRT-PCR did not show any change in endogenous steady-state FXN mRNA levels. We are currently investigating changes in FXN mRNA half-life by metabolic labeling of nascent transcripts, and measuring frataxin protein levels by ELISA in FACS sorted HEK cells.

Funding: This research was supported by a grant from the National Institutes of Health (NINDS) to SIB