34TH ANNUAL UBRP CONFERENCE ABSTRACTS & PRSENTERS

(in alphabetical order by last name)

NUMERICAL INVESTIGATION OF THE EFFECTS OF WING BRISTLES AND WING FLEXIBILITY ON THE FORWARD FLAPPING FLIGHT AERODYNAMICS OF THE SMALLEST FLYING INSECTS

***HRITHIK AGHAV*, LAURA MILLER**

We used computational fluid dynamics to investigate the effects of bristles and wing flexibility on the forward-flapping flight aerodynamics of the smallest flying insects. The immersed boundary method was used to solve the fully coupled fluid-structure interaction problem of a pair of flexible wings immersed in a three-dimensional viscous fluid. To determine the effects of bristles, three wings were considered that ranged from least to most bristled. The results suggest that at Re relevant to small insect flight, bristled wings generate nearly as much vertical force and thrust as solid wings while providing the benefit of lower wing mass. Five flexible wings with spanwise flexibility were considered to investigate the effects of wing flexibility, and a new parameter called deflection angle was defined to characterize their flexibility. Based on the results, it appears that at Re pertinent to tiny insect flight, adding a high degree of spanwise flexibility to rigid wings deteriorates their average vertical force and thrust to a large extent. For moderately spanwise-flexible wings, the results suggest that they generate nearly as much average vertical force as their rigid counterparts while providing the benefit of increased average thrust.

COMBINATORIAL INHIBITION OF INFLAMMATORY PATHWAY IN UV-STRESSED KERATINOCYTES

***YUCHEN (ELLA) AI*, PRAJAKTA VAISHAMPAYAN, SALLY E. DICKINSON**

Exposure to UV light from the sun is the major causative factor in skin cancer. In addition to causing DNA damage, acute UV exposure causes skin inflammation which can promote early tumor growth. We have recently shown that topical treatment of mouse skin with resatorvid, an inhibitor of the UV-induced inflammatory mediator TLR4 (toll-like receptor 4), blocks UV-induced non-melanoma skin carcinogenesis (NMSC) in mice. We have also shown that a similar innate immune stress sensor, RAGE (receptor for advanced glycation endproducts) is stimulated by UV exposure. In addition, acute UV exposure leads to stimulation of the immune checkpoint protein PD-L1 (Programmed Death Ligand 1) in keratinocytes, which is linked with blocking overstimulation of the adaptive immune response. Each of the above pathways is a potential target for pharmacological inhibition of UV-induced NMSC. In the current study, we used cultured keratinocytes harboring a luciferase reporter gene driven by the AP-1 transcription factor promoter element to examine whether pharmacological inhibitors of RAGE or PD-L1 can block inflammatory stress signaling in keratinocytes and whether combining these inhibitors with resatorvid would have an additive inhibitory effect. We have previously used this model to show that resatorvid significantly blocks UV-induced stress responses in these cultured skin cells. Our current results indicate that exposure to FPS-ZM1 (RAGE inhibitor) or BMS-202 (PD-L1 inhibitor) effectively reduced inflammatory stress response as measured by the luciferase assay. Additionally, treatment with either the PD-L1 or RAGE pathway inhibitors shows more inhibition when combined with resatorvid. Our results indicate that early intervention against PD-L1 or RAGE expression/activity could be a viable target for skin cancer photochemoprevention. Topical application of such small molecule inhibitors may provide novel treatment options for populations at high risk for NMSC. Combining agents known to reduce inflammatory responses to UV stress should also be considered for clinical testing.

MYOCARDIAL TISSUE EDEMA AND FIBROTIC ANALYSIS WITH CARDIAC MAGNETIC RESONANCE UTILITY IN A POST-MYOCARDIAL INFARCTION MODEL OF CHRONIC HEART FAILURE

***AMAL ANILKUMAR*, JACOB REF, ELI LEFKOWITZ, SHERRY DAUGHERTY, JORDAN LANCASTER, JEN KOEVARY, TUSHAR ACHARYA, RYAN AVERY, STEVEN GOLDMAN**

Purpose: Cardiac Magnetic Resonance (CMR) imaging is used to assess chronic inflammatory responses through the presence of edema. Myocardial infarction (MI) creates a pro-inflammatory state via paracrine mechanisms from ischemia-reperfusion injury (IRI). A Yucatan mini swine model has provided a surrogate to better understand the progression of ischemic heart failure (HF) and novel therapeutic candidates in translational science research because porcine coronary vasculature is similar to that of humans.T1 and T2 relaxation times are assessed by CMR T1/T2-Mapping sequences and global T1/T2 relaxation values were acquired to assess total myocardial tissue edema. Myocardial fibrosis presents us with a quantitative analysis of adverse remodeling that develops post-MI in the left ventricle (LV) due to IRI injury. Myocardial edema provides an approximation of the inflammatory response that occurs in the heart due to similar pathologies. The methods and analyses implemented in this study provide a basis to assess inflammation in longitudinal studies that assess cardiomyopathies or develop new treatments for HF.

Methods: Male Yucatan mini swine (N=9) underwent left anterior descending (LAD) coronary artery 90 min balloon occlusion/reperfusion to produce a MI. CMRs were obtained at baseline and 1-month post-MI. Short axis (SAX) MRI images were collected and post-processing analysis software (Circle Cardiovascular Imaging Inc., Calgary, AB Canada) was utilized to assess T1 and T2 relaxation time. Endocardial and epicardial contours were drawn on basal, mid, and apical T1-gated LAX images and T2-gated SAX images. Contours assessed global changes and changes involving the respective segments of the American Heart Association 16-Segment model in each swine.

Results: Average infarct size was 27 ± 4 %, T1 relaxation increased P (P<0.05) from 1078.3 ± 10.9 to 1151.4 ± 25.1 (no standardized hematocrit), T2 relaxation increased (P<0.01) from 55.8 ± 8.5 ms at baseline to 70.1 ± 11.5 ms at 1 month-post MI.

Conclusions: Both T1 and T2 relaxation increased significantly 1-month post-MI. The quantitative presence of fibrosis presents us with the clarification that LV dysfunction has been created due to adverse remodeling of the myocardium. The presence of edema can be attributed to the previously stated IRI, which causes changes in membrane water permeability and occurs due to endothelial cell death and basal membrane disruption within the myocardial wall. One present limitation of this assessment was the lack of a standardized hematocrit, which can be produced in more longitudinal assessments of CHF. Consistent assessment of myocardial fibrosis and edema can be used to assess treatments for early-stage or late-stage disease in other longitudinal studies of HF. Adverse inflammation due to myocardial injury or in response to potential treatment methods can be assessed with this modality of CMR analysis. Further assessment of the immune response in HF may lead to the development of new clinical treatments or protocols.

THE ROLE OF THE 2-AG SYSTEM IN MIGRAINE ALLEVIATION AND THE BLOOD-BRAIN BARRIER

SHREYA BALASUBRAMANIAN

BODY COMPOSITION CORRELATED WITH AGE AT DIAGNOSIS AMONG RENAL CELL CARCINOMA PATIENTS

***MICHAEL BECENTI*, ROB BLEW, JENNIFER BEA, FERRIS SAAD, MALCOLM TURMAN, KEN BATAI**

Clear cell renal cell carcinoma (RCC) is one of the 10 most common forms of cancer.
• RCC accounts for ≥70% of adults diagnosed with kidney cancer.
• RCC shows high diagnosis and poor prognosis among older patients.
High levels of adipose tissue have been shown to heighten the risk of other cancers 600. This risk increases with age. This study examines the relationship between body composition (subcutaneous 400 adipose tissue (SAT), visceral adipose tissue (VAT), and skeletal muscle (muscle tissue)) 300 and age among diagnosed RCC kidney cancer patients to determine if a specific range of 200 adipose/muscle tissue level is associated with early onset RCC or onset at an older age.

SCREENING FOR THERMOTOLERANCE IN WILD TOMATO PLANT ACCESSIONS

***OLIVIA BERTUCA*, KELSEY PRYZE, CEDAR WARMAN, RAVISHANKAR PALANIVELU**

Climate change diminishes crop yield by inhibiting pollen tube germination, transport of sperm to the ovary, and development of seeds and fruits. This then endangers global food security by limiting the supply of foods that are the product of plant reproduction. This project studies this critical process using *Solanum lycopersicum* tomato plants. Some accessions, or varieties, of tomatoes produce similar fruits under extreme heat as those in ideal temperatures. To identify thermotolerant tomato accessions, this project involves screening wild accessions of tomatoes by measuring the fruit weight and seed yield of plants pollinated at 25 degrees Celsius vs. 37 degrees Celsius. To date, 38 accessions have been screened and 5 produced fruits at both conditions. The relative fruit weight and seed yield were calculated for each accession to evaluate control and experimental fruits. Accessions CW0027 and CW0110 had a larger experimental fruit than the control fruit, which points to possible thermotolerance. Future work includes screening the remaining accessions, performing additional screens of high-performing accessions, and developing new assays to examine pollen grain viability at 25 degrees Celsius vs. 37 degrees Celsius. One such assay involves comparing levels of reactive oxygen species (ROS) of pollen grains stained and incubated at the control and experimental temperatures. Excessively high or low ROS levels indicate inviable pollen and can help identify the thermotolerant accessions in addition to the fruiting experiment. Screening for thermotolerant varieties of tomatoes will help combat climate change-induced food insecurity.

EXPLORING HPV L2 CAPSID PROTEIN'S ABILITY TO TETHER TO HOST CHROMATIN

***SHAAN BHULLAR*, ZACHARY WILLIAMSON, SAMUEL CAMPOS**

Oncogenic human papillomaviruses (HPVs) infect and replicate in differentiating epithelium. These viruses are responsible for causing roughly 5% of all cancers worldwide and over 90% of anal and cervical cancers. Once virions enter the basal cell layer, the minor capsid protein L2 spans endosomal membranes, facilitating intracellular transport of viral DNA (vDNA) to the Golgi. Once the host cell enters mitosis, the nuclear envelope and the Golgi body – which contains the L2-vDNA complex – can break into small vesicles. The Golgi-derived, vesicle-bound L2-vDNA complexes localize to the two mitotic chromosomes, ultimately resulting in penetration of L2-vDNA across limiting membranes, recruitment of PML bodies, viral gene expression, and infection of daughter cells in G1. Previous research has shown that there is a central region of the L2 protein that mediates the process of tethering the vDNA to the chromosomes during the prometaphase of mitosis. This central chromatin binding region (CBR) is predicted to fold into a beta-sheet structure. Mechanistically, the delivery onto and the tethering to the host condensed chromosome is not well understood. Additionally, the host cellular proteins involved in this process remain elusive. We are using two different assays to help better understand the biology of this delivery and tethering process as well as the structure of the chromatin binding region CBR of L2 protein. The first assay takes advantage of a BirA mutant enzyme called UltraID as a proximity biotinylation tool. By fusing this enzyme to the C-terminus of the L2 capsid protein CBR, it will permit the covalent

labeling of nearby proteins with biotin molecules, for subsequent isolation and identification by protein mass spectrometry. This can provide insight into potential host protein interaction partners L2 relies on during the delivery and tethering process. Furthermore, we will establish a microscopy-based functional assay for L2 CBR binding using EGFP fusions. Structural modeling has identified conserved hydrophobic residues within the CBR that likely play an essential role in tethering. We can confirm their importance by performing site-directed mutagenesis within this region and measuring the amount of chromosomal localization by quantifying and comparing relative amounts of chromosomal fluorescence between the mutants and the wild-type L2 protein. Ultimately this work will inform us about the structure and function of a piece of capsid machinery, critical to infection by oncogenic papillomaviruses and may provide targets for therapeutic or prophylactic interventions.

DETECTING BINDING BETWEEN CARDIAC MYOSIN SUBFRAGMENT-2 AND MYOSIN-BINDING PROTEIN C VIA TIME-RESOLVED FLUORESCENCE

***ARIANA BISTA*, TOM BUNCH, VICTORIA LEPAK, BRETT COLSON**

Hypertrophic cardiomyopathy (HCM) is a genetic disorder characterized by thickened heart muscle that can lead to arrythmia, heart failure, and sudden cardiac death. The disease has been linked to mutations in the contractile proteins of cardiac muscle. Specifically, mutations have been found in subfragment-2 of myosin (S2)—i.e., in the hinge region connected to the force-producing myosin heads (S1), which has been suggested to interact with the C0-C2 N-terminal region of cardiac myosin-binding protein C (cMyBP-C) in a phosphorylation-dependent manner. However, the mechanism by which C0-C2 modulates the S1-S2 hinge region to affect myosin structure remains unknown. To better understand where and how C0-C2 binds wild-type and mutant S2, we expressed and purified N-terminal human cardiac S2 fragments (i.e., ΔS2, the 126-residue C0-C2 binding region of S2) from bacterial cells and introduced site-specific cysteine mutations to surfaces along the ΔS2 dimer. We then attached a variety of fluorescent probes (i.e., labeling dyes) to the cysteines of ΔS2 and used time-resolved fluorescence (TR-F) to monitor the changes in probe lifetime upon C0-C2 binding. We observed changes in lifetime due to different dyes, different probe sites on ΔS2, and PKA-mediated phosphorylation of C0-C2. Most of the probes showed the largest changes in lifetime when localized near the N-terminus of ΔS2, the more flexible hinge component. Surprisingly, we do not observe large effects on the phosphorylation of C0-C2. Our results suggest that this TR-F assay is useful for detecting S2-MyBP-C interactions in order to better understand the role of these contractile proteins in muscle contraction and HCM disease.

REVISITING A NEURODEVELOPMENTAL DEFECT: FAILURE OF AXON ELONGATION, CELL MIGRATION, OR BOTH?

***ANAYSE BLAKLEY,* LINDA L. RESTIFO**

ISOLATING EXOSOMES FROM HUMAN TEARS

***REMINGTON M. BLISS*, BOHAN XING, BRIAN S. MCKAY**

Purpose: Exosomes are extracellular vesicles (EV) released by cells as either part of their normal physiology or due to pathology. Exosomes carry DNA, RNA, lipids, metabolites, and proteins and may serve as a communication system. Human tear EVs have not been previously studied but may be useful as a noninvasive biopsy for ocular surface diseases. EVs are not made and stored, rather they are created and released as an immediate response to the environment. An increased presence of EVs may indicate that cells are in a pathological state. Analyzing the concentration and size of exosomes may aid in diagnosing and measuring the effectiveness of treatment for various diseases. Here we aim to detect the presence and properties of tear EVs from healthy volunteers, specifically, characterizing the basic parameters of EVs such as size and concentration.
Methods: We collected tear samples from 6 healthy adults (N=6) whose only ocular conditions were either refractive error or post-cataract surgery more than 3 months prior. Both eyes were sampled, and the left eye data were taken for interclass agreement. Tears were collected on Schirmer strips that were immediately submerged into 1mL PBS with 0.1% azide and then promptly frozen at -18℃. EVs in the tears were eluted from the strip overnight at 4℃. Using differential ultracentrifugation (1000xg/4℃/5min, 25000xg/4℃/30min, 115,000xg/4℃/90min) we isolated the EVs. The physical characteristics (diameter and concentration) of the isolated exosomes were analyzed using ZetaView® ParticleMetrix nanoparticle tracking analysis.
Results: The mean EV size was 183.3nm, SEM +/- 6.2nm (range 151.6-211.5nm). The mean concentration was $5.35e^{7}$, SEM +/- $1.16e^{7}$ nanoparticles/mL, with a range of $7.8e^{6}$ – $1.2e^{8}$ nanoparticles/mL. Data from the left eyes were not significantly different from that from the right eyes, obtained independently. There was a strong correlation between mean EV size (ICC=0.87) and EV concentration (ICC=0.77).
Conclusions: Tear samples from healthy volunteers varied between individuals but were in general agreement. EVs derived from left and right eyes were similar, further supporting the effectiveness of our approach. The control data of EVs may offer insight into ocular diseases and may establish avenues for a noninvasive biopsy using tears to screen and evaluate such conditions.
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HOW THE BEETLE GOT ITS LEGS: ENGRAILED OR INVECTED (BUT NOT BOTH!)

***SUMMER BLUNK*, SIERRA O'SULLIVAN, HECTOR GARCIA-VERDUGO, LISA M. NAGY**

Engrailed and invected are paralogous transcription factors that regulate segment formation in Drosophila. In Drosophila, these paralogs are partially redundant: invected knockdowns are viable, while engrailed knockdowns are not, suggesting at least some functional differences between the genes. They are expressed in the posterior of each segment, as measured by an antibody that detects both proteins. They are found as a conserved gene cassette throughout hexapods, their conserved expression throughout arthropods led to an assumption that their function would also be conserved. Surprisingly, the loss of invected in Oncopeltus results in loss of the abdomen; engrailed knockdowns in Tribolium show variable degrees of segmental loss throughout the trunk. It also suggests that while the gene cassette itself is conserved, the functions of engrailed and invected are evolving independently in different lineages. To test these ideas, we created double and single knock-downs of engrailed and invected via embryonic RNAi. We found that the loss of both genes resulted in a range of phenotypes, with predominantly non-viable, asegmental, limbless embryos. The loss of either gene alone yielded significantly less severe phenotypes, all of which had limbs. No morphological abnormalities were found that were unique to either gene and the more severe phenotypes in these double knockdowns compared to the single knockdowns suggest functional redundancies. This work was funded by NSF MCB 1817873 to TAW; NSF MCB 1817173,1817485 and a UAz Faculty Challenge Grant to LMN.

THE IMPACT OF LEIOMODIN (LMOD2) ON ACTIN-MYOSIN INTERACTIONS

RYAN BOWSER

DEVELOPMENT OF ARYL DIAZONIUM ION (ADI) PROBES TO STUDY BIOLOGICAL SYSTEMS

ELIZABETH BROWNE

TERPENES FROM CANNABIS SATIVA MAY SYNERGIZE WITH SYNTHETIC CANNABINOID TO PRODUCE ENHANCED PAIN RELIEF IN CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

***THAI BUI*, ABIGAIL SCHWARZ, JOHN STREICHER**

There is no such thing as a perfect drug, and opioids are no exception. Opioids are the main medication prescribed for pain alleviation, but they are highly addictive and are responsible for most deaths caused by overdose. One proposed alternative to opioids is the administration of terpene compounds found in Cannabis sativa, which we previously showed had efficacy in relieving chronic pain and could synergize with opioids for greater pain relief. Extending from this work, we hypothesized that combining terpenes with a cannabinoid drug could also further enhance analgesic efficacy. We thus tested the ability of terpene compounds, namely betapinene, alpha-humulene, linalool, geraniol, and beta-caryophyllene, to synergize with a cannabinoid, WIN-55212, in a model of chemotherapy-induced peripheral neuropathy. To understand the analgesic effects of the simultaneous use of terpenes and cannabinoids, we induced peripheral neuropathy using paclitaxel in male and female CD-1 mice over seven days (2 mg/kg IP days 1, 3, 5, 7). On the eighth day, the mice were injected with 100 mg/kg of terpene and 1 mg/kg of WIN, IP. The control mice were injected with only 100 mg/kg of terpene, 1 mg/kg WIN, or vehicle control. We then tested mechanical sensitivity using Von Frey filaments. Mice injected with terpene alone showed no significant difference from mice injected with WIN alone. Additionally, mice injected with both WIN and terpene showed no significant increase in pain relief compared to mice injected with only terpene or WIN. All treated mice showed a significant increase in pain relief to the mice injected with the vehicle. The results suggest that the combination of cannabinoid and terpenes doesn't lead to enhanced pain relief, thus the administration of cannabinoid and terpene combined would not be an effective alternative to opioids.
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RETHINKING KINASE FUNCTIONALITY: A NOVEL RHEOSTAT MODEL OF PROTEIN KINASE A

***NICOLE CARMIOL*, MICHAEL PLANK, ANDREW CAPALDI**

The Protein Kinase A (PKA) pathway is highly conserved across eukaryotes, where it acts as a key regulator of cell growth and metabolism. Previous research on S. cerevisiae's PKA pathway generally assumes that the pathway's activation and resulting stimulation of cell growth only occurs in presence of glucose. Here we report that the model is inaccurate. Proliferation assays and phosphoproteomics demonstrate PKA is active in the presence of poor carbon sources (such as glycerol) and is activated to different levels depending on the quantity and timing of glucose stimulus, which directly correlates with proliferation rate. More generally, this data shows that the pathway elicits graded control over its signaling outputs and cell growth across a wide variety of carbon sources and cell growth, instead of acting as an "on/off switch" like some other kinases. Further research is in progress to determine details of PKA's role in different stages of the cell cycle. Analysis pipelines to quantify the data extracted

from DIC microscopy images have been developed (involving neural network processing, custom Python scripts, image analysis software, etc.), which report on budding indices and cell size measurements. Our findings on PKA impact the current view of kinase function, with some exhibiting a fine-tune control mechanism instead of acting as global switches for their signaling outputs.

TO SELF OR NOT TO SELF? INVESTIGATING THE IMPACT OF ROS-MEDIATED POLLEN REJECTION IN PLANTS

***SAMANTHA CASSITY*, NICHOLAS BIELSKI, ISAIAH TOTH, HAILEY BUELL, SANA SHAKOOR, WILLIAM M. PETERSON, LINDSAY KLAESS, MARK A. BEILSTEIN**

The production of viable offspring in plants is vital for global food security because seeds provide most of the calories we consume. Plant reproduction is governed by a highly selective series of reproductive barriers. One such barrier includes the ability to reject incompatible or foreign pollen at the stigma by increasing the production of reactive oxygen species (ROS) via the upregulation of respiratory burst oxidase homologs (RBOHs). The angiosperms *Capsella rubella* and *Capsella grandiflora* are relatively recently diverged sister species that differ in their modes of reproduction. *Capsella rubella* is self-compatible (SC), meaning that a single individual can reproduce with itself, while *Capsella grandiflora* is self-incompatible (SI), meaning it requires another *C. grandiflora* individual to reproduce. These two species provide a unique opportunity to investigate the use of ROS to distinguish between conspecific vs. foreign pollen and between self vs. non-self pollen, thereby providing invaluable insight into the intercellular communication that impacts reproduction. To manipulate ROS levels, CRISPR knockout (KO) and knockdown (KD) constructs were designed to target specific RBOHs in *C. grandiflora* and *C. rubella*. After confirming established mutant lines through genotyping experiments, pollen germination and pollen tube growth assays will be performed to determine the effects of the knockout or knockdown on the ability to recognize self vs. non-self pollen in *C. grandiflora* and conspecific vs. foreign pollen in *C. rubella*. These results will help determine the extent of conservation of specific RBOHs that regulate ROS production and how these RBOHs are involved in both inter- and intra- species barriers during angiosperm reproduction.

SYSTEMATIC IMAGING OF CAENORHABDITIS KILLING ORGANISMS

***LEAH CHANG*, LUIS ESPEJO, JONAH BALSA, ANNE HASKINS, ANGELO ANTENOR, SAM FRIETAS, VANESSA SILBAR, DESTINY DENICOLA, GEORGE SUTPHIN**

Aging is characterized by progressive cellular deterioration, resulting in an accumulation of cellular damage and eventual loss of physiological functions. As medicine and technology rapidly progress, modern society faces the trials of an aging population. Degenerative diseases and immune decline are some of the most prevalent obstacles to health in an aging society, making preventative medicine increasingly pertinent. By limiting the decline that comes with old age, the overall health of society will improve. Because immune decline is such a major facet of aging, by identifying the mechanism through which immunity decreases and developing a means to prevent it, we can vastly improve the overall health and lifespan of society. The roundworm *Caenorhabditis elegans* serves as a useful model organism for aging across many physiological systems, including innate immune function, due to its high reproduction rate and short lifespan. We developed an imagining system, Systematic Imaging of Caenorhabditis Killing Organisms (SICKO), to simultaneously track the lifespan of individual *C. elegans* while quantitatively measuring infection progression of fluorescently labeled bacteria in the *C. elegans* gut. SICKO opens the door for accurate, high-throughput data on the immune function of *C. elegans* over time and a much more detailed characterization of the interaction between individual animals and bacterial pathogens.

EVALUATION OF THE KALLIKREIN-RELATED PEPTIDASE 6 (KLK6) IN COLON CANCER CELL LINES

***JACKIE CHOI*, NATALIA IGNATENKO**

Colon cancer is the third most common cancer diagnosed in the United States. For all colorectal patients, regardless of cancer stage, approximately 64% survive out of a sample size of 100. Given the average rate of fatality, researchers have looked to a specific protein within the KLK gene family as a possible biomarker or therapeutic target: KLK6. The upregulation of KLK6 is observed in tumors of colorectal patients and has been linked to tumor metastasis. Due to this KLK6 overexpression, there is a possibility that KLK6 may be a suitable candidate as a tumor biomarker, a measurable substance whose presence is indicative of disease. This presentation discusses the techniques utilized during lab to study KLK6 in several cancer cell lines, from monitoring cell growth of colon cell lines with the different status of the KLK6 gene to running a western blot as a means of detecting KLK6.

BIOENGINEERED PROSTATE-ON-CHIP STUDY OF STROMAL DYSREGULATION MECHANISM IN PROSTATE CANCER

***TANJIA CLARKSON*, KAILIE SZEWCZYK, YITSHAK ZOHAR, CINDY MIRANTI, LINAN JIANG**

In 2022, prostate cancer (PCa) remains the 1st in new cases and the 2nd in death among men in the United States of America. The methods by which PCa metastases occur are poorly understood. Recent studies reveal that stromal cells play crucial roles in prostate tumorigenesis. The interactions between tumors and their underlining stroma, cancer-associated fibroblasts (CAFs), contribute to the development of an anti- or pro-tumorigenic microenvironment. Microfluid-based bioengineered organ-on-chips have emerged as a useful tool for studying cancer biology and related treatments. We have developed the first in vitro microfluidic human Prostate-on-Chip (PoC) model. Co-culture of cancer cells and stromal cells performed helped us indicate the development of CAFs and capture the growth of the cells over time. The PoC can be useful for studying tumor invasion and the process by which the cancerous cells and stromal cells perform simultaneously under given conditions.

MODELING THE FEEDING FLOWS OF *CASSIOPEA* JELLYFISH

***ALYSSA CONNOLLY*, MATEA SANTIAGO, LAURA MILLER**

Current trends in ocean warming have driven marine ecosystems to states of high stress and unprecedented invasions. While many species are suffering from these unnatural conditions, *Cassiopea* jellyfish are thriving amidst the weakened competition. As *Cassiopea* spread across global waters, it is becoming increasingly important to understand how their presence, and subsequent dominance, of a new area may lead to changes in the surrounding waters. *Cassiopea* are predominantly sedentary jellyfish that suction their bells on the ocean floor and use their pulsing bells and oral arms to continually drive water upwards. This process mixes the water column and allows the jellyfish to capture plankton and exchange nutrients. To model the magnitude and directionality of their feeding flows through the fluid-structure interaction of the bell and oral arms motion, the Immersed Boundary Finite Element (IBFE) Method is used. Prior work has neglected the prominent oral arms as a modeling

simplification, using only the contracting bell. This work is distinct in that fully three-dimensional models of the oral arms are included to represent a broad range of natural morphology. The resulting simulations track the jellyfish movement and fluid deformation over eight pulses of the jellyfish bell across seven distinct, biologically relevant oral arms models. The preliminary results show that the inclusion of the oral arms significantly impacts the vorticity and vertical velocity of the generated currents, creating a fully scaled model that reflects the feeding flows produced by *Cassiopea* jellyfish.

CYTOTOXICITY OF NANO-ENCAPSULATED PACLITAXEL VS. FREE PACLITAXEL ON PANCREATIC CANCER CELLS

LEYLA CORDOVA, **WENPAN LI, ZHIREN WANG, JIANQIN LU**

Pancreatic cancer is a devastating cancer with a very low survival rate. The pancreas produces essential hormones and enzymes such as insulin which regulates blood sugar levels. When a tumor or cancer is formed in the pancreas it can be within the exocrine or endocrine part of the pancreas. The type of pancreatic cancer that is used in this research is pancreatic ductal adenocarcinoma (KPC-Luc cell line) which affects the exocrine part of the pancreas. There are some treatment options for pancreatic cancer but the survival rate is still low. Chemotherapy and other treatments are not as effective in the remission and stunting of tumor spread. Using nanotechnology along with chemotherapy drugs, more effective tumor targeting can be achieved. To test how the cell viability of the nanoparticle delivery system contrasts with the free drug delivery system on pancreatic cancer, MTT assays were performed. In this experiment, KPC-Luc cells were treated with free paclitaxel (PTX) and a nano-encapsulated paclitaxel formulation (SM-CSS-PTX) in a 96-well plate using 7 different concentrations of each drug. An additional group that contains no drug acts as the control group. After the concentrations were added, the cells were allowed to incubate for 72 hours. Then the MTT solution was added and then analyzed by a microplate reader. It was determined that SM-CSS-PTX had the higher cell viability when applied to KPC-Luc cells compared to the free PTX. In future research efforts, testing the effectiveness of targeting between the two drug delivery systems will occur.

REAL-WORLD GOAL SETTING AND FOLLOW THROUGH IN YOUNG AND OLDER ADULTS

LAUREN CRUZ**, CHRISTOPHER X. GRIFFITH, CAITY CEGAVSKE, HANNAH BURNS, DR. JESSICA R. ANDREWS-HANNA, MATTHEW GRILLI**

Objectives: The ability to generate, plan for, and follow through with goals is essential to everyday functioning. Compared to young adults, cognitively normal older adults have more difficulty with a variety of cognitive functions that contribute to goal setting and follow-through. However, how these age-related cognitive differences impact real-world goal planning and success remains unclear. In the current study, we aimed to better understand the impact of older age on everyday goal planning and success.
Participants and Methods: Cognitively normal young adults (18-35 years, n= 57) and older adults (60-80 years, n= 49) participated in a 10-day 2-session study. In the first session, participants described 4 real-world goals that they hoped to pursue in the next 10 days. These goals were subjectively rated for personal significance, significance to others, and vividness, and goal descriptions were objectively scored for temporal, spatial, and event specificity, among other measures. Ten days later, participants rated the degree to which they planned for and made progress in their real-world goals since session one. Older adults also completed a battery of neuropsychological tests.
Results: Some key results are as follows. Relative to the young adults, cognitively normal older adults described real-world goals which navigated smaller spaces (p=0.01) and that they perceived as more important to other people (p=0.03). Older adults also planned more during the 10-day window (p<0.001). There was not a statistically significant age group difference, however, in real-world goal progress (p=0.65). Nonetheless, among older participants, goal progress was related to higher mental processing speed as shown by the Trail Making Test Part A (r=0.36, p=0.02) and the creation of goals confined to specific

temporal periods (r=0.35, p=0.01). Older participants who scored lower on the Rey Complex Figure Test (RCFT) long delay recall trial reported that their goals were more like ones that they had set in the past (r= -0.34, p=0.02), and higher episodic memory as shown by the RCFT was associated with more spatially specific goals (r=0.32, p=0.02), as well as a greater use of implementation intentions in goal descriptions (r=0.35, p=0.02).
Conclusions: Although older adults tend to show decline in several cognitive domains relevant to goal setting, we found that cognitively normal older adults did not make significantly less progress toward a series of real-world goals over a 10-day window. However, relative to young adults, older adults tended to pursue more socially-oriented goals, as well as goals that involved navigating smaller spaces. Older adults also appear to rely on planning more than young adults to make progress toward their goals. These findings reveal age group differences in the quality of goals and individual differences in goal success among older adults. They are also in line with prior research suggesting that cognitive aging effects may be more subtle in real-world contexts.

HIGH-RESOLUTION STRUCTURE OF THE NUCLEASE DOMAIN OF THE HNHDMV3 MUTANT OF HUMAN PARVOVIRUS B19 MAIN REPLICATION PROTEIN NS1

NICHOLAS CUSICK

LONGITUDINAL CHARACTERIZATION OF THE GUT MICROBIOTA IN THE DIABETIC ZDSD RAT MODELS

ANGELA DING, **SAVANNA WENINGER, FRANK DUCA**

The development of type 2 diabetes (T2D) is complex and hard to mimic in its progression in animal models. Unlike its counterparts, a newly developed rat model for diabetes, the Zucker Diabetic Sprague Dawley (ZDSD) rat, maintains an intact leptin pathway and expresses polygenic obesity that closely parallels the progression of T2D in humans. Here, we examine the progression of T2D and associated changes in the gut microbiota in male ZDSD rats and test whether the model can be used to examine potential therapeutics targeting the gut microbiota. Bodyweight, adiposity, and fed/fasting blood glucose and insulin were recorded over 24 weeks. Glucose and insulin tolerance tests were performed, and feces collected at 8, 16, and 24 weeks for microbiota analysis. 16s rRNA gene sequencing was used to examine the fecal microbiota throughout the progression of diabetes at 8 weeks (healthy), 16 weeks (prediabetic), 24 weeks (diabetic). At the end of 24 weeks, half of the rats were supplemented with 10% oligofructose, tests repeated. Cecal microbiota analysis was performed following 16 weeks of OFS treatment. Throughout the study, we observed a transition from healthy/nondiabetic to prediabetic and overtly diabetic states, via worsened insulin and glucose tolerance and significant increases in fed/ fasted glucose, followed by a significant decrease in circulating insulin. Microbiota analysis demonstrated alterations in the gut microbiota with shifts in alpha and beta diversity as well as alterations in specific bacterial genera throughout the development of diabetes. During the progression of healthy to prediabetic state, there was a significant decrease in *Lactobacillus*, while *Alistripes* and *Ruminococcus* presence increased and maintained elevated in the diabetic state. Reductions in Lachnospiraceae and Roseburia and increases in Blautia occurred only following progression to the diabetic phenotype. Oligofructose treatment improved glucose tolerance and shifted the cecal microbiota of the ZDSD rats during late-stage diabetes. These findings underscore the translational potential of ZDSD rats as a model of T2D and highlight potential gut bacteria that could impact the development of the disease or serve as a biomarker for T2D. Additionally, oligofructose treatment was able to moderately improve glucose homeostasis.

EARLY LIFE STRESS EFFECTS ON BEHAVIOR IN ZEBRA FINCHES

***GORAN DZUDZA*, KATHRYN CHENARD, RENEE DUCKWORTH**

Stressful conditions experienced during early life can have long-lasting effects on behavior. While laboratory studies experimentally inducing a stressful early life environment often show a strong effect on behavior, it is not known whether milder, naturally occurring stressors produce the same effect. In particular, environmental stress due to extreme temperatures and sibling competition is known to affect the body size and general health of avian species, however, the extent to which these potential early life stressors affect behavior later in life remains poorly understood. Here, we investigate this problem in a semi-natural outdoor colony of zebra finches. We determined whether offspring that experienced either extreme weather conditions or intense sibling competition showed differences in behavior in adulthood compared to offspring that did not experience these mild stressors. We used a panel of controlled behavioral trials to rate their aggression and various aspects of fearfulness. We found that prenatal stress, in the form of high ambient temperatures, was positively correlated with fearful behavior in male offspring. The relationship between early life stressors and aggression, however, showed no correlation. These results show that early life stressors, such as high incubation temperatures, can have significant carry-over effects on behaviors in adulthood and this has important implications for understanding behavioral development in a wide variety of species.

DEMOGRAPHIC HISTORY AND NATURAL SELECTION IN WILD MOUSE POPULATIONS

***OLIVIA FERNFLORES,* TRAVIS J. STRUCK, RYAN N. GUTENKUNST**

Much can be learned about a species' recent evolutionary past by fitting models to contemporary patterns of genetic variation. Here we focus on wild mice living in France, Germany, and Iran, because we can interpret findings about selection in these wild populations using information from lab mice. We use the population genetics software dadi to infer the demographic history (the pattern of population size, divergence, and migration over time) for these populations. This work is in preparation for inferring their distributions of mutation fitness effects (which controls the probability that a new mutation has a given effect on fitness).

NEUROIMAGING AND COMPUTATIONAL METHODS INFORMING INDIVIDUALIZED TARGETING OF TRANSCRANIAL DIRECT CURRENT STIMULATION (TDCS) FOR TREATMENT OF PRIMARY PROGRESSIVE APHASIA.

***NOAH FRAZIER*, KATLYN V. NICKELS, ANETA KIELAR**

Aphasia is a neurological disorder that compromises the ability to express and/or understand language. Primary progressive aphasia (PPA) is a neurodegenerative syndrome that can affect language production and comprehension. In PPA symptoms tend to emerge gradually due to atrophy of the brain regions that support language. PPA may seriously limit the independence of those with the disorder and have negative effects on their well-being. While the main treatment for aphasia is currently speech therapy, our study is currently investigating targeted noninvasive neuromodulation using transcranial direct current stimulation (tDCS) as a supplement to the behavioral intervention. In order to develop a stimulation protocol for individuals with PPA, we apply several analytical methods and neuroimaging approaches. To identify targets for the stimulation we acquire structural and functional MRI data from each study participant. Based on the information about lesion location and functional activation on linguistic tasks we determine which areas of the brain to stimulate in each individual. In addition, we implement computational techniques to simulate the current flow in the cortex. The current flow modeling is accomplished using the program SimNIBS (Simulation of Non-invasive Brain Stimulation) to further support determination of stimulation targets.

Together, these methods have a potential to optimize the neuromodulatory effect of tDCS and may improve outcomes for those receiving therapy for aphasia.

REAL-TIME ONLINE ANALYSIS OF SOIL ISOPRENE DIFFUSION: DRIVERS OF BIOTIC CONSUMPTION AND IMPLICATIONS FOR USING BIOGENIC VOLATILE ORGANIC COMPOUNDS AS BIOMARKERS OF SOIL HEALTH

***PARKER GEFFRE*, JORDAN KRECHMER, DAVID H HAGAN, EBEN CROSS, JOSEPH R ROSCIOLI, JULIANA GIL-LOAIZA, JOSEPH PALMO, LINNEA K HONEKER, LAURA K MEREDITH**

Volatile organic compounds (VOCs) are a diverse gaseous subset of the soil metabolome. Microorganisms cycle VOCs underground, where they play a variety of roles as signaling molecules, metabolites, and energy sources. However, soil VOCs remain overlooked - leaving a gap in microorganism nutrient cycling, and as such the potential impact these compounds could have on surrounding soil health. In this poster, we examine the dynamics of isoprene diffusion, a biogenic VOC (BVOC) commonly produced by some plants, and its observed retention in a series of subsurface dosing soil column experiments. By removing and analyzing soil cores at the beginning and end of the dosing period, alongside a novel subsurface continual real-time online measurement setup, we will explore the microbial drivers of isoprene consumption and the fate of its metabolites. By examining biotic consumption of isoprene in a controlled soil system, our work seeks to explore and validate how BVOCs could be used as predictive biomarkers in overall soil health by examining metagenomic, metatranscriptomic, and metabolomic shifts in tandem.

UNRAVELING THE DEVELOPMENTAL EXPRESSION OF TITIN ISOFORMS: NOVEX-3 AND CRONOS

***RAHUL GUPTE,* FELICIA HARVEY, NINA KOYILLA, EMILY CLARK, HECTOR GARCIA-VERDUGO, LISA M. NAGY**

Titin is the largest known protein and serves important functions in providing passive tension, cell signaling and structural integrity in sarcomeres, the smallest contractile units of muscle. Most studies have focused on the full-length isoforms of titin and its role in providing passive tension to muscle. However, there are two smaller low abundance isoforms called: Novex3 and Cronos, that are understudied and currently have no known function. In this study we aimed to establish developmental expression of the full-length, Novex3 and Cronos isoforms in cardiac and a panel of skeletal muscles. During the UBRP program I assembled a mouse muscle library of: left ventricle, to represent the heart; diaphragm, the most active skeletal muscle; extensor digitorum longus, a fast-twitch leg muscle; and soleus, a slow-twitch leg muscle at various stages of mouse development. I subsequently processed these muscles and extracted proteins for electrophoresis gels for large molecular proteins and did a preliminary analysis of the sarcomere proteins titin and nebulin. My initial findings show higher abundance of titin during early developmental stages is all muscles. In the future these samples will be used to do western blot studies and study the expression of Novex3 and Cronos titin. These studies will reveal important mechanisms in developmental regulation of these low abundance isoforms and may lead to important functional discoveries that are important for both understanding and treating (cardio)myopathies.

A STRIPE MYSTERY UNTOLD BY THE EVEN-SKIPPED ENHANCER

***BRIANA GUZMAN*, FELICIA HARVEY, NINA KOYILLA, EMILY CLARK, HECTOR GARCIA-VERDUGO, LISA M. NAGY**

The red-flour beetle *Tribolium castaneum* makes its segments one by one using a molecular oscillator, while *Drosophila melanogaster* uses a cascade of transcription factors to generate segments simultaneously. *even-skipped*, a gene common to both modes of segmentation, is a pair-rule gene in *Drosophila* and a key part of the oscillator in *Tribolium*. *Drosophila eve* has modular stripe and tissue-specific enhancers but whether the oscillations of *Tribolium eve* are regulated by similar enhancers is unknown. Another pair-rule gene, *hairy*, has been found to have stripe enhancers in *Tribolium*, so we hypothesize that *Tribolium eve* will also have stripe enhancers. Using the Motif Cluster Assignment Search Tool (MCAST) to find potential transcription factor binding sites, we identified five putative *Tribolium eve* enhancer regions. These regions were cloned into expression vectors and integrated into *Drosophila* and *Tribolium* via p-element transformation. We used live imaging and Hybridization Chain Reaction (HCR) to compare mCherry driven by the transgenes and endogenous *eve*. We find the 2.0 kb upstream beetle enhancer driven mCherry is co-expressed with endogenous *eve* in stripe 2, anal pad, and a few cells in the late CNS. When the same enhancer is expressed in the fly, we see co-expression in late-appearing, ventrally restricted stripes 2,3, and 4, as well as the anal pad. Putative downstream and intron beetle enhancers expressed in the fly show partial co-expression in late-appearing, ventral stripes 2,3,4,6, anal pad, and the developing heart. We speculate that stripe-specific enhancers are present in the beetle but have dispersed throughout the beetle *eve* locus and are not responding to the same upstream regulators as in *Drosophila*. Funding was provided by NSF IOS 1817485 and a UAz Faculty Challenge Grant.

INVESTIGATING THE ROLE OF STRESS GRANULES IN THE CELL CYCLE AND DNA DAMAGE RESPONSE

***AUNITA HAKIMI*, LUCAS HARRELL, NIKITA FERNANDES, J. ROSS BUCHAN**

Stress granules (SGs) are cytoplasmic assemblies of non-translating messenger ribonucleoprotein complexes (mRNPs) that form in response to numerous stresses. It is unknown what functions SGs play in cells, but recent affinity purification and proximity labeling experiments assessing SG composition suggest that SGs may regulate many processes in cells, including the cell cycle and DNA damage response (DDR). The long-term objective of our research is to reveal whether and how SGs regulate the cell cycle and DDR. Prior lab data using a human cell culture model revealed that a SG-assembly mutant (*G3BP1/2ΔΔ*) displayed abnormal cell cycle phenotypes, with a small percentage of them failing to arrest following an S-phase block. Furthermore, following release from an S-phase block, *G3BP1/2ΔΔ*mutants exhibited more DNA damage, which indicates they may be more prone to DNA damage and/or have defects in DNA repair. In this project, we will comprehensively assess the role of SG assembly on cell cycle regulation and the DDR by utilizing various SG inhibitory drugs, other known SG assembly mutants and SG inducing stimuli, in combination with cell cycle and DNA damage assays. By analyzing the consistency of cell cycle or DDR phenotypes amongst SG inhibited (or induced) cells, we will determine if SG assembly *per se*, or protein/inhibitor specific effects underpin observed cell cycle/DDR phenotypes. Future research will look at whether SG-inhibited cells fail to arrest at other cell cycle checkpoints and whether distinct subsets of cell cycle and DDR proteins accumulate in SGs triggered by various types of stress. Targeted disruption of cell cycle/DDR protein localization in SGs will also be explored. Understanding how SGs regulate the cell cycle and DDR could lead to novel therapeutic approaches for diseases such as ALS and cancer, the pathology of which is linked to aberrant SG formation and/or persistence.

CHARACTERIZING THE MECHANISMS OF RYANODINE RECEPTOR DYSFUNCTION UNDERLYING COCAINE-INDUCED BEHAVIORS

***GRACE HALA'UFIA*, LISA MAJUTA, MAURICIO SERNA, CODY DIEZEL, KARA BARBER, ARTHUR RIEGEL**

Over 100,000 drug overdose deaths have been reported in January 2022 (CDC). Compared to the estimated 95,000 overdose-induced deaths at the start of 2021, the problem of drug abuse remains a growing, nationwide crisis. Both human and preclinical rodent models establish that drugs of abuse, such as cocaine, stimulate neuronal hyper-excitation in the mesolimbic "reward system" consisting of the prefrontal cortex (PFC), nucleus accumbens (NAc), and ventral tegmental area (VTA). Drug-induced neuronal hyper-excitation results in excess intracellular calcium that we hypothesize causes dysfunction of the ryanodine receptor (RyR2) calcium channel. Ryanodine receptors (RyR2) are large tetrameric channels that quickly release calcium from the endoplasmic reticulum (ER) into the cytosol through a mechanism referred to as calcium-induced calcium release (CICR). With calcium bound, RyR2 is phosphorylated by protein kinase A (PKA), causing an adaptor protein FKBP12.6 to dissociate from a channel subunit, triggering a conformational change that allows for a large efflux of calcium into the cytosol. Under conditions of excess calcium, FKBP12.6 dissociation causes RyR2 to inappropriately leak calcium, which is a channelopathy associated with multiple diseases, including heart failure, neurodegeneration, and chronic alcohol-seeking behavior. Given its widespread pathological consequences, we suspect leaky RyR2s to be a key mechanism underlying chronic drug-seeking behaviors. Our primary goal herein is to characterize the presence of RyR2 in mesolimbic brain regions of rats that model both early and late-stage addiction. Previous work in our lab has shown that rats that receive either a natural reward or a drug reward learn to prefer pushing a lever that results in receiving a reward (active) over a lever that results in no reinforcement (inactive), which persists in extinction-like experimental conditions. To accompany this behavioral data, we have found that both drugs of abuse and natural reward upregulate phosphorylated RyR2 expression in the PFC in response to reward-predictive cues. In this present study, we continue studying RyR2 in the context of addiction by characterizing an antibody for RyR2 and exploring the NAc following chronic drug self-administration and drug-seeking (i.e., a recall test). We found the polyclonal Alomone anti-RyR2 antibody to be effective at a 1:100 concentration. Interestingly, we found that rats that have learned to self-administer either drugs of abuse or natural reward express RyR2 in the NAc core (n=6) at similar levels, as verified through a t-test of significance. However, it remains unknown if RyR2 phosphorylation is altered. Taken together, these results further justify the molecular investigation of RyR2 dysfunction in the context of addiction. Future experiments will focus on characterizing RyR2 phosphorylation in the NAc core and expression of FKBP12.6, an allosteric regulator of RyR2, in mesolimbic brain regions.

KINEMATIC COMPARISON OF CLINICAL ASSESSMENTS FOR ANTERIOR CRUCIATE LIGAMENT REHABILITATION

***ALEXIS HENDERSON*, KRISTEN RENNER**

Anterior cruciate ligament (ACL) injuries are well studied in the field of biomechanics and common among high school and college athletes. The single hop (SH), triple hop (TH), and stop jump (SJ) functional tests are often used by researchers and clinicians such as physical therapists to determine if an athlete is ready to return to sport. Typically, SH and TH are reported as distance traveled while SJ 3D mechanics have been widely reported. To date, the relationship between tasks is unexplored and may reveal clinically relevant information. PURPOSE: The purpose of this study was to explore the relationships between joint angles during these tasks with the hypothesis that the middle TH and first landing of the SJ (SJ1) would be similar METHODS: 20 healthy individuals completed 5-7 repetitions of SH and TH for distance and SJ. Ankle, knee, hip, trunk and pelvis sagittal plane angles and knee adduction were calculated for the SH, middle hop of the TH, SJ1 and SJ second landing (SJ2). Dunn-Sidak tests were used to detect differences between tasks (adjusted p-value<0.025). RESULTS: Joint angle means are reported in Table 1 for all tasks. All differences in joint mechanics are reported in Table 2. Dominant (D) and nondominant (ND) legs were determined by the participant kicking a soccer ball. CONCLUSION: Results indicate that the comparison between TH and SJ1 mechanics provides unique information regarding knee, hip, and pelvis mechanics. Thus, clinical populations that use SJ may benefit from analyzing the TH middle hop. There is also evidence that an increase in trunk angle is indicative of a change in direction of momentum within each task.

SULFORAPHANE (SFN) EXHIBITS ANTIVIRAL ACTIVITY AGAINST SARS-COV-2

***SHELBY HERRICK*, WEIFENG LIANG, LINGXIANG ZHU, ZIQI YAN, IVANA KRESO, DONNA ZHANG, YIN CHEN**

The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is still ongoing. Although vaccination rates are high nowadays, antiviral therapy that is readily available and easily administered to patients is still urgently needed to prevent severe disease, hospitalization, and death. So far, Molnupiravir and Paxlovid are the only two oral antiviral drugs authorized by the FDA for the treatment of COVID-19. SFN, a plant-derived chemical, particularly abundant in broccoli, cabbage, kale, and brussels sprouts, has been well-documented for its antioxidant and anti-inflammatory effects. It also acts as a potent activator of NRF2. SFN has been reported to exhibit antiviral activity against SARS-CoV-2 in vitro and in vivo via an NRF2-independent mechanism. However, the underlying mechanism through which SFN inhibits SARS-COV-2 is not clear. In our study seeking to understand the antiviral activities of NRF2 agonists, we found that SFN indeed inhibited SARS-COV-2 in Vero cells and human lung epithelial cells. However, different from the other report, the lack of NRF2 reduced SARS-COV-2 replication and enhanced the antiviral activity of SFN. Furthermore, using a fluorescence resonance energy transfer (FRET)-based assay, we found that SFN significantly inhibited the activity of non-structural protein 5 (NSP5), a main protease of SARS-COV-2. Therefore, the anti-SARS-COV-2 activity of SFN is likely mediated by both NRF2-dependent and independent mechanisms.

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CIARA HIMES

EXPLORING THE ROLE OF PROLACTIN IN ENDOMETRIOSIS-ASSOCIATED PAIN

***VERONICA HODE*, GRACE LEE, FRANK PORRECA, EDITA NAVRATILOVA**

Endometriosis is a gynecological condition characterized by chronic pelvic pain and infertility. Endometriosis affects women in the 25-40 age range and occurs when the tissue lining of the uterus, the endometrium, grows outside the uterus, usually on or around the organs in the pelvis or abdomen. Endometriosis can cause excessively painful and heavy periods, pain during intercourse, and abdominal pain. Though underlying mechanisms of endometriosis have not been elucidated, clinical evidence suggests the involvement of prolactin, a neurohormone associated with female reproductive functions, pregnancy, and lactation. Preclinical studies in mice also showed that increased prolactin levels might promote or trigger pain development in females. Hence, we hypothesize that prolactin may play an important role in endometriosis-associated pain. To understand the linkage of prolactin to endometriosis, we induced an endometriosis model in mice by implanting a donor's uterus fragments into recipient mice. Implanted mice developed increased mechanical sensitivity in the abdominal region, indicative of pain. We used immunohistochemistry to confirm the presence of endometriosis lesions, and ELISA analysis to measure serum prolactin levels. Our results showed that endometriosis lesions in mice had histological characteristics similar to human endometriosis tissue. Levels of serum prolactin in the endometriosis model were found to be higher in comparison to the naive group. Moreover, levels of prolactin within the endometriosis lesions were significantly higher than in the uterus. These results suggest a probable association between prolactin levels and endometriosis-associated pain.

COMPUTATIONAL PREDICTION OF BINDING POCKETS IN PROTEINS

***DAVID JURKOWITZ*, CONNER COPELAND, TRAVIS WHEELER**

Proteins interact with their environments (ligands, other proteins, etc.) at cavities in their targets bind to called binding pockets. In order to design drugs or other small molecules to affect a protein's function, it is necessary to know the location and architecture of its binding pockets. Traditionally, binding pockets have been identified through experimental structure-determining methods such as X-ray crystallography, NMR, or cryo-electron microscopy, of protein-ligand complexes. However, this data is sparse and is expensive and time-consuming to produce. Therefore, computational methods to predict binding pockets without individually studying them are needed. The two ways to accomplish this are by running molecular dynamics simulations on a protein with a known ligand, and by exploring the surface of a known or predicted protein structure and predicting where the pockets may be. The former option is also time-consuming, and the ligand must be known in order to find its binding pocket. The latter option is used by tools such as fpocket, but fpocket will return many unlikely pockets along with the more probable pockets for a given protein. This project's goal is to identify the true binding pockets for all human proteins from the collection produced by fpocket. This improvement is made by incorporating calculations of sequence conservation, since if a position or region of a protein is conserved across homologous sequences, it is likely to be necessary for maintaining the protein's structure or for accomplishing one of its functions. Multiple sequence alignments (MSAs) of homologous protein sequences were created from PANTHER protein family sequences. From the MSAs, relative entropy values were calculated for each amino acid of the human protein. These values served as a proxy for measuring sequence conservation. Potential binding pockets were computed using fpocket, and the relative entropy values were mapped to the amino acids involved in the pockets to arrive at the improved set of high probability binding pockets.

APPLICATIONS OF THE COUPLING ASSAY FOR T-CELL SPECIFICITY

***JAEMAN KIM*, MARK LEE, CALEB KIM, BRAD MOFFETT, MICHAEL S KUHNS**

T-cells help regulate the adaptive immune response through T-cell Receptors (TCR) and their interactions with antigenic peptides presented on major histocompatibility complexes (pMHC). However, pMHC-specific T cells have shown to be difficult to identify. Functional assays such as Limited Dilution Assay (LDA), Intracellular Cytokine Staining (ICCS), and Enzyme-Linked Immunosorbent Spot (ELISPOT) have been used in order to study pMHC-specific T cells, but they have shortcomings that make it difficult to study these cells effectively. The introduction of four-pronged pMHC molecules known as tetramers provided a novel way of enumerating and studying pMHC-specific T cells. By increasing the avidity between TCR and pMHC complexes, tetramers can overcome the off-rate issues of pMHC monomers and can effectively identify antigen-specific TCRs. However, only TCRs that have a high affinity to the tetramer can be recognized, meaning tetramers are unsuccessful when it comes to identifying low-affinity TCR-pMHC interactions. In order to combat this issue, the Kuhns lab developed the Coupling Assay for T-Cell Specificity (CATS), which utilizes cell lines that are modified to express MHC II molecules with tethered peptides that can recognize pMHC-specific T cells. Previous work in the Kuhns Lab has proven the viability of CATS and has demonstrated that the assay can recognize low-affinity TCR-pMHC interactions in polyclonal populations, overcoming the shortcomings of tetramer technology. In order to further improve this technique, Tag-it-Violet stained CD4+ enriched cells from monoclonal OTII mice were adoptively transferred into polyclonal B6 mice via retro-orbital injection. B6 mice were then immunized with an Ovalbumin (OVA) peptide, a cognate peptide to OTII CD4+ T cells, in order to see if adoptively transferred T-cells would respond to the immunization. M12 cells expressing full length I-A^b with tethered peptides were used in order to identify OVA activated T-cells. We concluded that CATS was able to identify T-cells activated by the OVA peptide in the adoptively transferred T-cell populations. We then tested to see if the TCR-pMHC interaction via CATS was able to be fixed by a fixation buffer and tested if

CATS was truly peptide dependent by comparing coupling rates unblocked and blocked MCC peptides (cognate). Results showed that CATS was in fact peptide dependent, as blocking the cognate peptide led to drastically lower coupling rates, and showed that CATS is able to be fixed, eliminating the risk of cells being uncoupled while analyzing data.

DELAYED GRATIFICATION IN PRIMATE ADOLESCENTS

***SUN WOO KIM*, ARCHER BOWMAN, ALEXIS MORRISON, CAMERON BOLLES, KATALIN GOTHARD**

The brains of primates undergo dramatic structural and functional changes during adolescence. One of the changes in the brain is the connectivity between the prefrontal cortex (PFC) and the amygdala. Both areas process reward, but the PFC plays a role in cognitive control and future planning, while the amygdala processes the emotional aspect of reward. Because of changes in connectivity and other environmental factors, reward processing during adolescence is more vulnerable to impairment, which potentially causes depression, anxiety disorders, eating disorders, and self-injurious behavior. Adolescents are more likely to act without thinking about future consequences while post-adolescents are more likely to act with consideration for future consequences. A better understanding of when and how adolescents gain the ability to control their impulsive behavior will inform techniques or treatments that support them through this difficult period and prevent the onset of mental disorders. To investigate reward processing in the adolescent brain, we work with rhesus macaques (*Macaca mulatta*) because the remodeling of their brain during adolescence shares many features with human adolescent development. In this project, we track the behavior of six monkeys (four peri-adolescents, A, B, C, and P; two post-adolescent controls, S and G) during a delayed gratification task. In this task, the monkeys choose between a small immediate reward (one banana pellet) or a larger, delayed reward (three banana pellets). The large reward is delivered at delays that are varied to find an indifference point where the small immediate reward is subjectively equivalent to the larger reward delivered with a particular delay. The indifference point is the delay time when the monkey chooses a small immediate reward exactly half of the time and a large, delayed reward the other half of the time. Monkeys A, C, and P have completed this task. The behavioral choices and the delay times did not have a linear relationship. Rather, the percentage of small immediate reward choices dramatically increased in a short range of delay time, which fitted a sigmoid function (R-squared values: A = 0.9250, C = 0.8720, P = 0.9511). Using the fitted sigmoid functions, we calculated the predicted indifference point (C = 11.3s, P = 7.3s, A = 6.2s). The indifference point of monkey C indicates that if the delay time for a larger reward is longer than 11.3 seconds, the monkey prefers the small immediate reward over the large, delayed reward. This means that with an 11.3 second delay, monkey C values the three delayed pellets and the one immediate pellet equally. When all the monkeys complete this stage of the experiment, we will compare the indifference points of peri-adolescents and adult controls to see if adolescents devalue rewards differently than adults. In addition to behavioral measures, we perform monthly physical exams to collect the weight, height, testicular volume, and sex steroid hormone levels of the peri-adolescent monkeys. We use these measurements to monitor the progression of the monkeys' adolescence. Testosterone levels are strongly correlated with anatomical measurements in some adolescents and uncorrelated in others. In the future, we will correlate changes in the indifference points of each adolescent with their physiological measurements to see how and when adolescent development influences the way their brains assign value to rewards.

DUST INGESTION IN CHILDREN STUDY

***JACQUELINE LARSON*, JENNA HONAN, PALOMA BEAMER**

The Environmental Protection Agency (EPA) funded the Dust Ingestion in Children Study (DIRT) to determine how much dust is incidentally ingested by children between the ages of greater than or equal to 6 months but less than 6 years of age during children's natural play. The University of Arizona partnered with the University of Miami and North Carolina Agricultural and Technical State University to collect data from three cities: Tucson, Arizona; Miami, Florida; and Greensboro, North Carolina. Each city is unique geographically, environmentally, culturally, and demographically, which will provide a range of children's

behaviors for analysis. Participants were recruited by holding a booth at community events and posting flyers in pediatric clinics. Over-the-phone surveys (n=450) were conducted to gather information on the child's general activities, the family's demographics, cleaning practices, housing characteristics, and any behaviors that may contribute to non-dietary dust ingestion. Of the 450 survey participants, 150 families consented to allow our research teams to conduct supplemental in-house visits. These field visits included audio- and visual-recordings of the child's activities in their own home for a duration of approximately four hours. Child participants were asked to wash their hands prior to the collection of an initial hand rinse sample, which was later compared to a final hand rinse sample to measure change in dust loading on the hands during the videotaping timeframe. Dust wipe samples, vacuum dust sock samples, and outdoor soil samples were collected to determine the amount and type of dust that is found in and around the home. Biometric measurements included the child's left- and right-hand measurements, height, and weight. The purpose of this study is to estimate non-dietary ingestion of soil and dust for children. Accurate knowledge of the ingestion rates can provide insight into subsequent exposure to chemicals that may be present in the soils and dust in and around the home and determine if there is a cause for concern for their health.

THE EVALUATION OF THE WATCHPAT^{TM} DEVICE AS A TOOL FOR THE NON-INVASIVE ASSESSMENT OF SYMPATHETIC NERVOUS SYSTEM ACTIVITY IN HUMAN SUBJECTS

***AVA LASATER*, JOSIE L. MAZZONE, E. FIONA BAILEY**

According to the American Heart Association, nearly half of American adults have above-normal blood pressure, and most are unaware of their condition. Hypertension, which is defined as chronically elevated blood pressure (systolic 130-139mmHg; diastolic 80-89mmHg), significantly increases the likelihood of developing cardiovascular disease and experiencing kidney problems, heart failure, and stroke. Blood pressure is regulated by the autonomic nervous system. Specifically, blood pressure is elevated in response to heightened sympathetic nervous system (SNS) activity via constriction of the blood vessels. The current technique for recording this activity is invasive and requires recording nerve activities using a microelectrode. These are difficult measures to obtain. Our research is focused on a non-invasive assessment of sympathetic activity. Our aim is to determine whether fluctuations in pulse pressure detected at the fingertip can serve as reliable estimates of SNS activity and whether pulse amplitude (PATTM) technology could be of use in the non-invasive evaluation of sympathetic nervous system outflow to blood vessels.

EXPLORING HYDROXYPROPYL-BETA-CYCLODEXTRIN MONOMER AND DIMER VERSIONS AND ITS EFFICACY IN IMPROVING ACUTE ISCHEMIC STROKE RECOVERY

ELIZABETH LE

ELECTROPHYSIOLOGIC EVALUATION IN A SWINE MODEL OF PERSISTENT ATRIAL FIBRILLATION

***ELI LEFKOWITZ*, JACOB REF, AMAL ANILKUMAR, SHERRY DAUGHERTY, JEN WATSON KOEVARY, JORDAN J LANCASTER, TALAL MOUKABARY, STEVEN GOLDMAN**

Purpose: Atrial fibrillation (Afib) is the most common cardiac arrhythmia in adults in the United States. The quivering, "irregularly irregular" contractions associated with Afib cause blood stasis within the atria, potentially leading to

thromboembolic events. Additionally, Afib may induce or exacerbate left ventricular (LV) dysfunction and result in heart failure. In order to assess the efficacy of new treatments for Afib, our laboratory developed an animal model of Afib. The induction of Afib into swine models can provide an assessment of the pathogenesis of left atrial and left ventricular dysfunction.
Methods: 12-month-old male Yucatan mini swine (n=5) underwent surgical implantation of a screw-in lead in the right atrial appendage via the left external jugular vein. Regular, rapid atrial overdrive pacing parameters (400-600 bpm, 8V) were set by a programmer (Medtronic 2090). The pacing was continued for approximately 3 months, or until persistent Afib was induced. To verify the cardiac rhythm, ECGs were continuously analyzed using a surgically implanted internal cardiac monitor (Medtronic LINQ). A majority of the rhythms were interpreted to be either sinus rhythm, a paced rhythm (denoting that the pacemaker is on), or Afib.
Results: The study is ongoing, and to date, out of five swine: two established persistent Afib, one spontaneously converted back to sinus rhythm, another died during anesthesia induction, and the last died due to pacemaker infection secondary to pacemaker erosion. This data demonstrates the difficulty of establishing a swine model of persistent Afib.
Conclusions: The use of pre-clinical animal models of Afib is necessary to understand the pathology and to develop new treatments. Our intent is to persist in developing this model to understand the changes in atrial cardiomyocytes after Afib induction and to potentially develop new treatments based on this data.

IDENTIFYING NOVEL REGULATORS OF TORC1 IN SACCHAROMYCES CEREVISIAE

BROOKE LINDEN

A SYNTHETIC GENETIC ARRAY SCREEN FOR INTERACTIONS WITH THE RNA HELICASE DED1 DURING CELL STRESS IN BUDDING YEAST

***HANNAH M LIST*, SARA B CAREY, ASHWIN SIBY, PAOLO GUERRA, TIMOTHY A BOLGER**

In humans, mutations in the DDX3X gene are linked to oncogenesis in medulloblastoma, viral replication, and genetic diseases; thus, understanding the mechanisms of the DDX3X protein product, a DEAD-box RNA helicase, is an essential step in understanding the pathology of related diseases. In the Bolger Lab, we use budding yeast as our model organism to examine the role of the translation initiation factor Ded1, another DEAD-box RNA helicase that is closely related to DDX3, in various stress responses. Here, we conducted a Synthetic Genetic Array to produce haploid double mutants between a ded1 mutant with a deleted C-terminal region (ded1-ΔCT) and the Yeast Knockout Collection, a library of nonessential gene deletions in Saccharomyces cerevisiae. We then performed an SGA analysis of these single and double mutants on media containing rapamycin to induce TOR pathway inhibition stress and identify potential genetic interactions. These interactions were scored for sensitivity or resistance, and select hits were experimentally verified via serial dilution growth assays of isolated single and double mutants in both pro-growth (YPD) and stress (YPD + rapamycin) conditions. Genes showing enhancement or suppression with the query strain were analyzed for GO term enrichment and we found that many of the hits fell into expected categories, such as signal transduction in the enhancers, while others were unexpected and did not have a clear link to Ded1. The results of this SGA help us to better understand the mechanism of Ded1 in the TOR pathway, particularly in response to TOR inhibition, while also continuing to develop a network of DED1 genetic interactions. With more comprehensive research in the future, I will examine several signal transduction hits, particularly phosphatases that negatively regulate the MAPK pathway by using a kanamycin resistance cassette to construct gene deletion mutants of hits of interest and further verify the SGA before exploring the genes' interactions with DED1 to elucidate further their mechanisms in the translational response to cellular stress.

EFFECT OF MATERNAL WESTERN-STYLE DIET DURING PREGNANCY ON ENDOCRINE PANCREAS TISSUES FROM FETAL BABOONS

***PAULINA LUNA-RAMIREZ*, SEAN W. LIMESAND**

Obesity has risen progressively reaching a 42.4% of prevalence, and it is now one of the leading causes of death worldwide. In the United States, the Western-style diet is considered a significant contributor to obesity due to its high-calorie and high-sugar content. Our objective was to measure morphological differences in the endocrine pancreas from fetal baboons whose mothers received a Western-style diet versus a control diet during pregnancy. Four pancreas sections were immunostained with insulin and glucagon antibodies, which were detected with fluorescent secondary antibodies. The pancreas sections were viewed with a fluorescence microscope and digital images (120 fields of view per animal) were captured for morphological analysis. The percentages of insulin and glucagon-positive areas were measured and are presented relative to the total parenchyma (epithelial) area measured by autofluorescence. There were no statistical differences (P > 0.05) between the Western-style diet and control-diet groups for pancreatic insulin (0.170 ± 0.016 and 0.429 ± 0.067, respectively) and glucagon-positive areas (0.072 ± 0.006 and 0.152 ± 0.062, respectively). Although marginal decreases in insulin-positive and glucagon-positive (total endocrine cell) areas were observed in fetuses in the Western-style diet group, our group sizes were too small to detect these differences. In conclusion, a maternal calorically dense diet may reduce pancreas endocrine cells and increase the risk for metabolic diseases in adulthood.

CIRCADIAN ACCLIMATIZATION OF PERFORMANCE, SLEEP, AND 6-SULPHATOXYMELATONIN USING MULTIPLE PHASE-SHIFTING STIMULI

***NATALIE MAK*, SHAWN YOUNGSTEDT, SALMA PATEL**

Misalignment between one's circadian rhythm and their environment may cause negative effects on physical and mental health, mental and physiological performance, and sleep. Evidence suggests exercise and melatonin can be combined with bright light to elicit larger shifts in circadian rhythms, but no study has yet to combine all these stimuli. The aims of the “Jet Lag Study" are to compare the effects of different treatments on circadian adjustment to simulated jet lag in a laboratory. Thirty-six participants will be invited to stay in our laboratory for 6.5-days and will be randomized to one of 3 treatments to determine the effects of light, exercise, and melatonin on shifting one’s circadian rhythm: (1) dim red light and placebo capsules, (2) bright light alone, (3) bright light, exercise, and melatonin. Acclimatization will be defined by shifts in circadian rhythms of aMT6s, psychomotor vigilance, Wingate anaerobic performance, mood, and sleepiness, and fewer impairments in these measures during the shifted schedule compared with baseline. We hypothesize that bright light alone and combined bright light, exercise, and melatonin will elicit greater shifts in circadian rhythms and fewer impairments in sleep, mood, performance, and sleepiness compared with dim red light and placebo capsules. We also hypothesize that combined bright light, exercise, and melatonin will elicit greater shifts and fewer impairments than bright light alone.

THE ROLE OF RSP5 ADAPTORS IN TDP-43 CLEARANCE AND DEGRADATION

***SOPHIA MARCINOWSKI*, LUCAS MARMORALE, J. ROSS BUCHAN**

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder in which patients experience a progressive loss of motor neurons that results in paralysis and respiratory failure. Over 95% of ALS patients have mislocalized cytoplasmic aggregates of a protein called TDP-43. TDP-43 is an RNA-binding protein normally found in the nucleus, but in ALS patients, TDP-43 is found in the cytoplasm where it forms likely harmful aggregates. Our preliminary data indicates that an enzyme called Rsp5 targets it to be brought to an acidic organelle called the lysosome for degradation via the endolysosomal/MVB pathway. Rsp5 requires adaptors to be able to recognize and tag TDP-43, but it is still unclear which ones are responsible for this. In this study, we aim to discover which adaptors play the most important role in targeting TDP-43 to the lysosome and if multiple adaptors could be involved. Using TDP-43 expressing yeast as an ALS model, with different Rsp5 adaptors individually knocked out from the genome, we assessed the impact on TDP-43 degradation. We used western blot tests to deduce how much the TDP-43 protein levels were altered in comparison to normal yeast cells. We found that only the Rim8 adaptor had a statistically significant increase in TDP-43 levels in the cell when deleted. However, when we combined *rim8Δ* with *ear1Δ* or *ssh4Δ* (other Rsp5 adaptors), we saw a synergistic effect. This work reveals the complex interactions between the cell and TDP-43 for it to be targeted and cleared. Understanding this process could identify ways to upregulate clearance of toxic TDP-43 protein, lead to better treatment options for ALS patients and help reduce or regress the symptoms.

RAPID, SENSITIVE DETECTION OF PFOA WITH SMARTPHONE-BASED FLOW RATE ANALYSIS UTILIZING COMPETITIVE MOLECULAR INTERACTIONS DURING CAPILLARY ACTION

***SAMANTHA MATA ROBLES*, LANE BRESHEARS, YISHA TANG, JACOB BAKER, KELLY REYNOLDS, JEONG-YEOL YOON**

Perfluorinated-alkyl substances (PFAS) pose an unmet threat to the public because they are not strictly monitored and regulated. Perfluorinated-carbon alkyl chains (PFOA), a type of PFAS, at 70 fg/µL is the current health and safety recommendation. Current testing methods for PFOA and PFAS chemicals include HPLC-MS/MS and molecularly imprinted polymers, which are expensive, time-consuming, and require training. In this work, PFOA and PFOS detection was performed on a paper microfluidic chip using competitive interactions between PFOA/PFOS, cellulose fibers, and various reagents (L-lysine, casein, and albumin). Such interactions altered the surface tension at the wetting front and, subsequently, the capillary flow rate. A smartphone captured the videos of this capillary action. The samples flowed through the channel in less than 2 min. Albumin worked the best in detecting PFOA, followed by casein. The detection limit was 10 ag/µL in DI water and 1 fg/µL in effluent (processed) wastewater. Specificity to other non-fluorocarbon surfactants was also tested, using anionic sodium dodecyl sulfate (SDS), non-ionic Tween 20, and cationic cetrimonium bromide (CTAB). A combination of the reagents successfully distinguished PFOA from all three surfactants at 100% accuracy. This low-cost, handheld assay can be an accessible alternative for rapid in situ estimation of PFOA concentration.

UNDERSTANDING POLICES THAT AFFECT CANCER CARE ACCESS FOR PEOPLE WITH INTELLECTUAL AND DEVELOPMENTAL DISABILITIES

***ESHA MATHUR,* FREYA ABRAHAM, CARLY CAMPLAIN, EMILE R. SAAD, HEATHER J. WILLIAMSON, JULIE ARMIN**

THE EFFECTS OF A PHTHALATE MIXTURE ON OVARIAN ANTRAL FOLLICLES IN-VITRO

MAILE MCSWAIN, **KARA MILLER, XIAOSONG LIU, ZELIEANN CRAIG**

DEVELOPING BISPECIFC ANTIBODIES FOR LOCALIZED DRUG RELEASE

CHLOE MIKOLAJCZYK, **MICHAEL KUHNS, DEEPTA BHATTACHARYA**

NANOPARTICLE-BASED VACCINES FOR CANCER IMMUNOTHERAPY

SOPHIA MOGHIMI**, BO SUN**

This study aims to evaluate the effectiveness of nanoparticle-based vaccines on different cancer models. Preliminary studies were carried out to determine the adjuvant combination and doses on melanoma and colon cancer models. Tumor growth and survival were monitored and calculated over a month. It came to our attention that the low bioavailability of soluble antigen peptides and potential adverse effects of soluble adjuvants would be the major barrier to current vaccine delivery approach. Therefore, a delivery system was developed for antigen peptides and two immune adjuvants delivered simultaneously with PLGA-PEG nanoparticles. These preliminary data will support the improvement of this vaccine delivery platform

THE EFFECT OF COMBINATION CHECKPOINT INHIBITOR IMMUNOTHERAPY ON SURVIVAL AND IMMUNE CELL POPULATIONS IN LYMPHOMA

AMANDA MOON**, KRISTY E. GILMAN, ANDREW P. MATIATOS, MEGAN J. CRACCHIOLO, DAN W. DAVINI, RICHARD J. SIMPSON, EMMANUEL KATSANIS**

Research on cancer immunotherapies has produced promising results, with checkpoint inhibitors specifically being a popular focal point. Combining several checkpoint inhibitors together can allow for a greater anti-tumor response, increased survival, and reduced tumor recurrence. We present novel combinations of innate and adaptive immune cell activating agents administered intratumorally in the A20 lymphoma model in mice. Mice receiving TIM-3 inhibitory antibodies and OX40 agonizing antibodies in combination with Poly(I:C) showed improved tumor control, with 90% of animals clearing tumors regardless of the addition of GM-CSF secreting cells. TIM-3+ OX40+ Poly(I:C) treated tumors had increased percentages of infiltrating CD4 and CD8 T cells. All treatment groups were able to achieve and maintain immunological memory, as mice which cleared their primary tumor were then immune to subsequent rechallenge with A20. These results show that the identification of novel combinations of checkpoint inhibitors is important in furthering cancer treatment efficacy and survival.

DEVELOPING A SCREEN FOR 3' UTR SCAFFOLDING OF PROTEIN-PROTEIN INTERACTIONS IN S. CEREVISIAE

***NICHOLAS MORTIMORE*, NICHOLAS P MORTIMORE, NIKITA FERNANDES, J. ROSS BUCHAN**

Protein interactions are fundamental to all cellular processes. However, relatively little is known about how specific protein interactions and complexes are established in the natural cellular environment, given limits on protein diffusion and a multitude of potential "off-target" protein binding partners. Recently, a novel mechanism for facilitating nascent protein interactions was discovered, deemed "3' Untranslated Region (3' UTR) Scaffolding." 3' UTR Scaffolding occurs when one protein binds to the 3' UTR of the mRNA encoding for its interaction partner and waits for the mRNA to undergo translation. Once translated, the two proteins are in proximity and efficiently interact with one another. To date, only a few examples of 3' UTR Scaffolding have been found. Here we describe a screen in S. cerevisiae (baker's yeast) to identify the role of many genes' mRNA 3' UTRs in mediating the interactions of their protein products. A better understanding of the prevalence of 3' UTR Scaffolding could have implications throughout all aspects of cellular biology and may identify novel means to alter protein interactions via targeting of mRNA 3'UTRs.

TESTING A GLYCOSYLATED ANGIOTENSIN 1-7 PEPTIDE TO TREAT COGNITIVE SYMPTOMS OF PARKINSON'S DISEASE

***JESUS A MOTA JR*, KELSEY BERNARD, PAIGE WENE, MANDI CORENBLUM, CHRISTOPHER R. APOSTOL, LAJOS SZABÒ, ROBIN POLT, MEREDITH HAY, LALITHA MADHAVAN, TORSTEN FALK**

Parkinson's Disease (PD) is a neurodegenerative brain disorder that causes motor symptoms, such as tremors, slowness of movement, and rigidity, but also cognitive symptoms, including memory loss. PD movement symptoms are due to the loss of dopaminergic neurons in the substantia nigra and Lewy bodies, cell inclusions containing aggregated forms of the protein alpha-synuclein, are the pathological hallmark. The etiology of PD is complex and not fully understood, and there is currently only treatment to improve motor symptoms. As there is no treatment for the cognitive symptoms, we are attempting to utilize a glycosylated angiotensin 1-7 peptide (PNA5), to treat PD. We are using PNA5 in hopes of reducing the inflammation in the brain as well as promoting neuroprotection, as has been shown in a mouse model of post-surgical cognitive decline. To test the effects of PNA5 on PD, mice expressing human alpha-synuclein are used. The Thy-1 alpha-synuclein mouse model is a tried and used model to study PD. We injected a group of male mice daily with PNA5 for 60 days starting when they are 4 months old until the mice have reached the age of 6 months. A series of behavioral tasks have been conducted at baseline and at 6 months of age in order to test the effects of PNA5 compared to a vehicle-injected group and a wild-type control group. Behavioral tasks consist of four commonly used tasks in the lab, novel object recognition (NOR), open field (OF), Y-Maze, and the beam task, and two new tasks; the marble task and puzzle box. The commonly used tasks test recognition memory, spatial memory, and motor function and are indicative of improved symptoms in the mice. The utilization of the puzzle box and marble task is still being determined as this task has not been used in our lab before. Analysis of behavioral tasks to test for any improvement of cognitive or motor symptoms is ongoing.

COVID-19-ASSOCIATED CYTOKINE STORM IN MECHANICAL CIRCULATORY SUPPORT: AN IN VITRO STUDY

***SAMI MUSLMANI,* KAITLYN AMMANN, MARVIN SLEPIAN**

Background: Catheter-based mechanical circulatory support (MCS) systems are increasingly utilized for therapy in advanced heart failure patients. The need for MCS has also grown in parallel with COVID-19-associated heart dysfunction. Following a COVID-19 infection, there is a rapid increase in levels of circulating pro-inflammatory cytokines referred to as a "cytokine storm". Such an increase in cytokines has been shown to disrupt the immune system drastically, resulting in a flood of inflammatory signals and potentially leading to organ failure and death. Proteins (e.g. cytokines) are sensitive to their physical and biochemical environment, undergoing conformational changes that can ultimately affect biological function. It is well recognized that MCS impart supraphysiological levels of shear stress on the blood and circulating components. 5 However, there remains a lack of understanding as to the impact and influence of MCS devices on cytokines, particularly COVID-19-associated cytokines. Here, we utilized an Impella 5.5 (Abiomed; Danvers, MA) to circulate a cytokine mixture (IL-6, IL-8, TNFα, IL-1β) representative of the COVID-19 "cytokine storm". We hypothesize that MCS-induced flow alterations - i.e turbulence and shear stress, will alter cytokine structure and binding ability as indicated by gel electrophoresis and ELISA binding, respectively.
Methods: "COVID-19 Cocktail": IL-6 (4.5ug/mL), IL-8 (4.5ug/mL) , TNFα (4.5ug/mL) and IL-1 (1.8ug/mL) in phosphate-buffered saline (PBS) was added to a 15-mL glass tube on ice. An Impella 5.5 was placed in the tube with the outflow cannula completely submerged and resting 0.5in above the bottom. The glass tube was sealed via a silicone stopper and chilled PBS was utilized as purge fluid. The pump was run at P-1 (approximately 12000 RPM) and fluid samples were taken at 1 hour of circulation, compared to uncirculated controls. Samples were either prepared for cytokine-specific ELISA or acetone precipitated and run on denaturing Tris-Tricine gel for 2 hours at 125V. Silver-staining was then performed on the Tris-Tricine gels to better visualize the cytokine bands.
Results: We found a significant increase in cytokine-antibody binding via ELISA after 1 hour of shear exposure, compared to the resting sample. Non-native gel electrophoresis of samples allowed for evaluation of molecular mass against protein standard (2-25 kDa). Notably, single bands of IL-6 and TNFα were visible in the resting samples; however, sheared samples showed double bands at the same location, indicating influence of shear on a portion of IL-6 and TNFα population size. Our findings suggest MCS could play a role in cytokine function and ultimately inflammation in a wide range of diseases. With further translation and defining mechanisms involved, these findings could help to inform improved MCS therapy.

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INCREASED ADIPOCYTE DIFFERENTIATION POTENTIALLY LEADS TO OVEREXPRESSION OF PROTEINS INVOLVED IN GLUCOSE TRANSPORTER 4 TRANSLOCATION

***JAKE NASH*, MAC MCGRAW, NOAH ROJAS, ANAY AMARO, MADISON GACKLE, MACIE GOODMANSON, KAELIE ZELMS, ATLEY MOBERLY, PAUL LANGLAIS**

Glucose Transporter 4 (GLUT4) mobilizes from intracellular storage depots to the plasma membrane upon insulin stimulation to mediate insulin-stimulated glucose uptake and maintain glucose homeostasis. We are currently studying how adipocyte differentiation leads to the increased expression of proteins involved in GLUT4 translocation to the plasma membrane and subsequent glucose uptake. Quantitative proteomics was performed to discover proteins overexpressed in mature primary white adipocytes compared to primary stromovascular cells. Quantitative proteomics analysis revealed 5,422 total proteins of which 604 proteins exhibited an increase in expression in the white adipocytes compared to the stromovascular cells. Analysis of the 604 increased proteins narrowed down the list to 41 candidate proteins hypothesized to be involved in GLUT4 -mediated insulin-stimulated glucose uptake. siRNA-mediated protein knockdown was performed on three candidate proteins, REEP6, NLRC3, and CAP2, followed by glucose uptake assay analysis, to assess for a possible role in insulin-stimulated glucose uptake.

OPTIMIZING FERRET BEHAVIORAL ASSAYS WITH THE INTRODUCTION OF BURROWING AS A NATURAL BEHAVIOR AND AUTOMATION IN DEEPLABCUT

***EVA OROZCO*, LAUREL DIECKHAUS, ELIZABETH HUTCHINSON**

Recent efforts to make animal studies of traumatic brain injury (TBI) more clinically translational have started a shift towards the use of species, such as ferrets, with greater neuroanatomical similarities to humans. The transition to ferrets warrants a re-evaluation of current behavioral assays used to quantify the severity of neurological disorders. Novel object recognition (NOR) is a current measure of memory used as an indicator of neurophysical change after experimental manipulation. Observations during NOR experiments suggest that while the assay provides valuable memory and learning data, incorporating more naturalistic behaviors (such as burrowing/digging) can potentially reflect a more comprehensive evaluation of a ferret's condition before and after brain injury. We hypothesize that burrowing will be altered after brain injury and produce additional information about a ferret's condition in complement to the established memory assessment, NOR. We conducted two baseline trials of NOR in a cohort of 8 female ferrets (co-housed) each consisting of a training session with two non-novel objects and a testing session with one non-novel object and one novel object. In one trial the ferrets were provided a pool and in the other, it was removed. The two trials produced discrimination ratios that were not significantly different across training and testing sessions (p=.58). Potential confounds include the use of a non-novel object that was not familiar enough to the ferrets and a 24-hour gap between training and testing sessions vs. the more common 1-hour gap. These confounds will be eliminated in future NOR assays to reduce the consequences of inconsistent experimental design. With those same NOR videos, we computed burrowing ratios by comparing the time spent burrowing with the total time. Burrowing ratios with and without a pool remained consistent within trials, across training and testing sessions, with no significant difference (p=.30 and p=.75). The burrowing ratios suggest ferrets engage in this behavior regardless of the task. Future directions include the collection of NOR/burrowing data after TBI. This data will allow us to address whether natural behaviors in studies evaluating well-being after experimental manipulation offer additional information that can supplement more conventional behavioral paradigms. We will also use and evaluate deeplabcut as a method to automate the analysis of ferret behavioral assays and reduce human error.

ROLE OF A HYPOTHETICAL GENE (TGME49_207210) IN *TOXOPLASMA GONDII* ENCYSTMENT

***CHLOE PARK*, CHANDRASEKARAN SAMBAMURTHY, ANITA KOSHY**

Toxoplasma gondii is an obligate intracellular parasite that infects virtually all warm-blooded animals and causes the disease toxoplasmosis. Key to *T.gondii*'s pathogenesis is its ability to differentiate from a fast-growing tachyzoite stage during acute infection to a relatively dormant bradyzoite stage that is contained in tissue cysts that are the hallmarks of a chronic infection. The bradyzoites within cysts can reactivate to cause serious pathology and death in the immunocompromised. Because of the critical role of stage conversion in pathogenesis and transmission, this study is aimed at identifying proteins that regulate differentiation in a *T. gondii* strain-specific manner. Using a recently generated RNA-seq data set from infected neurons, we identified a hypothetical gene (TGME49_207210) as being highly upregulated in type II strains which encyst faster and better compared to type III strains. Using CRISPR-Cas9, we deleted this gene from a wild-type type II strain and found decreased encystment in neurons for the knockout strain compared to the wild-type strain. Complementing the gene into the knockout partially restores encystment. A recent study has shown that the TGME49_207210 promoter contains binding sites for BFD1, a master regulator for bradyzoite differentiation. To confirm this, we infected neurons with ΔBFD1 parasites and found that TGME49_207210 expression was significantly downregulated in the absence of BFD1. Future studies will address finding the parasite proteins that interact with TGME49_207210 to enhance differentiation.

EXPLORING THE IMPACT OF CHEMICAL STRUCTURAL VARIATIONS ON DRUG METABOLISM: A COMPARATIVE ANALYSIS

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A main component of the drug discovery process is understanding the absorption, distribution, metabolism, and excretion (ADME) properties of each drug. The metabolism of a drug can help guide the understanding of safety and efficacy.Metabolic rates can help determine appropriate dosing regimens for drugs by providing information about how quickly they are metabolized in the body, and researchers usually do this through PK studies, which measure the ADME of a compound in the body. PK studies are important in drug development to ensure the safety and effectiveness of a compound for use in humans. This work aimed to evaluate the effect of structural changes in a chemical series on the metabolic rate in human liver microsomes. We tested the compounds through microsomal stability tests in human liver microsomes at four-time points (0 min, 15 min, 30 min, and 60 min). We proceeded to use LC-MS/MS analysis to measure this stability and see how much percent of the compound was left at these different time points. Then compared the different compounds to see which structural differences caused the greatest difference in metabolic breakdown rate. Results demonstrated that the most advantageous changes were various substitution patterns on an aromatic ring, the specific group being substituted on that phenol ring, and also varying lengths of alkyl chains. Small changes in structure can have a major effect on the properties of a compound. Decisions based on this research can be made on which compounds advance in a series and help guide the design of compounds. This is a vital stage in the process of drug discovery because it helps researchers figure out the most promising candidates and optimize their properties, which in turn streamlines the drug discovery process and provides higher success to bringing new drugs to the market.

SECONDARY PREVENTION OF CANCER PROGRESSION USING A NAMPT INHIBITOR

***ALLAN PAXSON*, JAIME GARD, DANIEL HERNANDEZ-CORTES PHD, KENDRA D. MARR PHD, ANNE E. CRESS PHD**

The human body contains three muscle types; skeletal, cardiac, and smooth muscle. Smooth muscle is involuntary, making it useful for functions of the digestive, reproductive, and urinary systems. We know that smooth muscle surrounds normal organs such as the bladder, prostate, and colon which are common sites for cancer formation. The smooth muscle capsule of these organs encloses the tumors that can progress to lethal metastatic disease. Escape from the smooth muscle capsule defines lethal cancers. A current unmet clinical need is to selectively eradicate aggressive disease or prevent the transition of non-aggressive into aggressive cancer. This study compares non-aggressive and aggressive prostate cancer cell lines to test their sensitivity to available FDA-approved drugs. Drug sensitivity (IC50 values) in 4 different prostate cancer cell lines was measured using a metabolic, colorimetric MTT assay. The assay measures the metabolic conversion of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), a tetrazole, to a purple product which is measured by absorbance at 540 nm 72 hours after drug treatment. This study tested 10 different drug concentrations for each of 6 different compounds. Through testing, we found that the FK866 NAMPT inhibitor compound proved to reduce cell viability of the DU145AA cell line (IC50: 7.35) which is a 3.74-fold difference compared to the DU145WT cell line (IC50: 27.46). Furthermore, collected data supported a sensitivity in the KM3g6 aggressive cell line to Bortezomib and drug resistance to Gemcitabine in the DU145AA and KM3j7 cell lines. By identifying these sensitivities within aggressive and non-aggressive cancer cells, we can begin to understand methods to stop smooth muscle invasion of cancer and prevent cancer metastasis.

COMBINED HEAVY METAL AND OSMOTIC STRESS IN *CAENORHABDITIS ELEGANS*

***WILL PETERSON*, EMILY TURNER, GEORGE SUTPHIN**

Aging is typically characterized by a decline in cells' ability to combat multiple forms of cellular stress such as oxidative, osmotic, or unfolded protein stress. Many prevalent age-associated diseases such as Alzheimer's and various types of cancers subject affected cells to multiple forms of cellular stress simultaneously. Through understanding the network of cellular responses to combined stress we can target specific pathways to better treat a variety of diseases. We looked specifically at osmotic stress via sodium chloride in combination with heavy metal toxicity via cadmium chloride which was previously shown to decrease lifespan in the model organism *Caenorhabditis elegans* to a greater extent when combined than individually. We used lifespan assays, fluorescently tagged markers, and RNA sequencing in conjunction with a new method for exposing animals to stressors sequentially rather than simultaneously for greater experimental resolution. Our data supports a model by which cadmium chloride inhibits the production of glycerol, an osmolyte responsible for combating the effects of osmotic stress, through inhibition of the enzyme GPDH-1. There are other potential points of interaction between the stress response pathways for heavy metal toxicity and osmotic stress which will be the target of future work to better elucidate the complete network of combined cellular response.

HISTOLOGICAL CHARACTERIZATION OF 3D MULTI-ORIGIN EPITHELIA

***ESHA RAJADHYAKSHA*, R. JACKSON, K. VAN DOORSLAER**

Human papillomaviruses (HPVs) can be separated into high and low-risk viruses based on their carcinogenicity. Specific types of HPV, such as HPV16 and 18, are responsible for nearly all cervical cancers and an increasing number of oropharyngeal cancers. When studying the viral life cycle, it is important to have an epithelial model representative of the different types of cells that these viruses infiltrate into and utilize for persisting. Culturing tissues utilizing the 3D raft technique allows us to generate the differentiated layers of the epithelia, which traditional 2D monolayer culture does not allow us to study. Current methods to study HPV's differentiation-dependent life cycle in 3D epithelial rafts typically utilize human foreskin keratinocytes (HFKs), however, the keratinized epithelia of HFKs may not be the most accurate representation for infections that occur at different mucosal sites throughout the body, with a special emphasis on the locations that carcinogenic HPV types infect: the tonsils and the cervix.We demonstrate the unique features that distinguish cervix, tonsil, and foreskin epithelia from each other utilizing immunofluorescent protein staining and RNA sequencing analysis. We hope to emphasize the importance of utilizing origin-specific epithelial cells when studying a particular pathogen/virus and aim to show how growing tissues ex vivo via the 3D raft culturing method allows us to accurately replicate characteristics from their origin. Overall, identifying universal and specific transcriptional and immunohistochemical markers will allow us to better understand and analyze HPV infections of different epithelial origins.

DEVELOPMENT AND CHARACTERIZATION OF CHOLINE-DICARBOXYLIC ACID IONIC LIQUIDS FOR DRUG SOLUBILITY ENHANCEMENT

***YASHWINI RAVINTHIRAN*, ABHIJIT DATE**

Ionic liquids (ILs) refer to a class of organic salts that are liquids at or near room temperature due to their low melting points. They have many advantages over traditional organic solvents as they are non-flammable, non-volatile, have high thermal stability, and increased solvating potential. The solvation properties of ILs depend upon the cation and counterions they are composed of. Choline cation-based ionic liquids have been widely studied in the field of drug delivery due to their biocompatibility, biodegradability, and low or non-toxic characteristics. Although choline has been well-established for ionic liquid use, long chain dicarboxylic acids have not been extensively explored in IL formation as a counterion in choline based ionic liquids. Here, we evaluated three hitherto unexplored dicarboxylic acids (azelaic acid, suberic acid, and sebacic acid) for

their ability to form ILs with choline at different molar ratios. Our studies show that all the chosen dicarboxylic acids could form ILs with choline at 1:1 and 1:2 molar ratios. The developed ILs were characterized using spectroscopic techniques such as NMR to confirm molecular interactions between choline and dicarboxylic acid. Finally, our pilot studies on the drug solubilization capability of these newly synthesized choline-dicarboxylic acid ILs showed that all the ILs showed ability to solubilize Nateglinide, a hydrophobic anti-diabetic drug and Naringenin, a poorly soluble natural polyphenol, at a concentration & gt; 50 mg/ml. In summary, our newly synthesized choline-dicarboxylic acid ILs could be used as a biocompatible solubilizer to improve oral and/or local delivery of hydrophobic drugs and natural products.

COMPARISON OF THE VOLATILE ORGANIC COMPOUND (VOC) GENERATION RATES FOR CONVENTIONAL AND GREEN BEAUTY SALON PRODUCTS AS A MEASURE OF WORKERS' EXPOSURE TO CONTAMINANTS IN THE WORKPLACE.

***CRISTOBAL REYES CUEVAS*, MARVIN CHAIRES, DENISE MORENO, CAROLINA QUIJADA, PALOMA BEAMER**

Volatile Organic Compounds (VOCs) are organic chemicals known to cause air pollution and to have health effects on humans and animals, including asthma and cardiovascular disease. After the identification of high emissions of VOCs in beauty salons in Tucson, Arizona with high employment of Latinx workers, the University of Arizona College of Public Health alongside the Southwest Environmental Research Institute sought to analyze individual beauty salon products used and their generation rate which will serve as a measure of the single contribution to the exposure that workers experience to these pollutants during their work shift. After selecting a list of conventional and used products from “La Promotora” Study’s chemical inventory and green products based on their online demand, the generation rates of the most utilized shampoos, hair sprays, and hair oils were compared to the generation rates of their green and alternative substitutes. The hypothesis is that green products would have lower VOC generation rates and thus could be recommended as an intervention to reduce VOC exposures in beauty salons in the parent study. Using a photoionization device in a system with a defined volume and controlled ventilation given by a conventional ice cooler, the rate at which these VOCs were introduced into the system – generation rate – from 18 beauty salon products was analyzed until they reached a steady state over a period of 6-8 hours after application of defined volumes onto cotton balls. Ultimately, the quantification of workers’ exposure to VOCs based on product usage and subsequent recommendation of least harmful products based on their generation rates can potentially reduce pollution and adverse health effects in small businesses and their workers who often lack appropriate resources due to several socioeconomic barriers.

SARS-COV-2 ANTIVIRAL INHIBITION POSSIBILITIES VIA SULFORAPHANE (SFN) INTERVENTION

VENESA ROMERO

SENSOR-BASED FRAILTY ASSESSMENT IN HOSPITALIZED COPD PATIENTS: PREDICTING POST-EXACERBATION OUTCOMES

***PAIGE RUDY*, MEHRAN ASGHARI, NIMA TOOSIZADEH**

Background: Chronic obstructive pulmonary disease (COPD) is a leading cause of death globally. Assessment of functional capacity can guide health management methods of individuals with COPD. However, typical methods of assessing disease severity are either inaccurate or impractical for bed-bound patients. We investigated the use of an upper-extremity functional (UEF) assessment and musculoskeletal arm model to predict adverse outcomes in hospitalized patients.
Methods: We recruited 156 individuals (aged ≥ 55 years) hospitalized for COPD-related exacerbations. Patients performed the UEF assessment involving 20-second rapid elbow flexion while the forearm and upper-arm sensors recorded the motion. Kinematic parameters (a previously validated UEF score) score patients from 0 (not frail) to 1 (extremely frail) based on slowness, weakness, exhaustion, and flexibility. We further calculated parameters representing muscle performance using an arm model with seven muscles. Significant relationships between UEF score and muscle model parameters and in-hospital and 30-day post-discharge adverse outcomes (extensive length of stay, re-hospitalization, death, and complications) were investigated.
Results: Of the 156 recruited individuals (age = 67 ± 7.4 years), 15 were excluded due to duplication or missing signals. Demographic parameters (age, sex, body mass index, and smoking status) and other survey scores (COPD assessment test and clinical frailty score) were not significantly different between patients with and without adverse in-hospital or 30-day outcomes (p > 0.10). ANOVA models showed significant differences in UEF score and musculoskeletal parameters, including co-contraction and mean flexion muscle force, between those with in-hospital and 30-day outcomes and those without outcomes (p < 0.05, effect size = 0.79 ± 0.084). Using the above two musculoskeletal parameters and UEF score, an AUC of 0.79 was achieved for 30-day (0.83 sensitivity, 0.68 specificity), and 0.74 for in-hospital outcome predictions (0.82 sensitivity, 0.52 specificity).
Conclusion: Results suggest that while demographics and questionnaires related to COPD progression were not significantly associated with the disease outcomes, a quick objective function test may efficiently predict both in-hospital and post-discharge outcomes. Findings also suggest that musculoskeletal model parameters in addition to kinematics may improve outcome prediction within the function test.

CO-MORBIDITY ASSOCIATIONS WITH RENAL CELL CARCINOMA GRADE

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Introduction: Obesity and excess adiposity are linked to the development of many cancers, including renal cell carcinoma (RCC). RCC patients also frequently present with other medical conditions and these co-morbidities have been shown to be an independent predictor of survival. The cancer cells found in high-grade RCC (grades 3 and 4) proliferate and spread much more rapidly than lower-grade cancer cells. Co-morbidities, such as obesity, hypertension, and diabetes, are risk factors, meaning they increase the risk of developing kidney cancer. However, associations between co-morbidities and tumor characteristics (aggressive tumor features) have not been well explored. This study aimed to observe the cross-sectional relations between co-morbidities and RCC grade at the time of diagnosis.
Methods: Patients diagnosed with RCC (N=60) from Banner University Medical Center in Tucson, Arizona completed either computed tomography (CT) or magnetic resonance imaging (MRI) scans. Adipose tissue was assessed using a single slice at the intervertebral space between the third (L3) and fourth (L4) lumbar vertebrae and SliceOmatic software (Tomovision, Magog, Canada). Minimum and maximum thresholds for tissue segmentation in CT scans were set at -150 and -50 Hounsfield units (HU) for visceral adipose tissue (VAT), -29 and 150 HU for skeletal muscle (SkM), and MRI thresholds were variable and set by the analyst to accurately capture all tissues. Previous diagnoses of hypertension and diabetes mellitus diagnoses (type I or II) were abstracted from medical records. Two-sample T-tests were used to compare any continuous and categorical characteristics. The association between co-morbidities and RCC grade was assessed by logistic regression, adjusting for age, sex, and race/ethnicity using increments of 10 cm2 for VAT, subcutaneous adipose tissue (SAT), and total muscle.
Results: This study included males and females of non-Hispanic white (N=21), Hispanic (N=30), American Indian (N=3), non-Hispanic black (N=4), and Asian descent (N=2). The mean age and BMI were 60.5 (12.1) years and BMI was 31.0 (8.3) kg/m2, respectively. Of the 60 RCC patients, 55.0% had high-grade RCC. A total of 71.7% of these patients had hypertension, while 38.3% had diabetes mellitus. RCC patients with hypertension were older (63.9±10.5 vs 51.7±12.0) with higher BMI and had significantly higher VAT, total fat, and skeletal muscle. RCC patients with diabetes had significantly higher VAT. The odds of presenting with high-grade RCC at diagnosis were increased among hypertensives (OR 5.12;95% CI: 1.11-23.69), but not among diabetics (OR: 0.26; 95% CI: 0.07-1.05). VAT, SAT, and skeletal muscle were not associated with high-grade RCC, in separate models.

Conclusion: While both hypertension and diabetes mellitus are known risk factors for RCC development, they had differential relations with RCC grade at diagnosis. Though adiposity did not directly influence tumor grade, it may have indirect effects via other co-morbidities Future models will examine more complex co-morbidity profiles, to build upon the individual relations examined herein.

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WHY DO LATE TALKERS NOT SAY ALL OF THE WORDS THEY UNDERSTAND?

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By the time that children turn two, most of them say about 200 words and begin to combine two or more words into phrases. As language acquisition progresses, children develop two vocabularies: an expressive vocabulary, which consists of the words that the child says, and a receptive vocabulary, which consists of words whose meanings the child understands. Late talkers are children who do not say enough words for their age; they have small expressive vocabularies. Children may learn to produce new words by focusing on words' phonological (sound) characteristics, such as their phonological neighborhood densities (i.e., how many words sound like them) and positional segment probabilities (i.e., how common their sounds are). In an effort to understand how late talkers may use words' phonological characteristics to learn new words, we looked at the receptive and expressive vocabularies of four late talkers. We compared the average phonological neighborhood densities, neighborhood density frequencies, positional segment probabilities, and biphone probabilities of the children's receptive vocabularies to those of their expressive vocabularies. We found that each of these phonological values was significantly greater for the expressive than the receptive vocabularies. This may suggest that late talkers are primarily saying words that have more common sounds. We plan to apply these analyses to a greater set of data to better understand how words' phonological characteristics and similarities to other words help late talkers learn to say them. Knowing this may be important in selecting words for treatment.

CLONUNG AND SEQUEENCING NOVEL LINC RNA PRODUCT IN FETAL SHEEP ISLETS

GENEVIEVE SCHOELEN

INTERMITTENT FASTING BOOSTS OPIOID ANTI-NOCICEPTION BY ENHANCING SRC KINASE SIGNALING IN THE SPINAL CORDS OF MALE MICE

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The use of opioids in the treatment of pain is widespread but comes with many drawbacks, including side effects like tolerance and addiction. Our previous research indicates that intermittent fasting (IF) can increase the efficacy of pain relief in morphine treatment while decreasing side effects like reward and tolerance. This pain relief was observed in male and female CD-1 mice

that were subjected to 7 days of IF, with daily 18-hour periods of fasting, followed by 6-hour periods of feasting on standard chow. Our current research is aimed at characterizing the molecular pathways behind this phenomenon. We thus performed a proteomic study in the spinal cords of IF mice, which implicated the SRC kinase pathway as a possible modulator of pain relief. Following this, mice were subjected to the standard IF protocol and then treated with the SRC kinase inhibitor Src-1I (10 nmol, intrathecal injection) or Vehicle, followed by morphine (3.2 mg/kg subcutaneous injection), and were assessed for anti-nociception using the tail-flick assay. The previously seen IF-induced increase in antinociception was eliminated by SRC inhibition in male mice though this effect was not observed in females. Further, mice having undergone the IF regimen and treated with the strong μ-opioid agonist DAMGO (10 nmol, intrathecal injection) displayed an increase in phosphorylated SRC in the dorsal horn of the spinal cord that was substantially higher in male mice than female mice. Despite this, Western Blotting on the spinal cords of mice having undergone the IF regimen and treated with DAMGO (10 nmol, intrathecal injection) showed an increase in phosphorylated SRC/total SRC in fasted mice that did not vary between the sexes. Combined, these results suggest that while fasting increases SRC phosphorylation in both male and female mice, the localized increase in SRC activation found in the dorsal horn of the spinal cord enhances morphine pain relief in male mice solely. Further discoveries in this mechanism could result in the development of improved pain management therapies, as well as uncover novel molecular circuits that link diet to opioid pain relief. This work was funded by institutional funds from the University of Arizona and the support of the UA Undergraduate Biology Research Program. The authors have no other relevant conflicts of interest to declare.

USING SIRNA TO UNDERSTAND DRUG RESISTANCE TO HDACI IN DIFFUSE LARGE B-CELL LYMPHOMA

***HAILEY STEENHOEK*, CATHARINE SMITH**

Histone deacetylase inhibitors (HDACi) are used clinically to treat peripheral T-cell lymphomas. However, they have little clinical activity against the much more common Diffuse Large B-Cell Lymphoma (DLBCL), and the mechanisms of resistance are largely unknown. Histone deacetylases (HDACs) are a family of related proteins, and all FDA-approved HDACi target multiple HDACs. Their clinical use is associated with various toxicities, especially when combined with other drugs. This has led to the development of HDAC-selective inhibitors. However, a major block to their use is a lack of knowledge concerning the functions of individual HDACs in regulating cell growth and survival. To address these gaps in knowledge, the Smith lab developed a preclinical, cell-based model system in which sensitivity to HDACi manifests as mitotic arrest and cell death while resistance is characterized by a reversible arrest in the G1 phase of the cell cycle without apoptosis. Their recent research using HDAC selective inhibitors has shown that selective inhibition of HDAC1 and HDAC2 leads to the resistant phenotype, and they hypothesize that the inhibition of HDAC3 is crucial to inducing cytotoxicity and cell death in DLBCL cells. Genetic depletion of HDAC3 expression in DLBCL cell lines would help confirm this hypothesis. This project was focused on optimizing the conditions for HDAC3 depletion in three DLBCL cell lines using siRNA. We first optimized transfection conditions using a positive control siRNA against Cyclophilin B. Next, we used 4 HDAC3 siRNAs to test their efficiency in reducing HDAC3 mRNA levels and determined that a combination of two was most effective. Lastly, we measured HDAC3 protein levels by Western blot to confirm selective depletion in the presence of the HDAC3 siRNA. Our results show that while HDAC3 mRNA was reduced by approximately 60-70%, HDAC3 protein was reduced by only 40%. While most proteins that regulate gene expression are short-lived and rapidly depleted within 24-48 h upon significant reduction of their mRNA, our findings suggest that DLBCL cells have a longer-lived population of HDAC3 protein that cannot be rapidly depleted.

AGE-ASSOCIATED DEFICITS IN SPATIAL AND WORKING MEMORY PERFORMANCE IN RATS

***CHRISTOPHER STERZINAR*, SAHANA SRIVATHSA, ELIZABETH CHURCH, OLIVIA GUSWILER, CAROL BARNES**

Memory is the process through which neurons encode, store and retrieve information about the world around us. Episodic memory pertains to past events and encodes context-based information such as spatial information. The hippocampus is an integral brain structure for encoding episodic memories and plays an important role in learning by integrating information with other brain regions. Another form of memory is working memory, a short-term memory system that involves temporary storage and manipulation of information in order to complete a task. The medial prefrontal cortex is critical in working memory by maintaining and updating information necessary for goal-directed actions. The hippocampus and medial prefrontal cortex are interconnected with each other directly and through indirect connections through the thalamus. Both regions are independently affected by aging, and past research displays that performance on tasks requiring medial prefrontal cortex and hippocampus function declines with age. The goal of this project is to identify and investigate the mechanisms that lead to these age-related deficits. We use three different behavioral tasks in order to analyze age-based changes between young (8 months, N=12) and old (22 months, N=13) male rats. The first task is the Morris water maze Task, in which rats are required to find a hidden platform in a pool of cloudy water through the use of distal cues which assess their spatial memory. Using a measure of corrected integrated path length taken by the rats to the platform, we observed significant differences in spatial learning between young and old rats. The second task is the Temporal Ordering Task, in which rats are familiarized with two different pairs of objects at different times in the same context and after a fixed interval they are tasked to recognize the least recent object. Old rats show significantly less discrimination favoring both objects equally while the young rats favored the less recent object more. The final task is the W-maze spatial alternation task. The W-maze task consists of two components: an "inbound" component testing spatial memory and an "outbound" component testing spatial working memory. Rats are placed at the base of the center arm on a W-shaped track and after receiving a liquid nutrient reward, are then tasked to go to a right or left outbound arm. The rat must then return to the center arm and then visit the outbound arm they did not initially visit – resulting in a continuous alternation of outbound arm visits. Old rats make significantly more errors than young rats on the outbound component even after reaching the learning criteria for the task. In the future, we will look at neural regions in order to identify further age-based discrepancies. This work was supported by the Undergraduate Biology Research Program, the BIO5 Institute, the Evelyn McKnight Brain Institute, and the RO1 grant AG072643

COORDINATION OF ACTIVE SENSING BEHAVIORS

***DALTON STORMO*, MELVILLE WOHLGEMUTH**

The natural world is a complicated and noisy place, and animals use many different methods to simplify the processing of stimuli available to sensory systems, such as sight, sound, or touch. One commonly employed method is active sensing, which is the purposeful use of motor systems to focus on relevant features of the environment. In humans, this typically involves coordinated eye and head movements. Another type of active sensing is the sonar system of echolocation in the bat, in which it emits a sound, and listens to the returning echoes, to build a 3D map of its environment. In order to investigate coordinated active sensing in the echolocating bat, we trained 3 big brown bats (Eptesicus fuscus) on a natural, 3D hunting task. We then characterized differences in sonar vocal control and ear/head position under different conditions. These different conditions come from many different factors, such as the bats' location in the room in relation to the target being hunted, the success of the hunt, as well as whether the bat was flying in a cluttered or uncluttered condition. We have focused on changes in sonar vocalizations, and changes in the distance between the outer ears (inter-pinna separation) because changes in vocal control and ear position greatly affect auditory localization cues. We find differences in sonar vocal control and inter-pinna separations across room conditions, as well as for trials when the bat successfully captures the target versus trials where the target was missed. Interestingly, these differences associated with capture success/failure could be seen several meters before contact with the target. Our results demonstrate how an animal coordinates multiple motor behaviors simultaneously to increase active sensing acuity, and suggest analogous processes may help other animals, including humans, in parsing the complicated sensory cues typical of the natural environment. Future research will investigate other motor movements associated with natural hunting behaviors and echolocation, including overall head movements as well as explore the relationship between these factors and the distance of the bat to the object of clutter, as opposed to the target.

OPTIMIZATION OF SAMPLE PREPARATION METHOD FOR NAPHTHALENE-DNA ADDUCT DETECTION IN MOUSE BLOOD, LUNG, AND LIVER

***INES STUDER*, SARRAH HANNON, XINXIN DING**

Naphthalene(NA) is a prevalent environmental contaminant. It is a common ingredient in mothballs and is generated in fire smoke and the combustion of fossil fuels. NA is classified by IARC as a possible human carcinogen; tumor formation occurs in the lungs of mice exposed to NA. The mechanism of carcinogenesis has yet to be elucidated. Genotoxicity from DNA adduct formation is a possible initiating mechanism. NA is bioactivated by cytochrome p450 and microsomal epoxide hydrolase enzymes into reactive epoxide and quinone metabolites. Published data has shown that the quinone metabolite 1,2-naphthoquinone (1,2-NQ), forms adducts with DNA in vitro1-4 and ex vivo5, 6. NQ-DNA adducts have been detected in vivo (Hannon, unpublished data) but the methodology has limited detection capability. The following describes the optimization of sample preparation methods for blood, lungs, and liver from NA-exposed wild-type (WT) mice. Samples are evaluated for NA-DNA adducts in two fractions: a stable fraction in which the NA metabolite remains bound to DNA and a depurinating fraction where the adducted nucleoside has detached from the DNA strand. Sample preparation includes RNA and protein removal, DNA isolation and enzyme digestion into single nucleosides, and subsequent liquid-liquid (LLE) and solid-phase extraction (SPE). Adducts are detected by liquid chromatography-mass spectrometry (LCMS) analysis. The adjustments described here minimized chromatographic baseline interference, the presence of extraneous peaks, and overall reduced ion suppression of the NA-DNA adducts through the removal of extraneous compounds. In conclusion, optimization of the sample preparation allows for improved sensitivity in the detection of NA-DNA adducts in vivo.

ANKLE JOINT POSITION SENSE TESTING FOR PROPRIOCEPTION EVALUATION AMONG HEALTHY YOUNG ADULTS

***ALEXIS SULLIVAN*, KARAM ELALI, NIMA TOOSIZADEH**

Proprioception is one's sense of self. More specifically, it is one's ability to sense where different parts of their physical body are relative to each other and objects around it at a given point in time. Proprioception is dependent on nerve fibers located in the muscle spindles. These nerve fibers relay information about the current position of muscles or the active movement of muscles (also known as kinesthesia). As people age, nerve fibers degrade, often due to the degradation of the myelin sheath of the nerve that facilitates action potential travel. The degradation of nerve fibers in older adults reduces proprioceptive capabilities in the legs, which makes it more difficult to maintain balance and leads to a higher risk of tripping and injury. Our study involves investigating the feasibility of using external muscle spindle nerve fiber stimulation using vibrations inputted on top of the lower leg muscles with external devices. To test whether this has an impact on proprioceptive ability, an angle matching study was conducted on a number of young healthy subjects and older high fall-risk subjects involving a test where a human subject has their ankle set to an angle, and without looking, must match that angle to the best of their ability, both with and without vibrations. Multiple vibration settings and different ankle angles were tested in randomized order for each subject. So far, we have tested with 10 young healthy adult subjects. Our initial findings have shown that there is no statistically significant difference in angle matching accuracy between different vibration settings in younger healthy adults. The largest difference in angle accuracy came from which angle was used to test. It was found that there was a lower percent difference between the initial set angle and the test subject set angle when using larger angles for testing, indicating that larger angles may be the better choice when testing for angle matching accuracy. In addition, it is found that angles above 5 degrees generally show comparable angle differences between passive (pre-set) angle and active (participant set) angle while 5 degrees shows a smaller difference in passive and active angle, both in dorsiflexion and plantar flexion tests.

THE ROLE OF ESTROGEN RECEPTOR IN THE PROTRUSIVE ACTIVITY OF ESTROGEN RECEPTOR-POSITIVE BREAST CANCER

***JILLIAN SWEETLAND*, SAMANTHA HILL, GHASSAN MOUNEIMNE**

The extracellular matrix (ECM) plays an important role in mediating the migration of estrogen receptor-positive (ER+) breast cancer cells. Durotaxis, or the directed migration towards increasing matrix stiffness, contributes to the proliferation, invasion, and ultimately, metastasis of ER+ breast cancer. Previous studies suggest that ER+ breast cancer is 'stiffer' than ER- breast cancers with increased fibrillar collagen content, and increasing matrix stiffness. However, the mechanism behind how ER promotes breast cancer migration within a stiff microenvironment is unknown. Here, we suggest a novel mechanism by which ER increases the protrusive activity of ER+ breast cancer cells in response to increasing stiffness. These results highlight the importance of matrix stiffness in cancer cell invasion and suggest that ER plays an important role in cell migration when paired with a stiff matrix.

QUANTIFYING THE ROLE OF AMINO ACID TRANSPORTERS IN CAENORHABDITIS ELEGANS AND THE POTENTIAL FOR XCT ORTHOLOG

NIALL THORNS

HAAO-1 INHIBITION ENHANCES *C. ELEGANS* OXIDATIVE STRESS RESPONSE

***EMMA THULLEN*, RAUL CASTRO-PORTUGUEZ, KAYLA RAYMOND, NIALL THORNS, SAMUEL FREITAS, EMILY TURNER, HOPE DANG, GEORGE SUTPHIN**

The kynurenine pathway produces NAD^+ from tryptophan. Previous work in our lab has shown that elevating endogenous levels of the kynurenine pathway metabolite 3HAA through either inhibition of HAAO-1 or 3HAA supplementation increases the lifespan of the nematode, *Caenorhabditis elegans*. HAAO-1 metabolizes 3HAA into a precursor for NAD^+. However, the mechanism of lifespan extension through this pathway is still unknown. Here, we investigated the relationship between *haao-1* and oxidative stress in *C. elegans.* Animals with reduced *haao-1* expression are resistant to multiple forms of oxidative stress. We find that *haao-1* animals have elevated endogenous reactive oxygen species (ROS) and activation of the NRF2/SKN-1 oxidative stress response pathway. Treating *haao-1* animals with the antioxidant n-acetylcysteine (NAC) rescues the increase in ROS, but only partially rescues activation of NRF2/SKN-1 in animals with *haao-1* mutations or RNAi. This finding demonstrates that there must be something in addition to ROS production in *haao-1* knockdown animals needed to activate *skn-1*.

ACTIVITY OF THE CENTRAL EXTENDED AMYGDALA IN MICE DEVELOPING ANOREXIA

***SAYUJYA TIMILSENA*, WESLEY SCHNAPP, HAIJIANG CAI**

Anorexia nervosa (AN) is a psychiatric condition characterized by extreme food restriction and is often accompanied by excessive exercise. Activity-based anorexia (ABA) is a well-established animal model that characterizes the key symptoms of human AN: life-threatening body weight loss, extreme physical activity, and insufficient food intake. The ABA model demonstrates that when restricted to a limited time of feeding in a day, mice will voluntarily continue running on a wheel, despite the caloric deficit, while also eating less than mice without a wheel. Previous experimentation has demonstrated that a specific population of neurons in two nuclei, the central nucleus of the amygdala (CeA) and the oval region of the bed nucleus of the stria terminalis (ovBNST), is necessary for the development of ABA. This neuronal subpopulation is distinguished by neurons that express protein kinase C-delta (PKC-δ). Here, to determine how the activity of the $CeA^{PKC-\delta}$ and $ovBNST^{PKC-\delta}$ neurons change with the development of ABA, we compared the levels of c-Fos expression in mice that were food-restricted with a wheel (FRW) to their controls, mice that were food restricted (FR) only. The protein, c-Fos, is an early response gene that is expressed after depolarization and can thus serve as a cellular marker for neuronal activity. Based on previous data showing that FRW mice do not develop ABA when $CeA^{PKC-\delta}$ and $ovBNST^{PKC-\delta}$ are ablated, we hypothesized that FRW mice developing ABA will have increased c-Fos expression in these neurons. Brains were extracted from FRW mice when they developed ABA (FRW ABA), as well as from FR mice on corresponding days (FR non-ABA). A small portion of the FRW group did not develop ABA (FRW non-ABA) but was still included for analysis. We performed immunohistochemistry to detect PKC-δ and c-Fos expression in the CeA and ovBSNT neurons. Quantification analyses display a significant increase of c-Fos expression in both $CeA^{PKC-\delta}$ and $ovBNST^{PKC-\delta}$ neurons of FRW ABA mice compared to that of FR non-ABA mice. Interestingly, the FRW non-ABA mice have comparable c-Fos expression levels to that of FR non-ABA mice. Such results indicate increased activity of $CeA^{PKC-\delta}$ and $ovBNST^{PKC-\delta}$ neurons as mice develop ABA and, thus, support the previous notion that this subpopulation of neurons in both nuclei modulates the development of ABA.

BATHROOM CONTAMINATION DUE TO 3 DIFFERENT HAND-DRYING METHODS

***ALEXIA VANCE*, JACK PICTON, JONATHAN SEXTON, KELLY REYNOLDS**

Hand washing and drying are essential for preventing the spread of viruses. However, it is unclear whether various methods of hand-drying lead to increased contamination of air and surfaces in the bathroom setting. Studies to date do not give a definitive answer as to the most optimal drying method to prevent contamination of the bathroom environment. This study assesses the spread of a viral surrogate during hand-drying by 3 different methods; paper towels, recessed, and unrecessed hand air dryers. Hands were submerged in water containing a high titer of Phi-X174 bacteriophage (10^6-10^7 pfu/mL). This was used to simulate poorly washed hands. 15 minutes following the hand drying event, air and surface samples were collected. Each drying method was completed 3 times. Samples included air at multiple locations, walls, floor, door handles, and trash cans. Samples were assayed using standard laboratory techniques and enumerated for comparisons. All 3 hand-drying methods in air samples delivered concentrations ranging from undetectable to 230 pfu/mL. The door handle site was 100% positive for the virus in all three hand-drying methods. After reaching the door handle with bare hands, the paper towel method showed an average concentration of $1.65x10^3$ pfu/mL on the door handle sample. Reaching the door handle with a used paper towel grasped in hand had an average concentration of 330 pfu/mL. The data is comparable between each method of hand-drying and the spread of the bacteriophage. This indicates that the virus may not be solely spread in bathrooms through hand dryers themselves. However, the spread of virus on door handles is higher when grasping a door handle after drying without a paper towel in hand compared to having a paper towel in hand.

RELATION OF SIGNAL TYPE TO PERCEIVED ROUGHNESS IN OLDER ADULTS

***UDBHAV VENKATARAMAN*, TAKESHI IKUMA, BRAD STORY, MELDA KUNDUK, ROBIN SAMLAN**

Signal type analysis of sustained vowels has been used to determine whether the periodicity of the acoustic signal is adequate for specific types of automatic acoustic analysis (Titze, 1995). Type 2 signals are those with subharmonic or modulating frequencies and Type 3 signals have no clear periodic structure. The presence of these irregular signals is known to be highly intermittent among speakers with vocal fold polys or unilateral vocal fold paralysis (Ikuma et al., in press). The aim of the current study is to identify the intermittency of non-Type-1 signals among older adults and the relation of these signals to perceived roughness and Voice Handicap Index (VHI) total scores. Method: Spectrograms and spectra of /a/ vowels for 122 adults aged 65 and older were observed and non-Type-1 segments were marked in the 2 seconds following a 0.2-second onset. The percent time for the non-Type-1 signal was computed. Roughness was rated from sustained vowels and connected speech. Results: Non-Type-1 signals were present in 33% of participants. When present, they accounted for an average of 34% of the production (range = 1-93%). The correlation of the roughness rating to the percent non-Type-1 signal was r=0.44. The correlation of VHI to the non-Type-1 signal was r=0.20. Conclusions: Non-Type-1 signals were present for approximately 1/3 of the participants. Roughness was moderately related to signal type while the VHI rating was not.

HISTOLOGICAL VERIFICATION OF TETRODE PLACEMENT IN THE MEDIAL PREFRONTAL CORTEX AND HIPPOCAMPUS OF RATS

***MEGHANA WARRIER*, SAHANA SRIVATHSA, ELIZABETH CHURCH, BRIDGET MALONEY, CAROL BARNES**

While memory and decision-making are cognitive processes that are an integral part of day-to-day life, they are also vulnerable to age-associated deficits. The medial prefrontal cortex (mPFC) plays a crucial role in decision making and the ventral hippocampus (vHC) is important in memory encoding and consolidation. These regions are connected via a direct monosynaptic projection from the mPFC to the vHC. In order to study the age-related deficits in memory consolidation and decision-making, we utilize a spatial working memory task that engages both the mPFC and vHC functions. Impairments to these brain regions lead to impairments in task learning (Kim & Frank 2009). In our study, we compare neural activity in both these regions in young (8 months) and aged (22 months) male Fischer 344 rats as they are trained on the spatial alternation task. In order to observe neural activity, we surgically implant the rats with dual bundle hyperdrives with 9 tetrodes in each bundle. Tetrodes are slowly lowered to their target depth after surgery. The tetrodes in the first bundle target the CA1 layer of the vHC and the tetrodes in the second bundle target the infralimbic and prelimbic regions of the mPFC. As the hyperdrives are implanted using stereotaxic coordinates, we need to verify the exact placement of the electrodes in the brain through histological methods after the experiment. We extract the brain and then section and stain the tissue to highlight different features. We utilize a combination of Cresyl Violet and Prussian Blue staining procedures. The Cresyl Violet stain binds to the Nissl bodies in neurons highlighting the different brain structures. The Prussian Blue stain marks iron deposits in the brain from the electrolytic lesions made by the tip of each tetrode. Utilizing these histological results, we have been able to modify the hyperdrive implant location to ensure that we collect data from the correct locations. By studying these two regions in tandem, we can learn more about how normative aging affects our ability to navigate tasks and store memory, with the eventual goal of applying this to models of human aging.

COORDINATION OF TRANSCRIPTION FACTOR ACTIVATION IN RESPONSE TO OXIDATIVE STRESS

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Reactive oxygen species (ROS) are byproducts of normal metabolic processes and at low levels, they have a vital role in cell signaling. However, high levels of ROS are highly cytotoxic, causing lipid and protein oxidation, DNA damage, and damage to other cellular components. Cells respond to high levels of ROS through the activation of various transcription factors (TFs). We have seen that in response to hydrogen peroxide (H2O2), the TFs p53 and FOXO1 are activated in distinct temporal phases:

FOXO1 is activated rapidly after H2O2 stress while p53 is repressed, this is followed by deactivation of FOXO1 and subsequent activation of p53. Single-cell ATAC-seq results have shown several oxidative stress-induced TFs that were enriched in distinct FOXO1 and p53 clusters in response to H2O2 treatment. Here, seven of these enriched TFs were screened for their correlation with the FOXO1 and p53 temporal phases using immunofluorescence imaging and single-cell quantification. We show that two TFs, NFAT and NRF2, were found to be directly temporally correlated with the activation of FOXO1 and p53 respectively. Peroxiredoxins (Prdxs) are a family of enzymes that reduce peroxides such as H2O2 and have been shown to be important mediators of redox-induced signal transduction. The oxidation state of Prdxs is directly linked to this biphasic response of TF activation suggesting Prdxs have a role in coordinating the temporal dynamics of this response.

EXPLORING A POTENTIAL ROLE FOR UL138 IN LDLR TRAFFICKING

MEIVEN YANG, **LINCOLN GAY, LUWANIKA MLERA, ALLISON STEEDMAN, FELICIA GOODRUM**

THE ROLE OF ZIP8 IN K562 CELLS

SUETMUI YU

IMPROVING DIAGNOSIS OF EPILEPSY WITH TRANSCRANIAL ACOUSTOELECTRIC BRAIN IMAGING: DEVELOPMENT OF A COMPLIANT COUPLING MATERIAL

IBRAHIM ZAKY**, RUSSELL WITTE**

Epilepsy impacts over 3.4 million people in the United States and many of these patients require surgery to control their seizures. Scalp Electroencephalography (EEG), which is the current method used, is not sufficient for accurately diagnosing epilepsy. Transcranial Acoustoelectric Brain Imaging (tABI) offers a more effective alternative. While noninvasive EEG has limitations in terms of spatial resolution and accuracy in estimating current source densities, tABI utilizes an acoustoelectric (AE) interaction signal which allows for current measurement with high precision at the focal region of the US beam on a millimeter scale. By combining ultrasound (US) delivery through the skull with radiofrequency sensing to produce electrical maps of the brain at high resolution, Transcranial Acoustoelectric Brain Imaging (tABI) technique offers a safe, accurate, and less expensive route to better diagnosing epileptic (ictal) signals in the human brain model. The Ultrasound probe is typically coupled to the tissue using ultrasound gel or humimic rubber, which provides a stiff interface and does not provide compliance between the probe and tissue, which may also introduce noise. When targeting a curved position on the surface of the skull, for example, the two-dimensional plane surface of the probe may further restrict movement. Similarly, in a heart model, the probe may affect the motion of the pumping heart. The goal of my UBRP project was to develop a more compliant coupling material to efficiently deliver ultrasound to the tissue for acoustoelectric imaging and assess its performance in a human head phantom or heart tissue. A more flexible material between the US probe and human skull rather than using a humimic adapter, could enhance flexibility and improve image quality if other sources of noise are controlled. A custom-designed, three-dimensional adapter was developed to fit onto the ultrasound probe and hold a thin-walled rubber balloon filled with degassed deionized water for acoustic coupling. The balloon, made of latex rubber, was sealed at both ends and checked for the presence of ultrasound-attenuating air bubbles before use. The 3D model was fixed onto the probe to hold the balloon securely in place

with coupling gel used to fill any gaps between the probe and the balloon. Candidate coupling materials were tested on a human head model with a real skull for tABI. We compared image quality using the latex balloons with that using other types of coupling media/devices. Specifically, we evaluated spatial resolution, signal-to-noise, and sensitivity of tABI through the skull for estimating and mapping local currents. The implemented solution has improved mobility and coupling. However, further, development is necessary to eliminate noise sources and enhance coupling. The causes of noise between the model and the US probe have been identified, and efforts are being made to minimize noise through additional development.

COMPUTATIONAL IDENTIFICATION OF PRECISION THERAPEUTICS FOR ALZHEIMER'S DISEASE-SPECIFIC TO ENDOCRINE AND APOE GENOTYPE STATUS

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Alzheimer's disease affects an estimated 6.5 million people in the US, ⅔s of which are women, yet highly efficacious, disease-modifying treatments for Alzheimer's disease (AD) are currently lacking. Among the strongest modify AD risk. Biological sex and apolipoprotein (APOE) genotypes exhibit potentially lower (male sex, APOE ε2 allele) and higher (female sex, APOEε4 allele) risk of developing AD. The neuro-immune system is also a key driver of chronological and endocrinological aging that occurs in the midlife female brain. In this work, we developed a sex- and APOE-specific precision therapeutic approach starting from transcriptomic signatures of 15 month acyclic vs 9 month regular APOEε3/ε3 and APOEε4/ε4 female mouse models. The integration of APOE-transcriptomic data with protein-protein and protein-drug interaction repositories allowed for the construction of an APOE-specific AD network. Finally, the development of a drug synergistic score accounting for topological properties of the network and off-target effects allows for the ranking of potential drug repurposing therapeutics that are specific for female and APOE genotype and endocrine transition state.