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LOUISIANA CANCER RESEARCH CENTER

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Poster Abstracts

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Exploring the Role of Endothelial-to-Mesenchymal Transition Related Transcription Factors in Sprouting Angiogenesis

Angiogenesis is the process of new blood vessel formation from existing vessels. It occurs either during the development of new tissues or during tissue repair. Endothelial cells (ECs), which line the lumen side of the blood vessel, are a key player in angiogenesis. ECs are normally quiescent but upon stimulation by growth factors like vascular endothelial growth factor (VEGF), they start to proliferate and migrate to form new vascular sprouts. However, after a mature blood vessel is formed, activated ECs completely regain their original identities to stabilize the blood vessel. Our lab hypothesizes that this involves partial endothelial-to-mesenchymal transition (pEndoMT), where ECs partially and reversibly acquire mesenchymal-like characteristics, like increased proliferation and migration. Interestingly, this pEndoMT process may be dysregulated in tumor angiogenesis during which we see leaky and uncontrolled vascular outgrowth.

SNAI1, SNAI2, and TWIST1 are transcription factors that play key roles in EndoMT progression. Additionally, OVOL1 and OVOL2 are regulators of SNAI1, SNAI2, and TWIST in the related process of epithelial-to-mesenchymal transition, and may similarly regulate EndoMT in EC. Previous studies have shown that both SNAI1 and SNAI2 are required in EC for tumor neovascularization in mice. Thus, there may exist a delicate balance between the expression level of pro- and anti-EndoMT factors to drive pEndoMT during angiogenesis. To determine the precise role of those factors in sprouting angiogenesis (potentially through pEndoMT), we generated SNAI1, SNAI2, TWIST, OVOL1 and OVOL2 overexpression lentiviral vectors, which will drive constitutive expression of each transcription factor in virus-transduced cells. Next, we determined the titer of the virus by puromycin selection, and we further validated transcription factor overexpression in transduced EC by quantitative PCR (qPCR).

Our future experiments include additional further validation of our overexpression vectors at the protein level by immunofluorescence. After that, we will perform the Fibrin Gel Bead Sprouting Assay with wild-type or lentivirus-transduced Human Umbilical Vein Endothelial Cells (HUVECS). The Bead Assay is a classic *in vitro* angiogenesis assay which involves coating plastic beads with HUVECs and embedding into a fibrin hydrogel matrix alongside primary fibroblasts. After a few days of culture in suitable growth conditions, coated ECs undergo sprouting angiogenesis. Different morphometric features including sprout number, branching, and length can be quantified for each bead. This assay will help us understand what effect (e.g., hypersprouting or loss of sprouting) the overexpression of EndoMT transcription factors will have on angiogenesis. Additionally, it will provide valuable insights into how pEndoMT may be dysregulated by the tumor microenvironment, and how this may contribute to tumor neovascularization in cancer.

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"Comparison of Cell Growth Between Healthy and Damaged Adipose-Derived Stem Cells in a Bioreactor"

Background: Every year over 11 million people in the world are affected by Burn derived injuries with 180,000 succumbing to their wounds. The American Burn association found that 450,000 people in the U.S. alone are injured due to burns and are in medically treated. Burn wounds are common and with a lot of major infrastructure in America revolving around corrosive and highly explosive materials that can cause burns. Stem cells help in wound healing when burn traumas happen as when a burn happens those cells that were killed must be replaced and stem cells are the one to replace those damaged or dead cells healing the area.

Objective: test how effective in growth (ADSC) adipose derived stem cells both healthy and damaged in an environment that mimics the human body when injured by a burn.

Methods: ADSC were cultivated in a static flask for a week to build up 100 thousand for the reactor, once incubated for the time the cell were then transferred to a RCCS4D bioreactor system in a 10ml (about 0.34 oz) HARV to be suspended in an in vitro setting that will mimic in vivo. The cells were incubated in 5% media to simulate a lacking condition as 10% media is standard in cell cultivation.

Results: the experimental group from damaged adipose tissue grew exponentially from the initial 100 thousand to 3.6 million ADSC's int eh span of 5 days in the bioreactor.

Conclusion: ADSC's from damaged tissue a quite capable of reproducing and even in a unfavorable environment are still quite capable of stimulating cell growth for repairs.

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"Nicotinamide Adenine Dinucleotide (NAD) Attenuates the Rate-Decreasing Effects of Oxycodone Withdrawal in Rats"

Background: Over 16 million people worldwide and over 2.1 million United States citizens are affected by Opioid Use Disorder (OUD) due to the over-prescription and underestimation of the addictiveness of opioids in the 1990s (Dydyk et al., 2024). When a person misuses or abuses opioids, the amount of nicotinamide adenine dinucleotide (NAD) naturally found in the body is depleted. NAD replacement stimulates cell regeneration to increase energy and neurological health without an abuse liability (McCrakin, 2022). Therefore, we implemented two protocols for inducing dependence in rats to test whether intravenously administering the small molecule NAD+ could effectively attenuate withdrawal and help transition individuals to abstinence.

Methods: Two cohorts of rats were first trained to lever press under a fixed-ratio 30 (FR 30) schedule for food reinforcers. After training one cohort of seven Long-Evans rats (4 male and 3 female), each rat received 3.2 mg/kg once daily, 3.2 mg/kg twice daily, and 10-18 mg/kg twice daily of oxycodone for a minimum of three weeks at each regimen. A second cohort of eight Long-Evans rats (4 male and 4 female) each received 10, 20, 30, and 40 mg/kg twice daily over four days and were then maintained on 40 mg/kg once daily. When responding stabilized after the initiation of these two protocols, spontaneous withdrawal was assessed by the cessation of chronic treatment for at least 24 hours, whereas precipitated withdrawal was determined by administering 0.32-3.2 mg/kg of naltrexone interperitoneally (i.p.) in increasing cumulative doses; both were assessed by disruptions in overall response rate (responses/second) and the duration of pre-ratio pausing (PRP) (seconds). After withdrawal was demonstrated consistently (indicating dependence), surgery was performed to implant a venous catheter and port. Twentyfour hours following catheterization, the chronic oxycodone regimen was restarted and responding was restabilized under the operant schedule. The capacity of NAD to attenuate withdrawal was then tested by permanently discontinuing the chronic oxycodone administration, and subjects were infused intravenously (i.v.) with either saline or 180 mg/kg of NAD+ each night for 10 hours for 10 consecutive days. Subjects responded under the operant task every afternoon during the 10 days to assess the disruptions in behavior.

Results: The chronic regimen used for the first cohort of rats reliably induced physical dependence, as the behavioral rate of responding decreased during both spontaneous and precipitated withdrawal. PRP was also increased. Subjects given 10 days of i.v. NAD+ for 10 hours recovered their pre-chronic non-dependent baseline of responding after 2 days of oxycodone cessation, while the saline group recovered their pre-chronic baseline after 4 days. PRP was restored more quickly in the NAD+ group than the saline group. Data is currently being collected with the second cohort.

Conclusion: In our previous rodent model of opioid-dependence, i.v. NAD+ infusions reduced the duration and magnitude of the rate-decreasing effects that occur during withdrawal from oxycodone. These results indicate that NAD+ may have promising potential as a treatment for dependence. The current experiments also tested a more efficient method of developing opioid dependence in rats, where subjects achieve dependence within a week. Going forward, this protocol should allow for a more rapid examination of the effects of NAD+ on withdrawal while decreasing the resources and time needed to achieve these valuable results.

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"TNF-α Concentration Curve Effect on Oral Squamous Cell Carcinoma In Vitro"

BACKGROUND: Metastasis, the spread of cancer cells from their origin to other places in the body, is a defining characteristic of malignant tumors and a leading cause of cancer-related death. This process involves cancer cells invading nearby tissues, entering the bloodstream or lymphatic system, surviving in circulation, and establishing secondary tumors in distant locations making another cancer site. Understanding and effectively managing metastasis are crucial in improving cancer treatment and advancing research. Tumor Necrosis Factor-alpha (TNF-α), a pro-inflammatory cytokine, plays a critical role in immune responses by regulating inflammation, apoptosis, and cell survival. Research with other cancer cells suggests that TNF-α can affect cancer cell migration. CAL27 and UPCI: SCC090 are types of oral cancer originating in the tongue, SCC090 is positive for Human Papilloma Virus (HPV).

OBJECTIVES: Observation of TNF-α effect of squamous cell carcinoma motility rate.

METHODS: A wound-healing assay is a laboratory technique used to investigate cell motility and invasion capabilities. Widely utilized in cancer research, migration assays assess metastatic potential, treatment effects, and the mechanisms of cell motility. We designed a concentration curve, with TNF-α concentrations of 0,1.0, 10.0, 14.4, 50.0,100.0 mg/ml across a 0, 6, 24, 48-hour periods.

RESULTS: Over a 24-hour period, TNF-α concentration had no significant effect on Cal 27 cells, but significantly lowered proliferate of SCC090 at concentration: 0.0, 1.0, 14.4, 50.0,100.0 ng. Preliminary experiment on TNF-α effect on motility rate of Cal 27 and SCC090 showed an overall lower rate of motility of SCC090 than Cal 27 over a 24-hour period with no statistical analysis performed due to low number of samples.

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"Role of DNA Methyltransferases in Enzalutamide Resistant Prostate Cancer"

INTRODUCTION: Prostate cancer is the most common malignancy in men and results in over 35,000 deaths annually in the USA. Androgen deprivation therapy (ADT) has been an effective strategy for reducing cancer cell growth and proliferation. Enzalutamide (ENZA) is a next-generation androgen receptor inhibitor approved by the FDA for the treatment of metastatic castration-resistant prostate cancer (mCRPC). ENZ-R is marked by adaptive cellular mechanisms that includes both cellular plasticity and the emergence of CRPC- NEPC and CRPC-DNPC phenotypes. While genomic alterations have been extensively investigated in ENZ-R, little is known about the plausible epigenetic mechanisms in this process. Recent studies from our lab and others have pointed to increased expression and activity of DNMT's (DNMT1, DNMT3a and DNMT3b) during prostate cancer progression, however, direct role of DNMTs in ENZ-R has not been evaluated. In this study, we aimed to determine the role of DNMTs in ENZ-R prostate cancer.

METHODS: LNCaP cells were obtained from ATCC Inc. The cells were cultured in RPMI-1640 (Corning #10-040-CV). All media were supplemented with 10% heat-inactivated fetal bovine serum (FBS), Cat# 10437028-Gibco; 1% penicillin-streptomycin, Cat# 15070-063-Gibco; and were maintained at 37°C, 5% CO_2 in a humidified incubator. LNCaP-ENZR cells were generated by culturing LNCaP cells in progressive concentrations of ENZ-R for over 3 months and were maintained in media supplemented with 5uM Enzalutamide. Cell growth and proliferation was measured in the IncuCyte® Cell Count Proliferation Assay. Clonogenic activity was measured by colony formation, and cell migration was assessed by wound-healing assay. Protein levels were measured by Western Blot analysis, while gene expression was evaluated by real time PCR

RESULTS: We observed that treatment of PCa (LNCaP Cells) with 5Aza-dC (5uM and 10uM) resulted in significant ($p<0.01$) inhibition of cell proliferation in a time dependent manner. The inhibition of cell growth was more pronounced than treatment with ENZA (5uM and 10uM). Moreover, treatment with a combination of both 5Aza-dC and ENZA was more effective in limiting cell growth and proliferation. We uncovered that DNA methylation (DNMT1, DNMT3A, and DNMT3B) was upregulated in ENZ-R cells. Our results also show that 5Aza-dC treatment of established ENZ resistant PCa Cells (LNCaP-ENZR cells) also reduced growth and proliferation significantly ($p<0.01$). Combination treatment of the cells with 5Aza-dC and ENZA decreased clonogenic activity. Results of cell migration are still in progress.

CONCLUSIONS: Our data demonstrated that the DNA methylation pathway is deregulated in ENZ-R Prostate cancer cell lines, and that targeting DNA methyltransferases sensitized the prostate cancer cells enzalutamide (current treatment). These studies suggest DMNT activity as a potential therapeutic vulnerability that can be exploited for limiting cellular plasticity, tumor progression, and therapy resistance in prostate cancer. Because DNMT inhibitors are currently approved for other malignancies, addition of these inhibitors to current treatment regiments could be readily explored in PCa.

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"Evaluating IL-11 and TGFβ1 Distribution Relative to Synovial Fibrosis Status"

Introduction: Synovial fibrosis (SFb), a painful contracture limiting joint motion and quality of life, is a hallmark of arthrofibrosis (AF), a common complication after joint repair. SFb is categorized by low (<41%), moderate (42-54%), and high (>54%) collagen deposition levels and is a significant challenge in osteoarthritis (OA) patients. Transforming growth factor beta 1 (TGFβ1) drives SFb and regulates essential cell processes. Interleukin (IL) 11, synthesized downstream of the TGFβ1-mediated JAK/STAT3 cascade, promotes fibrosis if dysregulated. Novomedix's (NMX) novel inhibitors selectively target IL11 without disrupting TGFβ1-mediated functions. These inhibitors effectively reduce IL11-driven collagen deposition in OA-derived fibrotic synoviocytes. To assess the potential of NMX for in vivo SFb treatment, this study analyzes IL11 co-expression with TGFβ1 in banked knee OA samples, hypothesizing a correlation between IL11 expression and SFb severity.

Methods: Eighteen de-identified knee synovium samples from patients with end-stage OA were categorized into low (n=7) and high (n=11) SFb cohorts based on pre-defined histological scores. These samples underwent paraffin processing, embedding, and sectioning for co-detection of TGFβ1 and IL11 by indirect immunofluorescence (IIF). Following deparaffinization, heat-mediated antigen retrieval in citrate buffer (pH 6.0), and protein blocking, sections were incubated overnight with anti-TGFβ1 (mouse monoclonal) and anti-IL11 (rabbit polyclonal) antibodies. Sections were then stained with anti-mouse Alexa 594 and anti-rabbit Alexa 647 secondary antibodies for TGFβ1 and IL11, respectively, along with DAPI nuclear counterstain. Samples were mounted and imaged using a confocal microscope (Olympus) at 200x magnification. Co-expression of TGFβ1 and IL11 was quantified through background-corrected signal analysis using Slidebook™ software. Statistical comparisons between low and high SFb groups for adjusted TGFβ1 and IL11 levels were made using a two-tailed Student's t-test (α=0.05).

Results: The mean expression of TGF1 observed in the synovium of patients classified with high SFb severity was 35% higher ($p = 0.0360$) compared to the signal measured from the low severity SFb group. Correspondingly, IL11 measures in the high SFb severity were registered at a 77% increase ($p = 0.0016$) from patients with less severe SFb. A moderate but significant correlation was calculated between TGFβ1 and IL11 ($R = 0.51$; $p = 0.0314$).

Discussion: Increased expression of IL11 relates to TGFβ1 in agreement with SFb severity. While this study does not prove causality, it suggests a relationship between IL11 and SFb, highlighting the diseased synovium as an effective target for NMX administration. The study is limited by sample size and doesn't account for confounding variables such as synovitis grade and presence of additional pro-fibrotic factors such as connective tissue growth factor. Further studies will investigate the effectiveness of NMX on aberrant collagen deposition, contraction, and myofibroblast differentiation rate of patient-derived synovial fibroblasts.

Conclusion: Evidence of TGFβ1-dependent expression of IL11 relative to SFb severity indicates the potential supplementation of NMX to assist manipulation under anesthesia and arthroscopic lysis of adhesions in the management of debilitating arthrofibrosis.

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"Tree-Inspired Deep Learning of Multi-omics Data in People Living with HIV"

People living with HIV face more comorbidities than the general population, including infections, cancers, and gastrointestinal issues such as liver disease. These comorbidities are further compounded by alcohol use. Despite antiretroviral therapy (ART) treatment, people with HIV experience persistent gut dysbiosis which is thought to contribute to the comorbidities associated with living with HIV and HIV-induced gut dysbiosis. Additionally, HIV-associated gut dysbiosis is thought to be compounded by alcohol use which is disproportionately prevalent in people with HIV. Further, HIV is correlated with an acceleration of the aging process. With ART treatment, some are thought to return to normal levels of aging whereas others continue to accelerate, and it is unclear what mechanisms are causing this precocious aging. Our goal was to use machine learning modeling to predict participant scores on the Alcohol Use Disorders Identification Test (AUDIT) and the 58-item deficit index (DI58) based on participant metabolome and microbiome as a means of assessing the predictiveness of multi-omics data for frailty and alcohol use in people living with HIV, and to identify multi-omic features potentially altered by alcohol use or related to DI58 pathogenesis. The dataset involved microbiome and metabolomics data of a cohort of 101 people living with HIV. We hypothesized that a machine learning approach integrating fecal microbiome and metabolomics data could accurately predict disease states and identify features relevant to disease states.

We adopted a late-stage approach to integrate the multi-omics data by utilizing an artificial neural network (ANN) whose unique architecture separately propagates the microbiome and metabolomics data and later concatenates the outputs of the respective sequences of layers through a final sequence of layers. However, initial modeling was heavily prone to overfitting. We used the tree ensemble method *xgboost* to determine which subset of features to selectively use based on their gain (the relative contributions the feature had on the predictions made by *xgboost)* to improve model performance. Notably, features such as *Prevotella* displayed significant feature importance in modeling with *xgboost* for both AUDIT and DI58. Other notable features included *Muribaculaceae* for AUDIT and *Ruminococcus* for DI58. These features were then used to train the ANN, and the model generalized well to non-training data for AUDIT and DI58. The training mean squared error (MSE) loss and validation MSE loss for AUDIT was 0.9356 and 0.973, respectively, and 0.8639 and 1.2478 for DI58, respectively.

While no longer overfitting, the models display slightly high bias and performance may be improved with the addition of more samples and features to the dataset and more robust hyperparameter/architecture tuning. Future project directions include testing for accuracy in the classification of frailty and AUDIT categories and determining the distributions of feature importance to the ANN across the cohort using SHapley Additive exPlanations (SHAP) values.

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"Sending out an SSO"

Abstract

Friedreich Ataxia (FRDA) is a progressive neurodegenerative disease caused by repeat expansion in the first intron of the FXN gene. FRDA is lethal, with the leading cause of death being heart failure due to repeat expansion. The number of repeats is directly proportional to disease severity. The repeats expand to the greatest length in the heart. If we can stop repeats from expanding in the heart, we could slow or even stop the progression of cardiomyopathy. Expansion leads to reduced expression of the FXN gene, which encodes the mitochondrial protein frataxin, responsible for iron-sulfur cluster formation and is essential to energy production. Cardiac cells require substantial amounts of energy to keep the heart pumping. Consequently, deficiencies in frataxin lead to cardiovascular problems in addition to neurological problems, as neurons and cardiomyocytes require the most energy.

Our long-term goal is to slow or even stop somatic repeat expansion in the heart. My project takes preliminary steps to establish a model for peptide mediated delivery. The DNA mismatch repair complex MutLγ (MLH1-MLH3) is responsible for DNA repeat expansion. MutLγ containing isoform 1 of MLH3 produces expansion and MutLγ containing isoform 2 of MLH3 does not. To slow repeat expansion, we aim to splice-switch MLH3 isoform 1 to MLH3 isoform 2. Oligonucleotides can be designed to induce splice redirection. Successful oligonucleotides are called Splice Switching Oligonucleotides (SSO). Our lab has developed an SSO that can successfully induce splice redirection. In this study, as a prelude to testing a cardiac targeting peptide, we linked the SSO to a well-known cell penetrating peptide (CPP), HIV-1 tat, to penetrate our model cells, HEK 293. As a positive control we used the vivo morpholino variant of the same sequence. We compared the Vivo, N-tat and C-tat linked versions. We isolated RNA 24 hours after dosing and determined MLH3 splice-switching by PCR product analyzed via gel electrophoresis. HEK 293 cells treated with a Vivo-SSO showed successful penetration and a dose-dependent increase in the amount of MLH3 splice-switched to isoform 2 as expected. HEK 293 cells treated with N-tat-SSO and C-tat-SSO show splice-switch to isoform 2 in high doses, providing encouraging information for future research.

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"Interplay between topoisomerase I and RNA polymerase of *Chlamydia trachomatis*"

Chlamydia trachomatis (Ct) is a gram-negative, obligate intracellular bacterium that is the leading cause of sexually transmitted bacterial infection worldwide. Ct has a unique developmental cycle, alternating between an infectious elementary body (EB) and a non-infectious reticulate body (RB). Precise signals that trigger changes in Ct development remain unclear, but two notable aspects are relevant: temporal gene expression and varied DNA supercoiling. The latter is sustained by the DNA topoisomerases (Topos). Previously, our lab utilized the recently-developed CRISPR technique to knock down Ct *topA* encoding topoisomerase I (TopA), which relaxes negative supercoils in bacteria. We observed that *topA* repression impaired EB-to-RB transition; conversely, expression of late genes was downregulated and early genes maintained their expression, highlighting the important link of DNA supercoiling and the developmentally regulated gene expression. RNA polymerase (RNAP) is responsible for transcription and consists of $\alpha_2\beta\beta'\omega$ subunits (core enzyme) and a promoter-specific σ factor in bacteria. We hypothesize that by directly interacting with the RNAP, TopA participates in the regulation of transcription during the chlamydial developmental cycle.

To begin to test this hypothesis, the objective of my study was to determine whether chlamydial TopA binds to the full-length chlamydial β subunit of RNAP, RpoB, *in vitro.* Two different strategies were employed. First, we co-transformed *E. coli* cells with two different expression vectors, one cloned with *topA* and a sequence of 6-histidine to its 3' and the other one cloned with *rpoB*. Second, the plasmid vector cloned with *topA* and a sequence of 6-histidine to its 5' or cloned with *rpoB* was individually transformed into *E. coli*. The expression of proteins of interested were induced by addition of proper inducers, followed by assessing the protein expression levels, purification of proteins, and the characterization of the complex of TopA and RpoB using chromatography, binding assay, SDS-PAGE, and Western blot analysis.

We confirmed the inducible expression of His6-TopA, TopA-His6, or RpoB in *E. coli*. Interestingly, we found that co-expression of TopA-His6 with RpoB resulted in a high level of RpoB expression compared to that in *E. coli* expressing RpoB only, implying the potential action of TopA in modification of gene expression likely via changing DNA supercoiling. Moreover, we provide evidence that His6-TopA is efficient in binding to RpoB and produces a stable protein complex *in vitro.*

Our results indicate a direct interaction between chlamydial TopA, and the RNAP mediated by its core subunit, RpoB, consistent with the role of TopA in affecting transcription elongation. How such direct interaction may affect the expression of highly transcribed genes at the site of transcription in *C. trachomatis* will be further studied.

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"Kappa Opioid/Dynorphin Expression in Adult Female Mice with Adolescent Opioid Exposure and Adult Chronic Intermittent Ethanol Treatment."

Adolescence is a very vulnerable period of transition to adulthood for humans in which the brain undergoes critical development and maturation physically, emotionally, and socially. Adolescent opioid exposure (AOE) directly affects the brain's reward system and results in elevated risk of prolonged opioid abuse. While opioids are prescribed for pain symptoms, alcohol is also used to self-medicate pain. In spite of efforts to cope with pain with alcohol use, alcohol use and withdrawal can induce hyperalgesia thus facilitating the cycle of future alcohol use. Chronic opioid use and withdrawals has also been found to increase pain sensitivity and potentially contributes to opioids' lack of long-term effectiveness.

The bed nucleus stria terminalis (BNST) is a sexually dimorphic structure located within the ventral forebrain that is involved in the anxiety and addiction circuitry and in pain. The oval and dorsolateral regions of the BNST are involved in the body's emotional processing and regulation and in stress response. The dynorphin/kappa opioid receptor (KOR) system is an important factor in BNST stress signaling also involved in addictive behaviors and pain. This experiment investigated the relationship between mechanical and heat sensitivity in female mice with combined AOE and adult intermittent ethanol vapor exposure (CIE) and the activation of dynorphin and KOR in the BNST.

Adult and adolescent alcohol exposure have been found to produce long-lasting mechanical hypersensitivity in male and female mice with persistent effects following AOE. When AOE was combined with CIE, we found that female mice given AOE+CIE produced more long-lasting mechanical and heat hypersensitivity than CIE alone. Interestingly, this difference was not seen in male mice. In the current work, female brains were collected following the behavioral analysis above. These brains were sliced and RNAscope in situ hybridization was utilized to detect the expression of c-Fos mRNA (indicating cellular activity), dynorphin (which is associated with stress, pain, and addiction), and KOR (which are activated by dynorphin) in individual cells across the oval and dorsolateral BNST regions. QuPath image analysis was employed to count the total number of cells and detect cells containing positive channels. Combined AOE and CIE exposure exhibited decreased expression of positive cFos mRNA, dynorphin mRNA, and KOR in the dorsolateral BNST compared to mice exposed to saline in adolescence and CIE, although these differences were not significant. Future studies will explore whether a larger cohort will provide significant differences of c-Fos, dynorphin, and κopioid receptor mRNA expression in the dorsolateral and oval BNST.

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"Modeling Sensitivity of Neuronal Firing Frequency to L-type Calcium Channel Activity"

Background: The substantia nigra is a part of the brain associated with learning, movement, and addiction, and more. Within the substantia nigra, there are different subpopulations of dopaminergic neurons which project to different areas of another part of the brain called the striatum. These different subpopulations are believed to have different functions and effects on behavior. Among two subpopulations in particular, DMS (dorsomedial striatum) projecting neurons and DLS (dorsolateral striatum) projecting neurons, one observed difference is a contrast in the sensitivity of firing frequency in both baseline and depolarizing inputs to inhibitors of the L-type calcium channel $Ca_V1.3$. In this experiment, the goal was to investigate how this channel acts as a linear amplifier in the DLS projecting neurons through the simulation of the L-type channel-specific blocker isradipine (ISR).

Methods: A Hodgkin-Huxley conductance-based single-compartment model of a neuron was used to simulate neuronal pacemaking and bursting. The project addressed two methods of investigation. Two scenarios were set up: one where the variation in frequency was attributed to the L-type channel, and one where it was attributed to sodium leak (NaLCN). For each scenario, we inhibited the L-type channel and observed whether a linear decrease in frequency occurred.

Conclusion: We concluded that the variation in frequency when driven by the L-type channel was consistent with the linear effect of ISR, while the variation in frequency independent of the L-type channel was not.

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Exploring the impact of alcohol and SIV on skeletal muscle mitochondria in western diet-fed Rhesus Macaques

Background: Skeletal muscle (SKM) is primarily responsible for all movements performed by the body. The contraction of the muscle leads to the movement of specific bones for different functions. Along with structural support, SKM also acts as storage for glucose, in the form of glycogen, in times of starvation. Human immunodeficiency virus (HIV, or SIV in macaques) weakens SKM, causing inflammation. Chronic binge alcohol (CBA) has also been linked to dysfunctions found in SKM through the altering of protein synthesis in SKM mitochondria. When combined, HIV and CBA can impair mitochondrial function in SKM, thus not meeting the biogenetic demand of the cell, and these detrimental changes can increase in the presence of high sugars and fats. Without functioning mitochondria, SKM does not receive the energy needed to perform everyday tasks. Therefore, this study aims to understand how CBA and SIV directly affect mitochondrial health in the SKM. **Methods:** SKM biopsies derived from vehicle (naïve, n=4), or SIV-infected, ART-treated adult male rhesus macaques (VEH/SIV+, n=2) administered CBA (2.5g ethanol/kg/day) with (CBA/SIV+, n=4) or without SIV (CBA/SIV-, n=6) were homogenized for analysis. RNA was then isolated and transformed into cDNA with the use of reverse transcriptase enzyme and a collection of buffers. The cDNA was normalized, and mRNA analysis was performed through polymerase chain reaction (PCR) with the use of primers to identify the quantity of the following mitochondrial-associated gene targets within each sample: PGC1a, TFAM, UCP2, NRF1 and PPARg. **Results:** Our preliminary results show that looking at the mRNA expression of the gene targets PGC1a, NRF1, TFAM, and UCP2, there was no significant differences in comparison to the vehicle control groups. The gene target PPARg, however, demonstrated a significant decrease in mRNA expression in the CBA/SIV- group, showing that CBA affects PPARg mRNA expression in SKM mitochondria. **Summary:** The study of the effects of CBA and SIV on the SKM of male rhesus macaques has proven to alter mitochondrial biogenesis and turn over through PPARg. However, this is an on-going study and as the sample size is increased, we will expand on the analysis and possible changes of the mitochondrial health-associated genes.

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Phytoalexin Glyceollin I Suppresses Viability in Her2+ Breast Cancer

For women worldwide, breast cancer (BC) ranks top 2 in both annual quantity of cases and deaths among cancers. Leading these statistics, developing new methods for combating breast cancer remains at the forefront of cancer research. Types of breast cancer are differentiated based on the presence of 3 receptors: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (Her2). HER2+ BC targeted agents such as trastuzumab, pertuzumab, and lapatinib, alone or in combination with traditional chemotherapeutics, have become the standard of care for this BC subtype, significantly improving patient survival rates. However, the development of resistance to targeted therapy represents a major obstacle in the treatment of HER2+ breast cancer, highlighting a critical need to identify novel therapeutic targets to treat resistant HER2+ breast cancer.

Isoflavonoids, an important class of natural compounds produced from numerous plant sources including legumes, have been identified to be beneficial for human health. A member of the legume family, the soybean, maintains a high isoflavonoid content, specifically daidzein and stress induced derivatives called glyceollins. Recent studies have demonstrated glyceollin activity against ER+ breast cancer due to inhibition of ER and its associated pathways. To date the impact of glyceollins on other breast cancer subtypes has not been fully explored. Here, we utilized a panel of Her2+ breast cancer cell lines (HCC 1954, Au565, SKBR3), as well as derived trastuzumab-resistant variants (Herceptin resistant SKBR3), to evaluate the effects of glyceollin treatment, alone or in combination with other targeted agents, on cell viability and clonogenicity. Additionally, we analyzed changes in downstream gene expression using qRT-PCR profiler array for Human Cancer Pathways to define potential glyceollin-targeted pathways involved in the regulation of Her2 breast cancer cell biology.

Results demonstrated that glyceollin was able to decrease cell survival and colony formation across cell lines. Further glyceollin suppressed expression of pro-oncogenic genes in Herceptin resistant SKBR3 cell lines. Our preliminary findings provide support for a novel approach using isoflavonoids in the development of targeted therapy for Her2+ BC.

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"Effect of Combinatory Treatment NPD1 + RvD1 on Behavioral After Experimental Stroke In Aged Male Rats"

Objective: The percentage of elderly patients who have stroke is significantly higher than that of young patients who have stroke. Therefore, it is important to study the effect of new treatments on neurobehavioral outcome in aged rats. This study focuses on the neuroprotective bioactivity of docosanoid mediators: Neuroprotectin D1 (NPD1), Resolvin D1 (RvD1), and their combination in experimental stroke. Recently, we have shown that NPD1 and RvD1 are neuroprotective in young male rats, but whether a similar effect occurs in aged male rats is unknown. Behavioral evaluation is important to confirm an initial deficit after middle cerebral artery occlusion (MCAo) surgery, chart progress, correlate with histopathology, as clinically relevant outcome measure, and to identify animals that need to be excluded from our study.

Methods: Aged male Sprague-Dawley rats (500-700g) were anesthetized with 1-3% isoflurane, 70% nitrous oxide, and 30% oxygen, mechanically ventilated, and subjected to 2h of MCAo by poly-L-Lysine-coated intraluminal suture. The composite neuroscore comprises six different neurological tests: postural reflex, visual forward placing, visual sideways placing, tactile dorsal placing, tactile lateral placing, and proprioceptive placing, which were evaluated on days 1, 2, 3, 5, and 7. The total neurological score could range from 0 to 12, with 0 being the normal score, and 12 being the maximal deficit. The individual scores ranged from 0 to 2, with 0 being the normal score, and 2 being the maximal deficit for each test. Rats were perfused on day 7, and their brains were sent to UCI for MRI imaging.

Results: Physiological variables were stable and showed no significant differences between groups. Combinatory treatment with NPD1 +RvD1 improved neurological variables on days 1, 2, 3, 5, and 7 compared to the vehicle.

Conclusion: Combinatory treatments with NPD1 plus RVD1 significantly improved neurological scores during 7 days survival period. Combinatorial treatment by NPD1 plus RvD1 affords synergistic neuroprotection in the post-ischemic brain when treatment is administered at 3 h after stroke onset. We are currently exploring the cell-specific and molecular mechanisms involved. These findings open avenues for ischemic stroke therapeutics.

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Development of a Smart Dual Acting Drug Delivery System (SDADDS)

BACKGROUND: The present-day challenge of delivering anti-cancer agents selectively to tumor cells to mitigate systemic toxicity has led to greater focus on drug delivery research using nanoscale carriers. Despite progress in preclinical studies, the therapeutic effects have not lived up to their expectations in the clinical setting. Though promising, these systems typically exploit passive delivery of a single therapeutic to the target tissue, for example, by the encapsulation of drugs in carrier systems followed by drug release under an external trigger. Our project addresses this issue through the design and synthesis of a Smart Dual Acting Drug Delivery System (SDADDS) consisting of monodisperse bifunctional nanocarriers capable of synergistic targeting of multiple drivers of cancer thereby overcoming current limitations to treating cancer. Triple negative breast cancer (TNBC), accounts for 10-15% of all breast cancers. TNBC is a multidriver disease that grows sporadically due to it having no selective actionable dominant target. This has caused no target therapy to be approved, thus making it an excellent model to explore the efficiency of SDADDS.

OBJECTIVES: This study aims to utilize two different modes of cellular targeting synergistically that would not only offer superior therapeutic *selectivity for tumor tissues*, but would also decrease chemotherapeutic toxicity due to *reduced drug dosage*. The SDADDS in theory should both target the overexpressed TAM receptors on tumor cells and deliver the therapeutic through nanomaterials to increase the bioavailability and decrease chemotherapeutic toxicity.

METHODS: The strategy for developing the proposed model is to first start by proving that the polyvalent targeting inhibitors on dendron A are efficiently able to attach to the overexpressed TAM receptors. In addition to efficiently attaching, they must be able to selectively bind to TAM(+) breast cancer cells over and TAM(-). Currently, we are synthesizing a fluorescent dendrimer to be functionalized with the TAM inhibitor for cellular binding and time lapse studies. We performed a two part synthesis, deprotection of the dendron with TFA followed by attachment of FITC dye to the deprotected dendron with TEA.

RESULTS: The fluorescent dendron was synthesized via a two-step process. First, the boc-protected terminal amines were deprotected using TFA and the product was confirmed by ^{1}H NMR and MALDI-ToF. The deprotected intermediate product was then conjugated with the fluorescent dye, FITC. The reaction was monitored via MALDI-ToF and showed successful quantitative attachment of FITC to both arms of the deprotected dendron. Product purification was attempted using size exclusion but was unsuccessful with no product being isolated.

CONCLUSION:The synthesis of the modified TAM inhibitors is underway and will be attached to dendron A once completed. We are in the process of developing purification methods for the dye conjugated fluorescent dendron which will ultimately be attached to dendron A using click chemistry and subsequently used in cellular binding assays.The long term objective is to make the system customizable so that it can target varying pathways that occur in different cancer type, thus allowing for the creation of personalized treatment for late-stage cancer patients.

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"Exploring the Mechanisms of Anticancer Agents with Improved Solubility Against Triple Negative Breast Cancer"

Triple-negative breast cancer (TNBC) presents significant challenges in treatment and prevention due to its aggressive properties, lack of actionable targets and high rates of resistance. A hallmark of TNBC is its dependence on aberrant glycolytic pathways, characterized by the Warburg Effect, where cancer cells downregulate oxidative phosphorylation in the mitochondria and preferentially produce energy through aerobic glycolysis in the cytosol. This reliance on aberrant pathways offers an opportunity to target TNBC with novel small molecular inhibitors. The Shen Lab investigates mitochondrial and metabolic targets for TNBC to develop innovative anticancer small molecules from synthetic compounds and natural products.

Oridonin, a natural diterpenoid derived from the Eastern herb Rabdosia rubescens, is known for its anticancer and anti-inflammatory properties. While these qualities are ideal for potential cancer therapies, oridonin has not been utilized in clinical settings due to its low potency and limited bioavailability. Our lab has previously developed a highly soluble derivative of oridonin, CYD0618, that has increased potency against breast cancer in both cell culture experiments and xenograft mouse models. In parallel studies, our lab also works on niclosamide, an FDAapproved synthetic anthelmintic drug that has promising anticancer effects but similar limitations like low potency and limited solubility in the gut. To address these issues, we developed a niclosamide derivative, HJC0152, that exhibits improved water solubility, bioavailability, and anti-proliferative effects against TNBC. While both CYD0618 and HJC0152 have improved potency and bioavailability compared to their parent compounds, the mechanisms of action of these derivatives against breast cancer remain understudied.

In this project, we evaluated the effects of CYD0618 and HJC0152 on TNBC viability and metabolism using MTT assays, ADP/ATP ratio measurements and Seahorse analysis. We determined that HJC0152 inhibits both glycolysis and oxidative phosphorylation but CYD0618 may target other oncogenic processes that are not related to glucose metabolism. In the future, our lab plans to identify the direct targets of both compounds using proteomics-based approaches like degradation-based protein profiling. The results of this project contribute to understanding the mechanisms of promising anticancer agents and provide a foundation to optimize these compounds for future clinical testing.

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"In Vitro Comparison of Oral Squamous Cell Carcinoma, HPV-positive and HPV- negative"

Oral squamous cell carcinoma (OSCC) is a type of skin cancer that affects the oral cavity. It is characterized by red or red and white, rough lesions. The etiology of OSCC includes use of tobacco products, heavy alcohol usage, and natural and artificial sun exposure over a significant amount of time. Human papillomavirus (HPV) is one of the most common sexually transmitted diseases. HPV is spread through skin-to-skin contact. While HPV has many strains that affect the reproductive systems, this infection can also develop in the mouth and on the tongue. HPV can potentially lead to the development of OSCC. Screening for OSCC for HPV-positive and HPV-negative is now becoming standard practice, and recent studies suggest that patients that are diagnosed with HPV-positive OSCC have a better prognosis than patients diagnosed with HPV-negative OSCC. Based on these current trends, it is important to investigate the differences between OSCC HPV-positive and HPV-negative for future treatments and disease prevention. To observe these differences, the studies compared two OSCC cell lines: HPV-negative Cal-27 cells and HPV-positive SCC090 cells. Based on current National Cancer Institute data on OSCC HPV-negative and OSCC HPV-positive prognosis, it was hypothesized that HPV-negative cell line Cal-27 would confer a growth advantage when compared to HPV-positive cell line SCC090. Qualitative and quantitative experiments were conducted over three days that included observation of cell growth morphology, cell counting, proliferation, and wound-healing assays.

Cal-27 and SCC090 cells were seeded at different densities in six and 96 well plates. Morphology observations and cell counting growth curves were recorded from six-well plates seeded at $5x10^5$, $1x10^6$, and $2x10^6$ over a three-day period. A MTT proliferation assay was performed on cells seeded at a $2.5x10^5$ density. The wound healing assay was performed in six-well plates with cells seeded at a $1x10^6$ density. The motility rate was recorded over a 24-hour period for each cell type. The results showed that the SCC090 cells grow in a mound-like form, and after three days at a higher cell density, they appeared to begin to grow on top of each other; in contrast, the Cal-27 cells maintained a monolayer confluency. The Cal-27 cells had a significant growth increase compared to SCC090 cells after three days, and after 24-hours, the Cal-27 cells had a significantly greater rate of motility than SCC090 cells.

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"Analysis of the Feasibility of the Pediatric Initiative Network (PIN) Cancer Risk Assessment System in Increasing Fertility Preservation Rates amongst Adolescent, Young Adult (AYA) Cancer Patients in Louisiana"

Background: With growing rates of survival amongst Adolescent and Young Adult (AYA) cancer patients due to innovations in chemotherapy, radiation, and stem cell transplantation, providers aim to provide preventative measures to improve fertility options from diagnosis to survivorship. Innovations in chemotherapy, radiation therapy, and stem cell transplantation have increased risk of gonadotoxic effects leading to infertility for AYA cancer patients. Thus, the American Society of Clinical Oncology and American Society of Reproductive Medicine created the Pediatric Initiative Network (PIN) cancer risk assessment systems for providers a comprehensive diagnostic and treatment information and guidelines for fertility preservation (FP) for AYA cancer patients. Based on diagnostic and treatment modality information from the PIN system, patients are categorized as; significant increased risk of infertility, minimally increased risk of infertility, or high level of increased risk of infertility. For the purpose of this study, oncology providers (pediatric oncologist, adult oncologist, radiation oncologists, surgeons, urologists and gynecologist oncologist) at the University Medical Center of New Orleans in New Orleans were provided fertility preservation education and resources for AYA cancer patients and families with significant and high levels of increased risk of infertility.The study examined barriers of fertility preservation within minority AYA cancer patients and examined fertility preservation referral rates to improve options from diagnosis to survivorship.

Objective: The aim of the study is to analyze how many AYA patients had the PIN system documented in their medical record and were referred for fertility preservation at time of diagnosis.

Methods: As part of the pilot data, a retrospective cohort study of AYA pediatric hematology oncology patients received care at Children's Hospital New Orleans between 2021-2023 and were examined for documentation of the PIN risk stratification system and referral rates for fertility preservation. A chart review was conducted to extract patient demographic information, cancer treatment modalities and fertility preservation referral rates and modalities.

Results: Data analysis is ongoing.

Discussion: Based on the study of the feasibility of the PIN system for AYA cancer South Louisiana, thus far, providers discussion on fertility preservation can emphasize the need of preventive measures from early onset of diagnosis. Further data analysis is needed to conclude the effectives of the PIN system for these patients.

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"Characterization of a Novel Marker for Neuronal Inhibition"

BACKGROUND: For many years, molecular markers such as Fos have been used to represent neuronal activation, and these markers have been instrumental in physiological research, specifically in the mapping of neuronal circuits. However, no equivalent marker existed for neuronal inhibition until 2023, when the phosphorylation of pyruvate dehydrogenase (pPDH) was identified as having an inverse correlation with action potential firing intensity in primary neurons. This relationship allows for the immunostaining of pPDH by monoclonal antibodies to act as a detection method for inhibition across the brain in in vivo mouse models. pPDH is a revolutionary tool in physiological research, allowing for the recognition of inhibitory pathways that were previously undetectable.

OBJECTIVE: This project explores this novel marker's ability to detect neuronal inhibition caused by various modalities including exposure to drugs known to cause profound inhibition in the brain such as isoflurane and alcohol, agonists of Kappa Opioid Receptors (KORs) which are inhibitory G-Protein coupled receptors, and social defeat stress (SDS). Our lab is interested in understanding neural mechanisms by which repeated SDS leads to increased alcohol consumption. The current working hypothesis is that SDS leads to activation of Dynorphin (Dyn) release from dorsal raphe Dyn neurons into the bed nucleus of the stria terminalis. The released Dyn then binds to KORs located on basolateral amygdala terminals in the BNST leading to neuronal inhibition. Here, we attempt to visualize this inhibition in BLA neurons after SDS and after systemic injections of a selective KOR agonist, U50,488.

METHODS: Mice were exposed to one of four conditions: exposure to the general anesthetic isoflurane, intraperitoneal (i.p.) injections of alcohol (2 g/kg), U50,488 (a KOR agonist, 10mg/kg), and exposure to repeated (10 sessions) brief SDS. Mice were sacrificed 30 minutes post exposure to these stimuli. Mice were perfused and brains were postfixed in paraformaldehyde followed by sucrose and 40μm thick sections were prepared. Sections were then processed for IHC to detect pPDH and cFos.

RESULTS: Exposure to isoflurane as well as systemic administration of alcohol (2g/kg) led to robust pPDH activation in many brain regions including the basolateral amygdala and several hypothalamic nuclei. Both alcohol and isoflurane potently activate GABA receptors in the brain and lead to profound inhibition, which is consistent with our data. However, systemic administration of U50,488 and SDS did not lead to significant changes in pPDH staining throughout the brain. Both U50, 488 and SDS lead to transient inhibition which may be difficult to capture with this method. Future studies will examine the inhibitory landscape in the brains of mice subject to voluntary binge alcohol consumption. In summary, our results indicate that pPDH is a useful tool to visualize strong patterns of neuronal inhibition mediated by alcohol and anesthetic exposure.

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HOW ENVIRONMENTAL RISK FACTORS DRIVE RACIAL DISPARITIES IN PROSTATE CANCER STAGES AT DIAGNOSIS

Introduction: Prostate Cancer is the most common cancer among men, contributing approximately 240,000 incidence cases per year that result in an estimated 28,000 deaths among US men mainly ages 65 or older.[1,2] Studies show that socioeconomic, environmental, and dietary factors are related to the racial disparities in prostate cancer outcomes between African American (AA) and Caucasian men (CA).[3] AA men suffer disproportionally more prostate cancer than their CA counterparts. AA men account for 73% higher rates of incidence and more than double the rates of mortality in comparison with CA men.[2] Increased rates in AA men come as their diagnosed at younger ages, with more aggressive tumors, advanced stages, and less effective treatments and poorer outcomes.[2,3] In literature, previous studies examined how racial disparities in prostate cancer such as diagnosis, treatment, and survival are affected by the socio/environmental risk factors like the social determinants of health and chemical exposures.[1-4] In this study, we investigate if environmental risk factors can be seen as potential mediators in the racial disparity of prostate cancer diagnosis, while also quantifying the specific variables of risk towards the observed disparity in prostate cancer stage.

Method: In our cross-sectional study design, a total of 24,647 AA or CA male patients who were diagnosed with prostate cancer between 2010-2018 in LA were included in this study. Among them 15,875 (64.40%) were white and 8,772 (35.59%) black. The research is based on data collected by Louisiana Tumor Registry from 2010-2018. This was merged with 2010 census tract level data along with CDC and Prevention and Agency for Toxic Substances Disease Registry's 2022 Environmental Justice Index data. The study compared the outcome variable (Stage at Diagnosis) against the predictor/exposure variable (AA vs CA patients) to quantify the potential socio/environmental mediators used (Marital Status, Insurance, Poverty, CDI, Ozone, Comorbidity, AceTot, Coal, TOTCR, etc). Using R studio, we first performed descriptive statistics on our data using Chi-Square, T-statistics, and Anova tests, and then conducted a Multiple Mediation Analysis (MMA) to determine essential factors that explain the observed racial disparity in stages.

Results: There is significant racial disparity in prostate cancer diagnosis stages (P-value=0.001). The included variables completely explained the observed disparity as the direct effect became insignificant (DE=-0.21, 95%CI (-0.034, 0.017)). We found the following significant mediators and corresponding relative effects: BMI (35.9%), marital status (31.9%), insurance (7.3%), poverty indicator (0.2%), comorbidity index (4.9%) along with environmental variables like proportion of population with asthma (7.1%), proportion of tract's area within 1-mi buffer of EPA risk management plan site (2.4%), railroad (1.6%), percentage of houses built pre-1980 (lead exposure) (0.9%), walkability (0.3%), and lifetime continuous toxicity exposure (7.3%).

Conclusion: Potential environmental risk factors were seen to have moderate influence (< 20%) towards the impact on explaining observed racial disparity in prostate cancer diagnosis. Factors of social and health related risk however provided substantial impact (> 80%) towards observed racial disparities in prostate cancer diagnosis. From this, future research should

continue to further investigate environmental risk factors in explaining the racial differences in prostate cancer outcomes.

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"Characterization of Voltage-gated Sodium Channels of Oral Squamous Cell Carcinoma HPV-positive and HPV-negative Cell Lines"

Metastasis, the process by which cancer cells spread from a primary tumor to distant sites in the body, is a hallmark of cancer and a leading cause of cancer-related mortality. The multi-step process involves local invasion, intravasation into blood or lymphatic vessels, survival in the circulatory system, extravasation into new tissue, and colonization to form secondary tumors. Each stage is regulated by molecular and cellular mechanisms, including changes in cell adhesion, degradation of extracellular matrix, and interactions with the tumor microenvironment. Research on molecular pathways is important for the development of biomarkers and pharmacological treatment targets. Recent advances in molecular biology and imaging technologies have elucidated key pathways and molecules involved in metastasis and upregulation of voltage-gated sodium channels (VGSC) in cancer cells. The upregulation of VGSC expression levels has been detected in different cancer types where channel activity has been associated with a variety of cellular behaviors integral to the metastatic cascade. Current findings from our research have showed distinct expression patterns of sodium channel subtype Nav 1.5 in a highly metastatic cancer cell line, MDA-MB-231. Investigating similar protein expression patterns in other cancer types can help to gain a better understanding of the role that sodium channels play in metastatic cancers. We therefore tested oral squamous cell cancer (OSCC) cell lines. There are many factors affecting the prognosis of oral cancer such as the stage of the cancer, where the tumor is located and if the patient has tested positive for human papilloma virus (HPV). Since HPV can play a factor in prognosis, we performed immunofluorescence an HPV- positive and HPV- negative cell lines to compare sodium channel expression patterns. Cell lines SCC090 HPV-positive and Cal 27 HPV-negative cells were seeded on coverslips in a six well plate and fixed. We performed indirect staining using primary antibodies, anti-Pan-VGSC (1:400 dilution) and anti-NaV1.5 (1:200 dilution), secondary antibody Alexa 488 (1:1200 dilution), and DAPI nuclear counter stain. Slides were imaged on a Nikon Eclipse Ti2 microscope with a 40x objective. Our results showed that both OSCC cell lines do express VGSCs and there is an observable difference in NaV1.5 expression patterns when Cal 27 and SCC090 are compared. Understanding VGSCs' role in cancer cells has significant clinical implications, from the development of biomarkers to novel therapeutic targets for the improvement of prevention and treatment.

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"IL4 and IL6 induce pro-survival reactivity and ALOX12 activation in human astrocytes."

Parkinson's Disease (PD) hallmark is the death of dopaminergic (DA) neurons in the Substantia nigra pars compacta (SNpc), a structure of the midbrain that is crucial for modulating the initiation of motor movement, among other specific cognitive and emotion processing functions. Astrocytes, major players in energetic neuronal support, synaptic maintenance as well as in ion and neurotransmitter homeostasis, contribute to the etiology of PD either by gain of toxic function or loss of survival support for the DA neurons. There is an inflammatory component in the PD pathology, with activation of microglial cells and astrocytes noticed in postmortem brains. Maresin 1 (Mar1), a bioactive lipid and derivative of Docosahexaenoic acid (DHA), is a signaling molecule that has been shown to exert its anti-inflammatory function on reactive microglial cells. Mar1 is synthesized by ALOX12, an enzyme expressed in astrocytes, neurons, and microglia. We hypothesized that astrocytes up-regulate and release Maresin-1 in response to certain cytokines to promote homeostasis. To test this hypothesis we exposed rat astrocytes in culture to cytokines (TNFα, IFNγ) in the presence or absence of Maresin-1 and recorded the nuclear translocation of NFkB/p65, a pro-inflammatory transcription factor; we measured the expression of markers of HMGB1, a stress marker, and IL6 and the activity of ALOX12, an enzyme in the synthetic pathway of Mar-1 in the presence of two interleukins 4 and 6, that were proposed to activate biosynthesis of other pro-survival factors in astrocytes. We found that TNFα and IFNγ induced the activation of NFkB/p65 and Mar-1 prevented the transcription factor nuclear translocation. In addition, IL4 increased the transcription of IL6 which induced the activation of ALOX12, leading to elevated production of 14-HDHA and 12-HETE from DHA and Arachidonic acid, respectively. These results suggest that IL6 is a cue that induces survival reactivity by activating the secretion of Mar-1 which promotes anti-inflammatory effects in astrocytes. In future studies, we will determine the mechanisms by which IL6 induce activation of ALOX12 and if that counteracts the inflammatory effects of TNFα and INFγ.

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"Validation and characterization of *Lphn3* gene deletion in a mouse model of ADHD"

Background: Attention deficit hyperactivity disorder (ADHD) is a highly heritable neurodevelopmental disorder characterized by deficits in attention, hyperactivity and impulsivity. Previous studies have found that ADHD is a major risk factor for addictive behaviors, such as alcohol use disorder (AUD). Individuals with ADHD have been shown to initiate alcohol drinking earlier and more robustly in adolescence than their non-affected peers which also accelerates the development of an AUD. AUDs are also highly heritable and in fact ADHD and AUDs share several genetic risk factors including the variants in the *LPHN3* gene. *LPHN3* encodes a cell adhesion G-protein coupled receptor (GPCR) known as latrophillin-3 which has a prominent role in forming and maintaining glutamatergic synapses. Constitutive deletion of *Lphn3* in rats and mice leads to impulsivity, attentional deficits, and hyperactivity compared to their wildtype littermates and is considered a leading ADHD preclinical model. We are interested in the relationship between ADHD and AUD and are assessing the effectiveness of neuronal and brain-region specific deletion of *Lphn3* as a model of ADHD and for studying the interaction of ADHD and alcohol. We hypothesize that neuron specific deletion of *Lphn3* using synapsin-Cre strategy results in reduced *Lphn3* transcript in neurons and behavioral changes that include increased hyperactivity, cognitive deficits, and increased alcohol consumption. Gaining insight into LPHN3's role in neurological functioning could lead to a greater understanding in the relationship between ADHD and AUD.

Methods: To obtain a pan-neuronal knockout of *Lphn3*, we crossed Synapsin-Cre mice with floxed *Lphn3* mice. We used fluorescent in situ hybridization (FISH) to determine if transcripts to *Vglut1* and *Lphn3* were present in our area of interest, the prefrontal cortex (PFC), and if *Lphn3* transcription was reduced in *Vglut1* neurons containing Cre recombinase compared to wildtype littermates. *Lphn3* wildtype (WT), heterozygous (HET), and mutant (MUT) conditional KO male and female mice were tested on behavioral task: elevated plus maze, open field, novel object recognition task, object in place recognition task, and Y-maze). *Lphn3* WT, HET, KO were also given access to alcohol during adolescence (PND30 to 60) using an intermittent 2-bottle choice method and alcohol dose consumed and alcohol preference were evaluated.

Results: Our FISH results indicate uninterpretable results related to genetic deletion of *Lphn3* in neurons and that troubleshooting of RNAscope procedures is necessary to evaluate our collected tissue. Behavioral results indicate behavioral changes in *Lphn3* KO and HET mice consistent with ADHD behaviors (increased hyperactivity, reduced recognition memory). Alcohol consumption and preference were not statistically different amongst genotypes.

Conclusion: Our pan-neuronal deletion of *Lphn3* leads to observable differences in behavior consistent with an ADHD phenotype, for instance increased locomotor activity and increased novelty seeking behaviors, however alcohol consumption during adolescence was not affected. Our proof-of-principle, FISH data are inconclusive and require further work improving our methodologies.

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"Predictors of Reunification for Children and Their Parents in Cases Involving Parental Substance Use in Louisiana"

Background: Substance abuse significantly inhibits a parent's ability to care for their children, with an estimated 50-80% of children in foster care having a minimum of one parent with a history of substance abuse [2]. These children spend more time in foster care, have lower reunification rates, and have greater rates of parental rights being terminated [5]. Research has found that a parent's completion of substance use treatment is a positive predictor for family reunification [3,4] and that two-parent households reunify more quickly [1]. Poverty in the family, along with the age and race of the children, have all been found to predict parental outcomes [1]. However, there is a scarcity of research regarding the demographics of parents with substance use disorders (SUDs) who reunified with their children.

Methods: Using data from the LSUHSC Infant Team, a Chi-Square test was used to test the hypothesis that a parent's SUD would be negatively associated with the rate of reunification, and a Binary Logistic Regression test was used to test whether White parents, older aged parents, employed parents, and partnered parents would have higher odds of reunification.

Results: Of the total of 178 fathers, 125 fathers had SUDs, and of these fathers, only 16 reunified with their children (13%). There was a statistically significant association between fathers' substance abuse problems and reunification. Further analysis found that there was not a significant association between the demographic variables and reunification for fathers with SUDs, but father's employment was approaching significance.

Of the total of 387 mothers, 247 had SUDs, and of these, 28 (11%) reunified with their children while 219 (89%) did not reunify, which was found to be statistically significant. Further analysis found higher rates of reunification for employed and older mothers with SUDs. Race and relationship status were not found to be significant predictors for reunification in this subsample.

Discussion: Substance use disorders are negatively associated with reunification for parents with children in foster care. When observing the commonalities in mothers who reunified, older (30+ years) and employed mothers had 3 times and 12 odds higher of reunification than younger or unemployed mothers, respectively. Accurate generalizations about fathers with SUDs cannot be made due to the small subsample size. Racial identity and relationship status were not found to be significant predictors of reunification for mothers with SUDs, but this may be due to the data's limited racial diversity (73% African American) and the limited number of mothers who were married or partnered (n=3).

Implications: Identifying commonalities between sub-groups of parents with SUDs who reunified provides important implications for clinical practice. A larger sample size may be helpful in better understanding the demographic factors that increase reunification for parents with SUDs. Targeted support for parents can then aid in increasing the likelihood of reunification, especially those that target educational and employment opportunities. The hope is that by paying closer attention to these demographics and providing extra assistance where needed, we can increase the likelihood of reunification for cases involving parental SUDs.

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"Establishing a 3-D multicellular Tumor Model to assess the impact of fatty acids activated immunosuppressor cells on Breast Cancer spheroid growth"

Background and Hypothesis: Studies have shown that post-menopausal women who are obese have a 20-60% risk of developing breast cancer compared to women with a healthy weight (body mass index < 25). Obesity is characterized by metabolic dysfunction, including excess of free fatty acids (FFAs) in circulation and low-grade chronic inflammation. Myeloid-derived suppressor cells, or MDSC, are immature myeloid cells that exhibit unusual behavior of immunosuppression associated with tumor immune evasion, promotion of angiogenesis and metastasis in cancer. It has been shown that the immunosuppressive function of MDSC is enhanced by the metabolism of lipids such as FFAs; however, it has not yet been explored the effect of FFAs on MDSC in facilitating tumor growth independently of their immunosuppressive features. We **hypothesized** that the FFAs Oleic (OA), Palmitic (PA) and Linoleic (LA) increase the expression of S100A8 and MMPs on MDSC and are associated with increasing the spheroid size of the mouse Luminal B cell line EO711.

Methods: To test this hypothesis, we induced mouse bone marrow-derived MDSC (mBM-MDSC) from bone marrow cells and cultured in presence of recombinant cytokines IL-6, GM-CSF, and G-CSF for 4 days. Exposure to the different FFAs, or bovine serum albumin (BSA) as the FFAs carrier control, was performed after 24h of initial induction of MDSC during the last 72h. After culture, the cells were collected and used for cell extract and for the coculture with 3D EO711 spheroids. Western blot was performed for MMP9, S100A8, and Arginase 1. To perform the multicellular 3D spheroid assay, EO711 cells expressing green fluorescent protein (GFP) were cultured in a 96-spheroid microplate. After spheroid formation for 3 days, the mBM-MDSC were added. Spheroid growth was followed for a 10-day period.

Results: Arginase 1 (Arg1), a marker of MDSC activation, was significantly affected by the FFAs. While OA and LA significantly increased the Arg1 protein expression, PA downregulated its expression. Contrary to Arg1, the expression of MMP9 and S100A8 were not dramatically influenced by the FFA treatment. Interestingly, coculture of EO711 with mBM-MDSC treated with PA, but not with OA or LA, did increase the size of EO711 spheroid. From the Spheroid Assay model, we learned that adding fresh media in the middle of the culture time disturbed the spheroids. Also, wells at the border of the plate should not be used for spheroids, instead for adding media to protect evaporation from following wells containing spheroids.

Conclusions: OA, LA, and PA have a different effect on MDSC features. PA decreases the expression of Arg1, a marker of immunosuppression, but enhances the ability of MDSC to increase tumor growth by undefined mechanisms. S100 proteins and MMP9 should be evaluated in the supernatant since no changes were observed at intracellular level of MDSC. Other factors derived from MDSC such as cytokines should also be analyzed. Additional optimization of the 3D system is needed including lower number of seeded cells (from 10,000 to 5,000) to avoid disturbances in coculture by adding media during the culture time.

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"Proteomics Analysis of PDAC and Pancreatitis Plasma Samples: Investigating Associations and Differences"

INTRODUCTION: Pancreatic cancer is currently the third-leading cause of cancer death in the United States. Roughly 90% of pancreatic cancer cases are pancreatic ductal adenocarcinomas (PDACs) and a vast majority present as advanced-stage with a 5-year survival rate of only 11%. Thus, early detection of pancreatic cancer is currently a crucial, yet challenging, goal. Having similar risk factors, pancreatitis has been associated with and is suggested to be an early manifestation or significant risk factor of PDAC. Therefore, comparisons between protein expression in PDAC and pancreatitis may lead to valuable insight into how the two are connected and the discovery of important biomarkers. Identified significantly expressed proteins and enriched pathways could be utilized or targeted in the diagnosis and treatment of pancreatic cancer.

METHODS: 30 human plasma samples from 3 different groups were randomized, prepared, and analyzed under standard protocol for extraction of proteins from plasma for peptide-based liquid chromatography-mass spectroscopy (LC-MS). 10 were collected from (1) a healthy control group with no diagnosis of pancreatitis or PDAC, 5 from (2) a group presenting with pancreatitis but no diagnosis of PDAC, and 15 from (3) a group diagnosed with PDAC. Label-Free Quantification (LFQ) intensity data collected from LC-MS for each sample group was processed and gap-filled using MaxQuant and Perseus. Pairwise comparisons (PDAC vs. Controls, PDAC vs. Pancreatitis, Pancreatitis vs. Controls) were performed using Student's T-Test to determine which proteins were significantly expressed across each pair. Significantly expressed proteins for each pair were mapped and analyzed using pathway enrichment analysis through Ingenuity Pathway Analysis (IPA) and Kyoto Encyclopedia of Genes and Genomes (KEGG).

RESULTS: LC-MS returned LFQ intensity data for 439 proteins across all 3 groups. Student's T-Test returned 27, 17, and 21 significantly expressed proteins that could be mapped on enriched pathways using IPA – between PDAC vs. Controls, PDAC vs. Pancreatitis, and Pancreatitis vs. Controls, respectively. Of the top 5 enriched pathways between PDAC vs. Controls and Pancreatitis vs. Controls, there were 4 common pathways, such as the Formation of Fibrin Clot (clotting cascade) pathway. The DHCR24 Signaling Pathway was also a top 5 canonical pathway between PDAC vs Controls and the Extrinsic Prothrombin Activation Pathway between Pancreatitis vs Controls.

CONCLUSIONS: As one of the leading causes of cancer deaths in the U.S., pancreatic cancer, mainly pancreatic ductal adenocarcinoma (PDAC), has a relatively low survival rate, which is only exacerbated by late diagnoses. Pancreatitis not only shares risk factors with PDAC but also may be an early indicator or risk factor for PDAC. In this study, LFQ-intensity data of plasma samples from healthy controls, pancreatitis patients, and PDAC patients were used to identify significantly expressed proteins and enriched pathways between sample groups. Further analysis of the identified significantly expressed proteins and enriched pathways may reveal potential biomarkers for early detection of PDAC by studying PDAC versus Pancreatitis.

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"The effects of EM1 on LPS activated microglia"

Purpose: Through the circulatory system gut dysbiosis commonly observed in obesity, hypertension, and aging. In this study, we determined whether a novel exercise metabolite (EM1) would attenuate LPS-induced activation of mouse microglial (BV2) cell line.

Methods: BV2cells were cultured and treated with 1µg of LPS (for activation) in addition to EM1 in different concentrations (0.1, 0.3 or 1 µM) for 24 hrs. The cell viability test was done to assess the effect of EM1 on the live/dead population of BV2 cells. Immunofluorescent cytology and micrograph analyses were performed to quantify the population of proinflammatory/activated (M1) microglia IBA1+MHC-II) and anti-inflammatory/ non-activated (M2) microglia (IBA1+CD206).

Results: EM1 at 1 µM significantly decreased ($p<0.05$) the number dead cells while and there was no difference in the number of live cells between cells. Results of immunofluorescent cytology are underway (and will be ready by Thursday this week).

Conclusion: We expect to find that EM1 at 1 µM will decrease LPS-activation of BV2 cells as well as improve their viability. If EM1 decreases LPS-activation of BV2 cells, the next logical step will be to investigate its effect on microglia activation, cognition, and mood-associated behaviors in an model of gut dysbiosis.

Keywords: BV2, LPS, Microglia, Immunofluorescence, inflammation

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"Evaluation of PFC-MdT circuit deletion on behavioral measures of cognition and alcohol consumption"

Alcohol Use Disorder (AUD) is a medical condition that has harmful effects on individual health and society. AUD is characterized by an impaired cognitive ability to stop or control alcohol use despite its reoccurring negative consequences. Chronic alcohol consumption creates a dangerous negative feedback cycle by compromising brain structure, resulting in executive dysfunction, and increasing the likelihood of continued alcohol preference and consumption. The prefrontal cortex (PFC) structure and function is critically connected with the cognitive processes often disrupted by alcohol consumption. AUDs have a higher likelihood of developing in adolescence, especially when the age of onset is early adolescence because of alcohol's impact on underdeveloped PFC neural circuitry. One circuit the PFC is intimately in communication with is the mediodorsal thalamus (MdT) which regulates impulsivity and cognitive control. We have previously found that mice given voluntary access to alcohol during their adolescence have behavioral deficits consistent with local PFC and MdT lesions on the objection in place recognition task. However, there is a lack of causal understanding on how this circuit specifically affects these same behaviors and impacts alcohol consumption in adulthood. Therefore, we hypothesize that deletion of the PFC->MdT shows a similar behavioral profile to adolescent alcohol and that it increases alcohol consumption during adulthood.

We tested this hypothesis by deleting the PFC-> MdT circuit through retrograde-AAV techniques and Cre/Lox systems, so PFC to MdT projecting neurons expressed caspase-3 versus control (red-fluorescence-protein to green-fluorescence-protein reporter). Because executive dysfunction is associated with alcohol preference, we used a voluntary intermittent access to alcohol, with increasing dosages, and an every-other-day drinking model in adolescent male and female mice to investigate whether disrupted PFC to MdT connection brings about altered behavioral preference to alcohol. Our behavioral data yielded unexpected results, as we observed reduced performance in PFC->MdT caspase-3 mice on the novel object recognition task, but increased performance on object recognition task and Y-maze. In addition, we did not observe differences in alcohol consumption between groups following increasing alcohol concentration, but we did observe increased alcohol consumption and blood alcohol levels in control mice compared to PFC->MdT caspase-3 mice. We analyzed and confirmed the virus's targeting using imaging of control viral expression red fluorescent protein (virus transduction) and green fluorescent protein (Cre-recombination). Our data leads us to a better understanding of how PFC function via the PFC->MdT pathway is affected by circuit deletion strategy. Further study can work towards better understanding these cognitive pathways involved with alcohol so that these circuits can ultimately be enhanced as a form of treatment and recovery in cases of AUD.

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"Do children who experience neglect differ significantly from children who experience other traumas relative to developmental delays?"

Background: Child Neglect, defined by Child Welfare (Department of Children & Family Services, 2024) is any inaction or action of a caregivers to meet their children's basic needs. Child neglect can significantly prevent children from achieving milestones crucial for their overall well-being by impacting socio-emotional development (Peterson, 2014). It can also lead to poor neurodevelopmental problems, including communication delays (Passmore, 2022). The current study aimed to examine whether children's experiences of neglect impacted their development within a sample of children who had been in the foster care system.

Methods: Using data from the LSUHSC Infant Team, 334 children in foster care were analyzed using the Pearson Chi-Square Test to test if neglected children had higher rates of developmental delay compared to others in the sample. Later, a subsample was analyzed using the One-Way ANOVA Test to explore the hypothesis that neglected children would score lower on a developmental screener, Deveraux's Early Childhood Assessment (DECA) compared to children exposed to other traumas.

Results: The first analysis showed no significant association between the type of trauma that a child experienced and the child presenting with developmental delays. Within the subsample, the One-Way ANOVA found a significant difference between the three groups. The Tukey post hoc test found that children who experienced Neglect had significantly lower DECA scores than children who were Physically Abused, with no statistical difference between any of the other pairings.

Discussion: The first analysis did not yield significant results perhaps because "developmental delays" was treated as a categorical (yes/no) variable. The three traumas analyzed (Neglect, Physical/Sexual Abuse, and Drugs in Utero) perhaps lead to different developmental rates that the analysis did not capture. The second analysis found that young neglected children had significantly lower DECA scores, highlighting neglect's severe impact on development. There was no significant difference in DECA scores between neglected children and those exposed to drugs in utero, perhaps due to home placement timing.

Implications: Prioritizing the prevention of neglect is imperative. Informing families about child development and the dangers of child neglect may aid in prevention. Providing support services for families who struggle with stress, addiction, and mental health issues will help these families

access the resources and assistance to foster healthier environments and relationships with their children.

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"Characterizing Genetic Mutations in Familial Lung Cancer: Insights & Implications"

Lung cancer (LC) is the leading cause of cancer-related deaths worldwide, often linked to smoking tobacco. However, less than 20% of smokers develop LC. It has been shown in literature that there is both an environmental component (smoking) and a genetic component that play a significant role in the development of LC, as indicated by disease aggregation and higher mortality rates in individuals with affected relatives with LC. Identifying genetic mutations is crucial for developing novel therapies to treat previously untreatable diseases. Researchers have identified significant genetic mutations that may be linked to susceptibility to LC in specific populations, yet more research is needed to confirm these findings. The goal of this study is to characterize the genetic mutations in individuals with LC who have a family history of the disease.

The research study of genetics of lung cancer recruited approximately 2,500 individuals with LC from a network of 30 Louisiana hospitals and from multiple states across the country. After screening, 800 participants were confirmed to have at least two cases of LC in their family. Out of those 800 study participants, only 21 people had previous mutation analyses by the certified laboratories, 18 of which received somatic mutation screening and three of which received germline mutation screening. Medical and pathology reports containing mutation analyses of the 21 study participants were obtained from hospitals in addition to demographic and environmental information from the families.

The analyses of the results found that approximately 24% of study participants from these familial LC families tested positive for the EGFR mutation and 0% tested positive for the ALK mutation. The four who tested positive for the EGFR mutation had a higher number of family members affected by LC compared to those who tested negative.

Limited information can be concluded due to the lack of a standardized list of mutations that LC patients are tested for. The mutation screening among individuals with LC helps with determining to administer the most appropriate therapies. This research confirms the findings of previous research, highlighting the need for continued research on the genetics of LC, and the need for a standardized mutation screening panel for all individuals with LC.

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"Mesoamygdala contribution to alcohol withdrawal-associated anxiety"

Background and Objectives: Humans with alcohol use disorder (AUD) often experience anxiety during withdrawal (WD), which is associated with greater risk of relapse. Previous work has demonstrated activation of central amygdala (CeA)-projecting ventral tegmental area (VTA) neurons during alcohol WD in a rat model of alcohol dependence, raising the possibility that this circuit contributes to WD-associated behavior. We tested this by using a chemogenetic strategy to inhibit the VTA-CeA circuit and evaluating anxiety-like behavior during WD.

Methods: We used a dual virus approach to selectively transfect CeA-projecting VTA neurons with an inhibitory DREADD and modeled alcohol dependence using a chronic intermittent exposure (CIE) to ethanol vapor paradigm. Following 4 weeks of vapor exposure, rats were tested for anxiety-like behavior in an elevated plus maze (EPM) during WD. To inhibit the VTA-CeA circuit, DCZ (0.1 mg/kg, i.p.) was administered 30 minutes prior to behavioral testing. Brains were then sectioned to confirm virus placement and stained to confirm inhibition of cells using phosphorylation of pyruvate dehydrogenase (pPDH).

Results: We first assessed whether vapor exposure parameters are related to behavioral outcomes. Average BACs across cohorts were aggregated and used to determine a threshold for inclusion in future studies, defined as the lower quartile (≤130 mg/dL). We applied this threshold to our analysis of rats following chemogenetic inhibition of the VTA-CeA circuit during WD. Preliminary data suggests that inhibition of the VTA-CeA circuit in alcohol dependent rats tested during WD may rescue increased anxiety-like behavior, although this did not achieve statistical significance. To confirm cell type-specific inhibition following DCZ administration, we stained VTA-containing sections for pPDH, a marker of cellular inhibition. We found a greater number of pPDH+, mCherry expressing (i.e., virus-containing) VTA neurons of Gi-expressing rats compared to inactive virus controls.

Discussion: Our data indicate that inhibiting the CeA-projecting VTA circuit may rescue increased anxiety-like behavior associated with alcohol WD. Further tests with a larger data set to perform a full statistical analysis is necessary to verify the current findings. Additionally, future experimentation is necessary to see whether CeA-projecting VTA neurons have collateral projections which may influence behavior. Ongoing work is utilizing a brain clearing and whole-brain imaging strategy to see if CeA-projecting VTA neurons project elsewhere in the brain. If other regions are identified as being involved, these previous experiments can be repeated with site-specific drug and virus administration to test the role of these regions. Our ultimate goal is to better understand the neurobiology underlying alcohol withdrawal-associated increases in anxiety, potentially allowing for improved therapeutic options for individuals with AUD.

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"α-syn PFF Triggers Stress Responses in Human Astrocytes That Induce senescence"

The substantia nigra pars compacta (SNpc) is a structure of the midbrain that is crucial for modulating the initiation of motor movement, among other specific cognitive and emotion-processing functions and is composed of a group of neurons that fire rhythmically at a rate of 2–10 Hz. This characteristic makes SNpc vulnerable to metabolic stress, characteristic that is altered in Parkinson's Disease (PD) patients. In normal conditions, astrocytes sustain neuronal function. Recently, it came to our attention that there is a wide spectrum of phenotypes that astrocytes may acquire depending on the signaling they encounter, but the most striking observation is that astrocytes become progressively impaired under protein-misfolded pathological conditions. We hypothesize that astrocytes exposed to neurons undergoing degeneration related to α-syn aggregates become reactive and Maresin-1 revert this status. To determine the changes in phenotype, we exposed rat and human astrocytes in culture to α-synuclein preformed fibrils (α-syn PFF) in the presence or absence of Maresin-1 and recorded the nuclear translocation of NFkB/p65, a pro-inflammatory transcription factor; the expression of markers of senescence stress and inflammation and the activity of ALOX12, an enzyme in the synthetic pathway of Mar-1. α-syn PFF induced the upregulation of the senescence marker CDKN2B/p15INKB along with the activation of p65 and the increase transcription of HMGB1 and IL1B. Mar-1 addition decreased all four parameters. In addition, the activity of ALOX12 was decreased by α-syn PFF, suggesting that the aberrant form of α-syn may induce not only senescence but also an impairment of the astrocytes to secrete the pro-survival bioactive lipid Mar-1. Altogether, the results point to an induction in the impairment of the astrocytes by the α-syn PFF instead of promoting an inflammatory phenotype. In future directions we will focus on the mechanisms by which α-syn PFF induces senescence and the link between this cellular process and the decrease in the synthesis of Mar-1.

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"Evaluating In Vivo Efficacy of Enzymatic Biofilm Dispersal from Orthopaedic Implants"

INTRODUCTION: Biofilm related implant complications affect 0.5-2% of all orthopaedic procedures, highlighting the urgent need for effective cleaning methods. Recent in vitro studies have shown that bromelain enzymatic debridement can effectively break down biofilms on infected implants, but its efficacy in vivo has yet to be investigated. Through utilization of the nucleic acid stain Sytox Orange, we can assess the bioburden on both infected and uninfected explanted in vivo orthopaedic implants. By fluorescently labeling sessile bacteria in biofilm, this stain allows for a comparison of bacterial burden in vitro as well as in vivo. This study aims to gain insights into bromelain's capability to remove biofilms outside of culture through an in vivo model.

METHODS: Stainless steel bone pins were incubated at 37℃ in tryptic soy broth with 10% fetal bovine serum and inoculated with methicillin-resistant Staphylococcus aureus (MRSA) for 120 hours inside a 12-well plate. Biofilm washed pins were first infected in the same fashion and then soaked in 1000 µg/mL bromelain for 20 minutes followed by phosphate buffered saline (PBS) rinse. Uninfected, infected, or infected and bromelain washed pins were placed in zinc-buffered formalin fixative to demonstrate the in vitro condition of the biofilm. A second set of pins prepared as above were placed into the intramedullary space of adult Sprague-Dawley rats for seven days. After seven days, the pins were explanted and placed in fixative. All pins were washed with PBS after fixation. The pins were stained with Sytox Orange (Thermo) for 10 minutes and then washed with PBS. Subsequently, the pins were mounted on slide spacers with antifade media and mounted on a slide. The pin threads were imaged at 100x magnification using confocal microscopy. The bacterial burden was quantified by size exclusion relative to host cells for explanted pins using SlideBook 5.0 software (3i). Statistical comparison between pins exposed to in vitro or in vivo conditions was done using Prism 10.0 (GraphPad) with one-way ANOVA and $\alpha = 0.05$.

RESULTS: The in vitro infected pins (n=6) exhibited a mean bacterial burden of 560.83 colony forming units (CFU), while the in vivo infected pins (n=3) displayed a higher mean burden of 873.7 CFU (p=0.3944). The pre-washed pins (n=3) had a mean bacterial burden of 819.3 CFU compared to the in vitro bromelain-washed pins (n=4), demonstrating a vastly lower bacterial burden of 96.5 CFU (p=0.0195).

DISCUSSION: The data indicate that while bromelain shows promise as a viable option for cleaning infected orthopedic implants, further exploration beyond in vitro culture studies is necessary. Although the bromelain washes effectively reduced the bacterial burden in vitro, it was insufficient in maintaining low bacterial levels in vivo. Additionally, a larger sample size is required to validate the credibility of our findings. Stricter sterile techniques must be implemented in future research to prevent extraneous contamination.

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"Identifying Dietary Protein Intake Using Fecal Nitrogen Quantification"

INTRODUCTION

Individuals who aim to gain muscle mass often consume more dietary protein, but little is known about its effect on gut microbial metabolic pathways. A previous cross-sectional study from our lab discovered that higher protein intake correlated with increased colonic nitrogen and purine and pyrimidine metabolites. Nitrogen that reaches the colon comes from dietary protein and purines (nucleotide portion of DNA/RNA). The majority of dietary nitrogen comes from dietary protein. The relationship between protein intake and its effects on colonic nitrogen and nucleotide metabolism warrants further investigation. To explore this correlation, our lab is studying the nitrogen content of stool samples collected from healthy individuals before and after increased protein intake. However, fecal matter's complexity and its various components, such as fibers and lipids, make it difficult for nitrogen to be quantified. There are no available methods for quantifying fecal nitrogen content, so we developed a method to ultimately use as a biomarker of nitrogen intake.

PURPOSE

This project aims to develop a biomarker for detecting the nitrogen content in participants' stool samples.

METHODS

The following method was developed and tested. A fecal sample was collected, and a 5g aliquot mixed with 10 ml of distilled water. The sample was quickly frozen in liquid nitrogen and stored in a -80°C freezer until freeze drying. In batches, samples were freeze-dried on a lyophilizer (Labconco FreeZone 6 7753022 Kansas City, MO) for 48 hours. Once dried, the sample was transferred to a stainless-steel milling vial and ground on a Bead Ruptor 96 Cryo (Omni International, Kennesaw, GA). The sample was sent to LSU Agricultural Center (Baton Rouge, LA) for nitrogen analysis using the Dumas method (J AOAC Int. 2007 Jan-Feb;90(1):6-20).

RESULTS

The newly developed method successfully detected nitrogen in fecal matter (39,370 ± 63.68 mg/kg).

CONCLUSION

This newly developed method demonstrates that nitrogen can be successfully detected in fecal matter, suggesting it can be used as a biomarker for dietary protein intake.

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"A Mitochondrial Uncoupler, BAM15, Inhibits Liver Tumor Promotion in the Context of a High Fat Diet Enriched in Saturated Fat"

Liver cancer ranks as the third deadliest cancer globally and is on the rise due to the obesity epidemic. The incidence is higher in males than females. BAM15, a mitochondrial uncoupler, has demonstrated protective effects against weight gain in obesity models in mice. Our objective was to assess the effect of BAM15 on tumor promotion caused by a high saturated fat diet in mice. We also aimed to determine the role of Pparα, which is a transcription factor stimulating the expression of rate-limiting enzymes of fatty acid oxidation. Since bone marrow is enriched with adipocytes in adulthood, we finally assessed the role of BAM15 on bone turnover.

Experimental Design: Wild type (wt) C57Bl/6J and Pparα knockout (KO) mice were injected intraperitoneally with 20 mg/kg diethyl nitrosamine on postnatal day 13. From weeks 4-10, mice were fed a high saturated fat diet with cocoa butter as a saturated fat (CB diet). At 10 weeks of age, mice were either continued being fed the CB diet or were fed a cocoa butter diet supplemented with 0.1% (w/w) BAM15 (BAM diet). The mice were sacrificed at 30 weeks of age with recording of visible liver tumors and collection of serum and tissues. From the serum, severity of liver tumorigenesis was determined by ELISA of the tumor stem cell marker alphafetoprotein (AFP) and liver injury by a kinetic enzymatic assay of alanine transaminase (ALT). Serum markers for bone synthesis (Procollagen 1a1) and bone resorption (collagen crosslinks CTX-1) were assessed by ELISA. Gene expression was determined by RNA isolation and qRTPCR assays.

Results: For both wt and Pparα KO mice, the BAM diet led to significantly ($P < 0.05$) lower body weight and weight of gonadal fat pads. In males, the BAM diet significantly decreased the liver weight and hepatic steatosis. The number of tumors per mouse was significantly higher in male than female mice fed the CB diet. In male mice, the BAM diet led to significantly lower numbers of tumors, and significant decreases in serum AFP content and serum ALT activity. Surprisingly, knockout of Pparα did not stimulate hepatic steatosis. Yet, Pparα KO male mice fed the CB diet had higher ALT levels, but significantly lower AFP levels than wt males. In wt mice, the BAM diet had no effect on procollagen 1a1 abundance but caused a significant decrease in serum CTX-1 content in both sexes. Gene expression in femoral bone marrow of two marker genes of adipocytes (*Fabp4* and *Pparg*) was unchanged by the BAM diet.

Conclusion and Discussion: In addition to protection from obesity, BAM15 inhibits liver tumor promotion caused by a high-saturated fat diet, particularly in males. Pparα has a dual effect, with knockout of the gene promoting liver injury, but reducing the tumor severity. Finally, BAM15 may inhibit bone resorption without a decrease of bone marrow adiposity.

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"Comparative Analysis of Cognitive Function in Patients with Parkinson's Disease and Multiple Sclerosis"

Multiple Sclerosis (MS) is an autoimmune disorder where the immune system attacks the protective myelin sheath that covers nerve fibers, causing communication problems between the brain and the rest of the body. MS can cause permanent damage or deterioration of the neurons in the central nervous system. The exact cause of MS is unknown though symptoms can include pain, tremor, changes in vision, fatigue, difficulty moving, and cognitive decline though these symptoms can vary from one patient to another.
Parkinson's disease (PD) is a neurological disorder that is caused by the death of cells in the substancia nigra that relay the neurotransmitter dopamine primarily to basal ganglia, a group of brain regions important for motor and other functions. Symptoms of PD include tremor rigidity and bradykinesia. Parkinson's treatment focuses on managing symptoms. MS and PD are neurological disorders characterized by progressive neurological decline which can then leads to cognitive impairment.

Patients with Parkinson's disease (PD) and multiple sclerosis (MS) were included in our study to evaluate their cognitive performance. They completed cognitive screenings using several neuropsychological assessments. The Montreal Cognitive Assessment (MoCA) examined language, memory, and attention. The Symbol Digit Modalities Test (SDMT) screens for information processing speed, attention and visual scanning. Lastly, the King-Devick Test evaluates eye movement and attention.

Food Insecurity Associated with Infection of Hepatitis C and B Viruses in the United States

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Background: Hepatitis C virus (HCV) and Hepatitis B virus (HBV) infected populations are rising in the modern day, with approximately 1 million and 1.2 million new cases per year, respectively. Additionally, food insecurity (FI) is also growing, with an increase in the rates from 10.4% to 13.5% between 2021 and 2022. Past studies have shown a link between FI and HCV-HIV coinfected populations. However, the association between FI and HCV/HBV is still unclear. Past studies provide evidence for a link between FI and injection drug use (IDU), which is one of the primary paths for spreading HCV and HBV infection. This evidence suggests a possible association between FI and HCV/HBV infection. The objective of this study is to investigate the association between food insecurity and HCV/HBV infection.

Methods: The data used for this study was derived from the National Health and Examination Survey (NHANES) 2015-2020. To be eligible for this study, respondents had to be adults over 20, have no missing values for HBV or HCV infection status, the primary outcomes, and have not received the HBV vaccine. The total sample size was 9797. The primary predictor in this study was food insecurity. Other potential confounding factors we considered in the study included age, gender, education level, race, smoking status, drinking status, marital status, and income, measured using the family income to poverty ratio. Analyses were conducted using the Statistical Analysis System (SAS). With SAS, the predictors associated with HCV/HBV status were tested using the chi-square test. FI associated with HCV/HBV status was evaluated using logistic regression while adjusting for other factors.

Results: The food-insecure individuals had a higher chance of HCV or HBV infection than food-secure individuals (4.3% vs. 2.5%). The bivariate analysis indicated that the association between food insecurity and HCV/HBV was statistically significant ($p < 0.001$). Other variables that were statistically significant in association with HCV/HBV were gender ($p < 0.001$), age ($p < 0.001$), smoking status ($p < 0.001$), and family income ($p < 0.001$). Furthermore, the multivariable logistic regression provided evidence that HCV/HBV-infected individuals were more likely to be food insecure (odds ratio (OR) = 1.35, $p = 0.041$), male (OR = 1.62, $p = 0.004$), old adults (OR = 3.03, $p < 0.001$), middle-aged adults (OR = 2.65, $p = 0.004$), current smokers (OR = 3.11, $p <0.001$), and poor (OR = 1.93, $p < 0.001$).

Conclusions: Food insecurity was found to be associated with higher rates of HCV/HBV infection. In addition, smoking status, age, and family income were significantly associated with HCV/HBV infection. Our findings provide valuable information for the prevention of HCV/HBV infection and incentivize steps to be taken against food insecurity to reduce HCV and HBV infection.

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"Targeted Activation of Cannabinoid 2 Receptor to Attenuate Painful Synovitis"

Background: Osteoarthritis (OA) causes painful joint stiffness, reducing quality of life and imposing a significant economic burden. The functional limitations associated with OA arise from cartilage degradation, inflammation, fibrosis, osteophytes, and muscle weakening around the joint. The synovial membrane produces joint fluid and is a significant source of sensory nerves that intensify pain during inflammation (synovitis). Synovitis severity is graded based on the amount of inflammatory cell foci in the synovial subintima. Underpinned by the critical need for innovative, longer-lasting anti-inflammatory therapies, non-surgical treatments for OA include opioids, steroids, and hyaluronic acid, which pose risks such as addiction and accelerated disease progression with short-term relief. Interleukin (IL)-1β promotes enzyme-mediated cartilage degradation and initiates inflammation in OA. The cannabinoid 2 receptor (CB2R), highly expressed in synoviocytes, has been associated with anti-inflammatory responses. JWH133 is a CBD analog designed for targeted activaiton of CB2R with 200-fold higher binding affinity than endocannabinoids and CBD. We aim to assess the response of inflammatory synoviocytes to JWH133 compared to CBD. We predict that JWH133 will more effectively reduce the concentration of IL-6 secreted by IL-1β-stimulated synoviocytes compared to CBD.

Methods: Synovial tissue from banked knee OA patients (n=15), stratified into high (n=10) and low (n=5) self-reported pain groups based on microscopic synovitis scores, was processed for histological assessment (H&E) and CB2R immunofluorescence (IIF). CB2R IIF signal was quantified using confocal microscopy and normalized to cell number and tissue area using Slidebook™(3i). Cultured human fibroblast-like synoviocytes (HFLS) were serum-starved, stimulated with IL-1β (4 ng/mL), and treated with 20 μM CBD or JWH133 (dissolved in Cyrene). IL-6 levels in conditioned media were measured by ELISA and normalized to total protein content. Statistical analyses included Student's t-tests for histological comparisons, Pearson's correlation to assess associations, and one-way ANOVA to evaluate in vitro treatment effects with $\alpha = 0.05$.

Results: The mean CB2R expression in the synovium of the low inflammation group (7.62% ± 1.2%) was significantly higher ($p=0.0009$) than in the high inflammation group (3.23% ± 0.41%). An inverse, moderate, yet significant correlation between synovitis and CB2R expression was found ($R = -0.051$; $p < 0.001$). Compared to the cells pre-stimulated with IL-1β and treated with Cyrene vehicle, IL-6 levels were 44% lower in the HFLS pre-stimulated with IL-1β and treated with 20 μM of JWH133 ($p = 0.0198$). CBD decreased IL-6 by 24.65% compared to stimulated Cyrene vehicle controls but without calculated significance.

Discussion: The inverse relationship between CB2R expression and synovitis highlights a therapeutic need in patients with heightened painful inflammation. JWH133 has shown superior efficacy in suppressing IL-6 production compared to CBD, positioning it as a potential anti-inflammatory agent for synovitis. Ongoing evaluation of knee OA patient-derived synoviocytes will further elucidate its therapeutic potential. Future studies should investigate its broader effects on inflammatory pathways, mechanisms of action, and interaction with pain receptors in the synovium. Because the anti-inflammatory effect of CB2R helps modulate cartilage-degrading proteases and fibrous collagen deposition, JWH133 must be evaluated in animal models of OA.

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"Socio-economic Factors and Racial Disparities That Influence Stage Diagnosis of Cervical Cancer"

Cervical cancer remains a significant public health challenge in Louisiana, ranking 10th in incidence and 5th in mortality rates across the United States. Despite the availability of primary preventative methods, the state experiences high rates of cervical cancer among women. Early detection through HPV and Pap Smear tests is critical, yet data from the Louisiana Tumor Registry (2016-2021) reveals that over 50% of women are diagnosed at regional and distant stages, where 5-year survival rates drop to 56% and 16%, compared to 88% for localized stage diagnoses.

Socio-demographic barriers significantly impact the stage at diagnosis. This study focuses on identifying underserved areas and populations to support Louisiana's cervical cancer elimination initiative. It evaluates the influence of urban-rural residency and poverty status on the stage at diagnosis for non-Hispanic Black (NHB) and non-Hispanic White (NHW) women. Findings indicate that NHB women are more likely to be diagnosed at advanced stages than NHW women, regardless of metro or non-metro residency. Additionally, women in non-metro areas, particularly NHB women, are less likely to receive a localized stage diagnosis. High-poverty regions correlate with fewer localized diagnoses, with NHB women in these areas faring worse than their NHW counterparts.

This research underscores the need to prioritize NHB, non-metro, and high-poverty populations to enhance early detection and improve survival rates. It highlights the necessity of targeting non-metro and high-poverty regions in Louisiana's cervical cancer elimination efforts.

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"Activation of DYN- and KOR-expressing Neurons in Bed Nucleus of the Stria Terminalis in Adolescent Alcohol-Induced Hyperalgesia"

Introduction: Adolescent alcohol can lead to long-lasting changes in brain function by impairing healthy adolescent brain development and increasing the likelihood of developing an alcohol use disorder (AUD) over the lifetime. Most alcohol use begins during adolescence, usually in a binge-like fashion, and adolescent alcohol use is a major predictor of AUD. One behavior that contributes to alcohol use and potentially the development of AUDs is pain. Alcohol use is known to reduce acute pain, yet chronic alcohol withdrawal can produce hyperalgesia. There are also sex differences in pain sensitivity and alcohol's effect, but the interaction of these factors has not been explored. The bed nucleus of the stria terminalis (BNST) is a highly sexually dimorphic brain region involved in negative affective states, alcohol withdrawal, and, more recently, pain. BNST neurons are very heterogeneous, expressing numerous neurotransmitters and neuropeptides. One of these stress-related neuropeptides is Dynorphin. Dynorphin (Dyn) is involved in pain, AUD, and mood regulation, primarily through the action at the kappa opioid receptor (KOR). The purpose of this study is to access the activation of Dyn- and KOR-expressing cells in the BNST of male and female mice that show adolescent intermittent ethanol (AIE) induced hyperalgesia.

Methods: To replicate adolescent binge drinking, both male and female C57BL/6 mice were given either AIE vapor exposure or air exposure. Adolescent mice were given a daily injection of either pyrazole + saline (Air-control) or pyrazole + ethanol (AIE group) to impair the metabolism of ethanol. Mice underwent two four-day cycles of AIE on postnatal day (PND) 28 to 39. This involved 16-hour periods in vapor chambers followed by 8-hour periods in normal animal housing, which allowed for the reliable obtainment of blood ethanol concentrations in the 150–185 mg/dL range. Von Frey and Hargreaves occurred at five different time points to detect hyperalgesia in mice. Mechanical and heat sensitivity was assessed 24 hours, 7, 14, 21, and 28 days post-vapor exposure. The brains were extracted 30 minutes after the last session of Von Frey and flashed frozen. Brains were then sliced on the cryostat collecting the BNST region and used for RNA in situ hybridization (RNAscope), which allowed for quantification of c-fos, Dynorphin, and KOR expression in the BNST. C-fos can be used as an indicator of cellular activity and quantified in the presence or absence of Dyn and KOR expression.

Results: AIE induced prolonged hyperalgesia (up to 28 days) in the von Frey test, decreasing paw withdrawal thresholds compared to air control in both male and female mice, with no observed sex difference. However, in the Hargreaves test, female air and AIE groups showed no differences, whereas male mice exhibited significant differences between groups across all time points. Using RNAscope, we were able to determine that female mice have higher c-fos in all cells along with higher c-fos expression in Dyn and KOR cells in the dorsal lateral BNST (dlBNST) than males with no significant effects of AIE treatment. In the oval BNST, we also see greater overall c-fos expression and c-fos/Dyn expression in female mice than in male mice. However, we did not find a significant difference in cell activation between mice exposed to air

and AIE. For future directions, this study may be replicated with a larger cohort to determine whether the results are consistent.

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"Crotonylation of the Oncoprotein c-Myc: A Novel Post-Translational Modification Modulating Its Cancer Promoting Activity"

Cancer, a global health concern, is one of the top two leading causes of death worldwide.[1] Dr. Lu's laboratory at Tulane University has been studying the signaling pathways of cancer biology, focused on the molecular and biochemical mechanisms for cell proliferation and tumorigenesis involving the p53 and c-Myc pathways. We were specifically interested in understanding the role of unique post-translational modifications of proteins such as p53, a tumor suppressor, and c-Myc, an oncoprotein, in the regulation of the transcription factors' functions and cancer biology. Short-chain fatty acids (SCFSs) are produced by the gut microbiome and have been shown to have anti-cancer effects due to their ability to suppress tumor growth and cancer cell metastasis.

Post-translational modifications (PTM) are processes that involve covalently modifying the amino acid chain of a protein by adding a chemical group to it after the protein is synthesized. Since the amino acids are very rapidly removed and taken off to respond to stimulus, they are relatively unexplored. Protein crotonylation is an important PTM of lysine. Histone or non-histone lysine can be crotonylated in the presence of crotonic acid in vitro cell culture. Crotonic acid (CA) has been shown to modify histone proteins and some non-histone proteins like p53.[2]

Crotonylation of c-Myc was identified by our lab using a combination of western blots with a pan-crotonylation antibody, a technique that separates and identifies specific proteins, and high-resolution mass-spectrometry (HR-MS), which allows for the detection of analytes and determination of elemental and isotopic compositions of a sample.[3] PCR generated K to R mutants identified two sites that ablated the western signal (2R mutant). HR-MS identified six additional sites, and the 8R mutant was generated for this as well.

The addition of CA to cancer cells caused a decrease in c-Myc activity and cellular growth, increased chemosensitivity, and decreased stem-cell formation. The mutant, however, has increased proliferation and colony formation capacity. Quantitative chain polymerase reaction (q-PCR) used to measure gene expression of downstream cell-cycle related c-Myc targets also shows increased activation by the mutant. Mechanisms to explain this phenotype are being investigated by Immunoprecipitation (IP), a process used to purify antigens using specific antibodies. Lentivirus is also being generated for c-Myc and its mutants to generate stable-cell lines to further investigate phenotypes, such as proliferation, colony formation, migration, and spheroid formation. In conclusion, we identified a novel post-translational modification on the onco-protein c-Myc and early work shows a modulatory effect on the protein's cancer-promoting activity.

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"Overcoming Multidrug Resistance in Breast Cancer: Targeting MRP Proteins for Enhanced Therapeutic Efficacy"

PURPOSE: Chemotherapy remains one of the major treatment options for metastatic breast cancer. Resistance to chemotherapeutic agents is a major reason for cancer treatment failure. We plan to utilize nanotechnology to overcome chemoresistance mechanisms. It's been reported that down-regulating the nuclear expression of MDR proteins (P-gp, MRP, BCRP *etc.*) by siRNA could increase the delivery of cancer curing drugs (*i.e.* doxorubicin) to drug resistant breast cancer cells. However, unless those siRNA-encapsulated nanoparticles are targeted specifically to cancer cells, they will have hardly any impact; rather they will create notorious undesired side effects to normal tissues. We plan to overcome these problems by developing a targeted nanocarrier delivery system for siRNA into breast cancer cells. Our hypothesis is that conjugating nanoparticles with a cancer cell specific aptamer should enhance the knockdown of multidrug resistant genes, which will increase the delivery of Dox (*i.e.* doxorubicin) into breast cancer cells leading to enhanced cellular toxicity and antitumor effect as compared to unconjugated nanoparticles. This study is intended to know whether silencing the expression of P-gp or MRP-1 by aptamer-labeled siRNA nanoparticles could enhance the delivery of doxorubicin into breast cancer cells in culture. **METHODS:** For targeted delivery, Aptamer-A6 has been used which can bind to Her-2 receptors on breast cancer cells. The particles were prepared by high pressure homogenization (HPH) using different amount of DOTAP, cholesterol, PLGA or PLGA-PEG and Mal-PEG. After siRNA encapsulation, the particles were incubated with aptamer-A6 for surface labeling. The liposomal particles were characterized for their size, surface charge and cytotoxicity. The delivery of P-gp siRNA or MRP-1 siRNA into 4T1-R cells has been assessed by immunofluorescence, PCR and FACS analysis. The doxorubicin accumulation into the cells has also been observed before and after the knockdown of MDR proteins by immunofluorescence and FACS analysis. **RESULTS:** This study has shown that the uptake of Dox by Dox-resistant 4T1-R is significantly less than Dox-sensitive 4T1-S which is partly attributed to the higher expression of drug-efflux pump (*i.e.* ABC transporter proteins P-gp, MRP-1, BCRP *etc.*) on the surface of the resistant cells. The targeted knockdown of P-gp or MRP-1 has been enhanced when the particles carrying P-gp siRNA or MRP-1 siRNA, respectively were labeled with aptamer. Concurrently, the uptake of Dox into the Dox-resistant 4T1-R breast cancer cells has increased significantly when the MDR proteins were knockdown by appropriate siRNA-encapsulated aptamer-labeled nanoparticles. **CONCLUSIONS:** This preliminary study concludes that aptamer functionalization of the nanoparticles could enhance the knockdown of MDR proteins which increases the delivery of doxorubicin into the breast cancer cells. **ACKNOWLEDGEMENT:** This work is funded in part by the Louisiana Cancer Research Consortium, NIMHD grant number TL4GM118968, NIGMS grant number UL1GM118967 and R25GM060926, CUR from Xavier University of Louisiana, LBRN and NSF.

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"Determining the Efficacy of the Gardasil-9 Vaccine in HIV-Positive Individuals"

Background: Cancer in the anal and cervical regions is caused by specific genotypes of the human papillomavirus (HPV). The Gardasil-9 vaccine is an effective vaccine that prevents HPV infection as well as decreases the chances of cancer development in the anogenital tract - specifically targeting oncogenic HPV genotypes. The vaccine has been tested to be effective in HIV-negative individuals as they have been observed to produce an antibody response to the vaccine. However, there are relatively few studies conducted that evaluate the vaccine response in people diagnosed with HIV. A higher prevalence of HPV infection, as well as pre-cancerous and cancerous lesions, are seen in the HIV-positive population so it is unclear if this is due to lack of efficacy of the vaccine or other outside factors. This study's goal is to test for HPV genotypespecific infections in the anogenital tract in well-controlled HIV individuals, as well as test the antibody response to the HPV vaccine. This project is interested in a diverse racial and socioeconomic background of individuals who have not received or fully received the Gardasil-9 vaccine.

Methods: Following the informed consent of eligible individuals for this study, a cotton swab was used to collect cellular materials from the anogenital region. Genomic DNA from the samples was extracted by lysing the cell to free the DNA from the cell nucleus and then washing it with appropriate buffers to have the DNA suitable for storing before testing. The extracted samples were nano-dropped to measure the DNA concentration using the 260/280 specific absorbance range. For testing, a polymerase chain reaction (PCR) was utilized with primer set MY09/MY11, commonly used for identifying the presence of HPV, regardless of genotype. An additional βglobin primer set, PC04/GH20, was used to ensure human DNA was present. After completing the PCR, the product was analyzed through gel electrophoresis. The DNA fragments were separated by the base pair length of the specific primer sets - detecting a band around the 450 bp range signified a positive result.

Results: Approximately 105 samples were collected, and out of those, 48 samples have been extracted and tested for HPV. From the tested samples, 34 were male and 13 were female, and the e age ranged from early 30s to late 40s. The individuals from the tested samples consisted of a majority Black/African American population (81%), with the remaining being of a white population. In total, 21 samples (44%) were positive with HPV, and of those, more than half did not receive the HPV vaccine prior to enrollment.

Discussion: This study will continue to enroll this at-risk population. The sponsor company, Merck will be testing the collected samples for serum antibodies against the 9 types of HPV in Gardasil. The high rates of HPV positivity at 44% underscores both the need for HPV

vaccination and follow up screening to prevent anogenital malignancies in people living with HIV.

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"Socioeconomic Disparities in Environmental Conditions at Playgrounds in the Greater New Orleans Area"

Background: Socioeconomic disparities in the environmental quality of playgrounds are a widely recognized environmental justice problem across the United States. In the Greater New Orleans area, little is known about the levels of pollution in public parks. Tremé, a once culturally rich neighborhood, has transformed into a shadow of its former self due to the construction of the I-10 Claiborne Corridor in the late 1950s. This study quantified pollution risk factors for children at playgrounds in the Claiborne Corridor.

Objectives: This study aims to characterize air and soil pollution at select public playgrounds in the Greater New Orleans area, and to assess if playgrounds in minority communities have a disproportionately higher burden of pollution.

Methods: We measured traffic-associated environmental pollutants at four playgrounds, including fine particulate matter (PM2.5), carbon monoxide, and ozone concentrations in ambient air. We also measured soil lead concentration levels. Soil samples were tested for lead with a SciAps XRF Analyzer. We used Airbeam sensors to measure PM2.5 and Aeroqual S500 monitors to measure ozone and carbon monoxide. EJ Screen was used to map health outcomes associated with air and soil pollution.

Results: We found substantially higher pollution levels in playgrounds located in minority populated neighborhoods. The average soil lead concentration level in Lemann Park was 240 ppm, which exceeds the EPA guideline of 200 ppm. The highest soil lead levels we observed were in Hunter's Field, where 6 samples exceeded 200 ppm and four samples exceeded 400 ppm, with a maximum of 624 ppm. Soil samples taken near the interstate had consistently higher lead levels than samples taken in areas away from the interstate, which suggests that interstate traffic pollution was the source of the lead pollution. In addition, the average PM2.5, ozone, and carbon monoxide levels were higher in the Claiborne Corridor parks in comparison with measurements we made in Pontiff Playground, a suburban park located far from interstate highway pollution sources.

Conclusions: Children are exposed to higher levels of pollution than adults, and they are more susceptible to health effects from these exposures. For example, children with growing and developing lungs breathe in significantly more air per unit body weight than adults. Regarding lead exposure, children have a greater ratio of skin surface area to body mass than adults, leading to more significant dermal absorption rates. Furthermore, many children engage in pica, where they place inedible objects into their mouths, including dirt and soil. Exposure to lead during early stages of development can result in permanent intellectual disabilities and behavioral disorders, while exposure to pollution can result in health conditions such as asthma, chronic bronchitis, COPD, lung cancer, and heart disease.

Based on our findings, children in Claiborne Corridor neighborhoods face disproportionately high exposure to pollution. More federal, state, and local actions must be taken to remediate pollution and reduce exposure in these communities.

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Could members of the maternal vaginal microbiome aid in the optimal maturation of their newborn infant's gastrointestinal microbiome?

The bacterial metabolite Indole-3-lactic acid (ILA) has an important role in maturing, and preventing inflammation in, a newborn infant's gastrointestinal (GI) tract. ILA is a predominant metabolite produced by *Bifidobacter longum* subsp. *infantis* (*B.infantis*), a major 'pioneer' bacterium in a healthy infant GI microbiome. These *Bifidobacter* make ILA by metabolizing tryptophan, an essential amino acid found in high concentrations in breastmilk. My lab recently observed a significant concentration of ILA in the vaginal secretions of some, but not all, young reproductive age women. We hypothesize that vaginal bacteria, transferred to newborns at birth, could aid in the optimal early development of their infant's GI tract. The objectives of my study were (1) first to determine if there was an association of ILA with specific vaginal bacterial species in this clinical cohort and (2) second, to determine if ILA concentrations found in the vaginal secretions can ligate the aryl hydrocarbon receptor (AHR), which is reported to play a key role in maintaining homeostasis and preventing inflammation at mucosal sites.

For objective (1) I found that (i) ILA abundance was significantly increased in the vaginal secretions of participants with a vaginal microbiome categorized as community state type I (CST I, an optimal CST which is dominated by *Lactobacillus crispatus*) compared to participants categorized with CSTs defined by low *Lactobacillus* abundance, CST IV-A and CST IV-B (p-value < 0.001). Analysis of subCSTs found that participants categorized as CST I-A (highest *L.crispatus* dominance) had significantly greater ILA abundance compared to participants categorized as CST III-A (*L.iners* dominance) ($p = 0.02$), CST III-B ($p = 0.01$), CST IV-A ($p < 0.001$), and CST IV-B ($p < 0.001$). *L. crispatus* relative abundance was found to be positively associated with ILA abundance ($p < 0.001$). For objective (2) I became proficient at cell culture, expanding and utilizing the HT29-Lucia AhR Cell line (InvivoGen), which is an epithelial cell line engineered to express endogenous AhR, and which allows the screening of potential AHR ligands by measuring secreted Lucia luciferase reporter protein in the culture supernatant. I determined that ILA ligated the AHR receptor, with activity in the concentration range found in the vaginal secretions of women with *L. crispatus*-dominant CST I.

This preliminary data suggest that (i) ILA may be a metabolic marker of a healthy vaginal microbiome, potentially produced by *Lactobacillus crispatus*, and ii) having read a recent study of mothers and their infants indicating that members of mom's vaginal microbiome are transiently shared with their infant, this raises the possibility that an optimal, lactobacillus dominant microbiome in mom may play a role in seeding their infant's gut, and potentially contribute to early ILA production in the infant.