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“Examining Louisiana Mammography Facilities for Medicaid Coverage Gaps”

Breast cancer is second only to lung cancer for the leading cause of cancer deaths and has the highest cancer incidence rate for women in the state of Louisiana. As a result, breast cancer has become a major concern to health professionals around the state. The main way to lower these rates is to provide diagnostic breast screening, such as mammograms; detecting the cancer early can increase the chances of survival. Our target population is women, ages 40-64, with incomes at or below 250% of the federal poverty level (FPL). Recent changes to the Medicaid insurance plans in Louisiana have led to an expansion of coverage, allowing more women to have access to mammography services throughout the state. Despite more women being insured there are still coverage gaps. The goal of this research project is to locate any gaps where women cannot find access to mammography services covered by Medicaid insurance.

To begin this project, the first step was to locate all facilities that provide mammography services in Louisiana and place their information on an excel spreadsheet. Afterwards, each facility or provider was contacted to confirm whether or not Medicaid insurance was accepted. Finally, all of the locations of mammography facilities that accept Medicaid insurance were placed on a map using ArcGIS mapping software.

After analyzing the maps, different trends were shown. There were many clusters of facilities in the southern region of the state, focusing around the New Orleans, Baton Rouge, and Lafayette areas. In the northern, more rural part of the state, there were very few clusters of facilities. In the far Southeast and Southwest portions of the map, the region surrounding Winn Parish, as well as downward from Avoyelles to Iberville Parishes lacked coverage. Women in these areas would have to travel over 10 miles to reach a mammogram provider. This may be a barrier to screening due to transportation being an issue.

In conclusion, nearly all of FDA approved mammography sites in Louisiana accept Medicaid insurance. However, there are still areas where women would need to travel over 10 miles or even out of their parish's borders to reach a facility. When comparing these results to the uninsured rate and the incidence rate, we can see areas where adding a mammography provider that accepts Medicaid is essential. Adding a mobile mammography service and setting up imaging centers in a central location are possible solutions to closing these gaps. Hopefully, these changes will lower both the incidence and mortality rates in Louisiana.

Ashley N Augustus

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Mentor: Tiffany Wills, Ph.D

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“Effects of Chronic Adolescent Ethanol Exposure on Glutamatergic Receptors and Plasticity in the BNST”

Adolescent alcohol consumption is one of the strongest predictors of future alcohol dependence. In rodents, adolescents display distinct responses to acute ethanol, and exhibit both short- and long-term neural changes in response to chronic ethanol. The bed nucleus of the stria terminalis (BNST) is a critical region for mediating the negative reinforcing effects of alcohol withdrawal that often lead to relapse. Thus, understanding how plasticity in the BNST is regulated by ethanol is vital to the development of effective therapeutic strategies to treat alcohol use disorders. Synaptic plasticity in the brain typically involves NMDA receptors (NMDARs). The effect of ethanol on NMDAR function depends on its duration of action; such that acute ethanol inhibits NMDARs while chronic ethanol generally leads to enhanced function. The NMDAR complex is composed of two obligatory GluN1 subunits and two other subunits, either from the GluN2 or GluN3 subfamilies. Alterations in the expression of NMDAR subunits as a consequence of chronic ethanol treatment has been described in other brain regions. Moreover, in adults, other forms of glutamatergic plasticity (e.g., group 1 mGluR-mediated long term depression [LTD]) are also affected by chronic ethanol exposure. The aim of the current study was to examine the effects of adolescent alcohol exposure on glutamatergic transmission in the BNST. Male and female adolescent (P30) C57 mice were given a daily injection of either pyrazole or pyrazole + ethanol to impair alcohol metabolism. Mice were then placed into a chamber filled with either volatilized ethanol or water. After 16 hours of exposure, mice were removed from the chambers and returned to standard animal housing. This process was performed in two 4-day cycles of intermittent (16 hours on, 8 hours off) ethanol or water vapor—separated by a 3-day withdrawal period—to produce a blood alcohol concentration ~ 200 mg% in ethanol-exposed mice. Five hours following the final vapor chamber session, brain slices and tissue punches containing the BNST were collected so that the consequences of adolescent alcohol exposure on glutamatergic transmission could be assessed using electrophysiology and western blot, respectively. Electrophysiological recordings revealed that chronic ethanol exposure during adolescence caused greater GluN2B-NMDAR inhibition and NMDAR-mediated plasticity in male but not female mice, suggesting up-regulation of GluN2B-containing NMDARs during withdrawal. Interestingly, preliminary western blot analyses diverge from these findings, revealing no change in NMDAR subunits (GluN1, GluN2B, and GluN2A) in the BNST of male mice. One explanation for this disparity could be the lack of specificity offered by the western blot approach; thus, future studies will focus on evaluating synaptic fractions. Regarding the effect of adolescent ethanol exposure on metabotropic plasticity, results from slice recordings revealed altered group 1 mGluR-mediated LTD in the BNST of female but not male mice. Future western blot studies will set out to determine if proteins involved in this signaling cascade (mGluR5, Arc, GluA2) are altered as a result of adolescent ethanol exposure.

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Dr. Dean Smith:

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The Future of Accountable Care in Louisiana

The United States has one of the most fragmented healthcare systems in the world. Fee for service payments previously dominated the healthcare market and encouraged patient volume rather than quality care. The lack of coordination of care has resulted in wasteful practices and excessive spending with no improvement in patient outcomes. With the cost of healthcare constantly rising in the United States, legislators have been looking for ways to reduce spending and improve the quality of care patients receive. Accountable Care Organizations are possible solutions to these problems. Accountable Organizations are groups of providers that coordinate the care of a specific population of patients. The healthcare providers in the organizations agree to assume financial responsibility for their patient population's health outcomes. If they successfully increase the quality of care and reduce cost, the providers split the shared savings. If they fail to increase quality and/or reduce costs, they share responsibility of the losses as well. The pioneer ACO model was launched in 2012 with the Medicare population. Over the performance years, the amount of ACOs have grown tremendously and the reported data shows that they have been effective in reducing healthcare costs. ACOs are now being looked at as a solution to reduce Medicaid spending in different states as well. The aim of this project is to determine if Louisiana should be attempting to implement Medicaid ACOs. Methods for this research question include in depth literature reviews of CMS.gov, individual ACOs reporting websites, and data of Medicare ACO results of the past performance years. Current information on states that have already begun to experiment with Medicaid ACOs was also reviewed and taken into consideration.

The findings of this project show that ACOs have been successful in reducing healthcare costs in States like Massachusetts, Oregon, and Colorado in the Medicare populations and show promise of reducing cost among Medicaid populations as well. Current ACOs reduce healthcare costs by 2 to 5 percent. However, there still needs to be more performance data published along the performance years before Louisiana makes a definite decision to implement them fully. Each state's requirements for Medicaid are unique, so the success of ACOs in one state does not mean that it will guarantee success in Louisiana. Continued observation and small scale implementation in targeted populations and areas of Louisiana is the safest way to implement Medicaid ACOs.

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“The Microvascular Chemokine Response to Infection by *Candida auris*”

Introduction. Bloodstream infections involving *Candida* are one of the most common etiologies of fungal sepsis and a major cause of morbidity and mortality (30-day mortality rate of 54%). *Candida albicans* is the most predominant organism implicated in fungal infections of the bloodstream; however, non-*albicans Candida* species are collectively responsible for more infections than *C. albicans*. A drug-resistant *Candida* species named *Candida auris* has recently been identified as the cause of candidemia in several locations worldwide, including the United States. *C. auris* poses a major health threat due to its inherent resistance to all three major classes of antifungal drugs. The Johnston Lab has previously shown that *C. albicans* induces the rapid and widespread loss of vascular integrity and subsequent hyperpermeability through interactions with the endothelium that require the up regulation of inflammatory mediators, including the CXCL family of chemokines. The aim of this project is to compare the chemokine response to *C. albicans* and *C. auris* infections in three microvascular endothelial cell types.

Materials and Methods. We used clinical isolates of *C. albicans* and *C. auris* in co-culture experiments with microvascular endothelial cells, including a cell line (HMEC-1) and primary cells from neonatal dermis (MVEC-neo) and adult cardiac tissue (MVEC-cardiac). EC were serum-starved for 2 hours prior to infection with *Candida* and incubated at 37°C for 5 hours. Cell culture supernatants were subjected to ELISAs (R&D Systems) for quantification of chemokine release. Total RNA was purified from cell lysates and used for synthesis of cDNA (Applied Biosystems). cDNA was then subjected to quantitative reverse transcription polymerase chain (qRT-PCR) analyses using fluorophore-labeled TaqMan probe and primer sets (IDT DNA, Bio-Rad). Additionally, endothelial cells were co-cultured with *Candida* on glass coverslips and stained for protein expression by fluorescent immunocytochemistry (ICC). Lastly, HMEC-1 cells were transfected with CRISPR-Cas9 Double Nickase plasmids (Santa Cruz Biotech) in an attempt to knockout the genes encoding both CXCR2 and c-Src.

Results. Previous work in the lab has shown that *C. albicans* induces significant increases in the Human Umbilical Vein endothelial cell (HUVEC) expression of the CXCR2 ligands CXCL1, CXCL2, and CXCL8. These results were comparable in the HMEC-1 cell line. In this report we show that while the mRNA expression of all three ligands is also increased in primary microvascular endothelial cells by *C. albicans*, the fold-induction is much lower. Interestingly, the basal levels of CXCL1 protein secretion were significantly higher in MVECs. Surprisingly, the emerging pathogen *C. auris* seemed to have no effect on gene expression or protein secretion in any cell type tested. These results highlight the importance of choosing the correct model system for in vitro endothelial analyses. Additionally, we hypothesize that *C. auris* likely utilizes a unique infection strategy for infection of endothelial cells, as compared to *C. albicans*.

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Investigating the Resistance and Sensitivity of *Rhizopus delemar* Strains on Various Drugs and Utilizing the CRISPR/Cas9 Endonuclease to Create Auxotrophic Mutants

In 2008 and 2009, five immunocompromised children at New Orleans Children's Hospital contracted fatal, hospital-acquired mucormycosis due to contamination of bed linen with *Rhizopus delemar* spores.¹ *R. delemar* is a filamentous fungus that has both sporous and hyphal growth stages. *R. delemar* spores are multi-nuclear, which complicates the genetic transformation and transformant selection process. In addition, this organism exhibits an innate, high resistance to conventional drugs used with dominant selectable markers for transformation. Despite being the leading causative agent of mucormycosis, *R. delemar* is relatively understudied and lacking in available genetic tools. Therefore, the goal of this study was twofold: 1) characterization of the growth of two wild-type clinical *R. delemar* strains FGSC9543 and CDC8219 on common antifungal drugs. 2) creation of plasmid constructs containing guide RNA (gRNA) insert and the CRISPR/Cas9 endonuclease in the vector backbone for use in biolistic transformations of *R. delemar* to generate an auxotrophic *ura3* mutant.

1) Six different strains of *R. delemar* strains were examined: wild-type FGSC9543 and CDC8219, and 5-flouro-orotic acid (5-FOA) -resistant strains that originated from the wild-type strains designated CDC8219 f1, f3, f5 and FGSC9543 f4. However, these mutants were not created using the CRISPR/Cas9, but by spontaneous mutation or putative epimutations through selection on 5-FOA. Results concluded that all of the *R. delemar* strains displayed similar growth on each respectable drug, with the exception of the CDC8219 f1 strain, which seemed to be dramatically more sensitive to all of the drugs tested. CDC8219 f1 also exhibited a stable *ura⁻* auxotrophic phenotype in contrast to the other 5-FOA-resistant strains.

2) gRNA for the CRISPR/Cas9 endonuclease was created by phosphorylating and annealing the complementary sense and antisense primers specific for a given CRISPR target site and ligated into a Cas9-containing plasmid (pmCAS9) utilizing two inverted BsmB1 restriction sites. *E. coli* harboring plasmid ligation products were selected and DNA plasmids were obtained by miniprep and screened by restriction digest. Positive DNA plasmids were then sequenced to confirm gRNA insertion. The resulting plasmid p1970/71 and p1928/29 were used as gRNA to simultaneously target two CRISPR sites to create a deletion within the *URA3* coding sequence by biolistic transformation and selection on 5-FOA.

Preliminary biolistic transformations of p1970/71 and p1928/29 gRNAs into CDC8219 has yielded several potential transformants on 5-FOA-containing medium. Since the spores are multi-nuclear, the putative transformants will be passaged multiple times to enrich for any CRISPR/Cas9-induced *URA3* gene deletions. Genomic DNA will be extracted and analyzed by PCR, Southern blot analysis, and DNA sequencing to identify and confirm any *URA3* gene deletions.

1. "Mucormycosis Outbreak Associated with Hospital Linens" LIFE. May 6, 2014.

Kaitlyn, G, Calabresi

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Drs. Alison Quayle, Ashok Aiyar & Chris Taylor:
LSUHSC Microbiology, Immunology, & Parasitology**“Evaluating genetic variants of *Gardnerella vaginalis* for indole consumption and their effect on immune clearance of *Chlamydia trachomatis*”**

Chlamydia trachomatis (CT) infection is a sexually transmitted infection that affects roughly 4.2% of women and 2.7% of men worldwide. Although CT infections are typically treated with antibiotics, some infected persons can clear the infection via an immune response that triggers production of the cytokine IFN γ . IFN γ acts against CT infections by triggering the enzyme indoleamine 2,3-dioxygenase to catabolize tryptophan, thereby depriving CT of this essential amino acid.

The efficacy of IFN γ against CT is determined by the composition of the vaginal microbiome, which varies between individuals. Individuals with Bacterial Vaginosis (BV) have vaginal microbiomes that contain large levels of anaerobic bacteria, some of which can produce the metabolite indole. CT can express an enzyme called tryptophan synthase that uses indole to produce tryptophan, thereby escaping tryptophan starvation imposed by IFN γ . Indeed, the majority of women in whom immune clearance of CT is successful do not have BV. Nevertheless, some women with BV do successfully clear CT without antibiotics. One bacterial species that associated with BV is *Gardnerella vaginalis* (GV). While no strain of GV can produce indole, there are some strains, with a TrpB gene, which can consume indole to synthesize tryptophan for GV's use. We hypothesized that increased competition for indole by these GV strains could result in the inhibited growth and eventual clearance of CT.

To evaluate the possible effect of GV with the TrpB gene on CT clearance, clinical samples known to have CT (either cleared or treated) were evaluated for presence of GV with the TrpB gene by PCR. Primers were validated against GV with or without TrpB. Of the 62 samples tested, 29 had positive results for GV with the TrpB gene. These samples were previously analyzed to pinpoint the bacterial makeup of the vaginal microbiome and were sorted into 3 Community State Types (CSTs) based on their bacterial makeup. The data was evaluated using bioinformatics to find correlations between CT, BV, CST membership, and presence of GV with the TrpB gene.

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“Correlation between gene expression and methylation signatures with biochemical recurrence in prostate cancer in Colombian men”

Prostate cancer (PCa) is the most prevalent cancer in men worldwide and the second most lethal. Ethnic disparities exist in PCa with minority groups, namely African American and Hispanic/Latino having the worst outcome. Statistics from the American Cancer Society indicate that 1 in 7 men will be diagnosed with prostate cancer within his lifetime, however it is still not fully understood how genetics influences the prognosis of prostate cancer. The goal of the present study was to investigate the correlation of gene expression and gene methylation with the prognosis of prostate cancer in Hispanic/Latino

Non-tumor and tumor tissues were extracted from FFPE blocks of radical prostatectomy samples (179 from 90 patients). Information for each patient was also provided, including PSA follow up and biochemical recurrence (BCR) status within five years after the treatment. RNA-seq was used to determine gene expression levels and the Infinium MethylationEPIC kit was used to determine global methylation. Results were analyzed in DESeq2 under R-Studio (gene expression) and in GenomeStudio v2011.1 (methylation). We compared the samples in terms of both tumoral status (tumor vs. non-tumor) and biochemical recurrence risk (low, intermediate, or high).

Gene expression analysis revealed that the greatest differentiation occurred in the samples with high risk of BCR, with 9 genes showing significant differences (p -value < 0.05) including *ABCC11*, *CLGN*, *RPS6KA3*, *FAR2P2*, *POTEE*, *CYP4F30P*, *POTEJ*, *FAM95B1*, *CYP4F62P*. Methylation analysis showed a differential methylation between tumor samples of BCR positive (BCRPos) and BCR negative (BCRNeg) samples in the *ABCC11* (5 CpG sites), *CLGN* (1 CpG site), *RPS6KA3* (6 CpG sites) genes. On a global scale, 128,139 genes (226,148 CpG sites) were significantly different between tumor samples of BCRPos and BCRNeg samples with a $p < 0.05$, while 7,881 of these genes (10,100 CpG) were significantly different with a fold change ≥ 2.0 . Those genes were associated with several diseases, the most significant being neoplasms of glandular and epithelial type. When we compared the methylation level of tumor and non-tumor tissues, we found 5,685 genes differentially expressed in BCRPos and 977 in BCRNeg. Interestingly, the genes in the BCRPos are associated with several types of cancers, showing neoplasms of glandular and epithelial type as the most significant, which is in agreement with the previous analysis. It is noteworthy that the 9th significant disease in the list ($p < 10^{-80}$) is prostatic neoplasm. On the contrary, genes in the BCRNeg were not associated with neoplasms.

In summary, gene expression and methylation analyses are good tools to separate BCR positive from BCR negative individuals and to identify new targets for future interventions.

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“Adequacy of Health Care Advance Directive in Patients Admitted to the Intensive Care Unit”

Introduction: With the development of advanced medical technology, it has become increasingly important for patients to use advanced directives, such as a Do Not Resuscitate (DNR) order or living will, to stipulate the methods of care that they want at the end of life. This is particularly important in patients requiring intensive care. Eleven percent of healthcare providers still use chest compressions if a patient with a DNR order sustains cardiopulmonary arrest (Puma, J. L.). This study aims to determine what percentage of patients admitted to the intensive care unit (ICU) have advance directives, and whether having an advance directive impacts the number of potentially futile procedures that patients undergo.

Method: This study is an ongoing retrospective chart review of patients admitted to ICU at the University Medical Center of New Orleans. Of the 5000 subjects who met the eligibility criteria, 700 charts have been reviewed to determine whether or not an advance directive was present. For all patients, we recorded whether or not the following procedures were actually performed: intubation, cardiopulmonary resuscitation with chest compressions, cardiopulmonary resuscitation with medications, antibiotic administration, intravenous (IV) fluid, parenteral feeding tubes, infusion of pressors, tracheostomy, other surgical procedures, and dialysis.

Results: 80.3% of subjects reviewed have an acknowledgment of advance directive in the chart. 61% were male. 39.3% were aged 55-64 and 23.9% were 45-54 years old. 68.2% of the study population was black and 30.5% was white. There was statistically significant difference in the mean number of procedural interventions performed on patients with advance directive compared to those without ($t(621) = 3.998$: $p < 0.0001$). There is a correlation between presence of advance directives and antibiotic administration ($p=0.007$, $LR=0.011$) and intravenous fluid ($p=0.004$, $LR=0.007$). The presence of advance directives also impacted the final disposition ($p=0.019$, $LR=0.027$),

Conclusion: Most patients admitted to the ICU have advance directives, regardless of age, however advance directives are more likely to be found for middle aged patients. Patients who had advance directives were more likely to receive antibiotics and IV fluid administration, and the presence of an advance directive impacted final disposition. Completion of the data analysis of the complete cohort of 5,000 patients will likely yield more statistically significant correlations.

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Mentor: Ben Kelly, Ph.D.
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Assessing the Toxicity of Phenol-Derived Compounds against Mammalian Cells

Chagas disease, which is caused by infection by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*), affects approximately 10 million people in Latin America and 300,000 people in North America. Despite the widespread scale of this disease, the treatment options are limited to Benznidazole, the most commonly used medication, and Nifurtimox, the alternative option. The current treatments are, however, inadequate because they lack efficacy in treating chronic infections and provoke toxic side effects in patients, including kidney and liver failure, bone marrow suppression, and skin rashes. Additionally, a lack of alternative treatments could render patients defenseless in the event that parasites become resistant to current medications. Therefore, it is essential that new, low toxicity treatments are developed to limit the health risks associated with the current treatments for Chagas disease.

Prior to this study, a novel phenol-derived compound library was screened *in vitro* against mammalian cells infected with *T. cruzi* and compared to Benznidazole. Three compounds that displayed greater or similar antiparasitic activity to Benznidazole were selected for further examination. In this study, these three compounds were screened against uninfected mammalian cells, and cell vitality was observed over time. The purpose of this study is to analyze the cytotoxicity of antiparasitic compounds in order to identify a treatment that is both effective against parasites and less toxic than current treatments to host cells. Compounds that display these qualities will be assessed for therapeutic efficacy in a murine model of Chagas disease, in future studies.

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“Sensory Screenings in Infants and Toddlers: The Need to Refine Technology by Expanding Developmental Sensitivity”

Frequent vision and hearing screenings are essential to diagnosing, treating and preventing hearing and vision loss in infants and preschool children. Proper visual and auditory functioning are essential to the cognitive development of children, increasing success in an education and social setting. The Human Development Center at LSU Health New Orleans has partnered with Early Head Start Child Care, a federal program designed to equip children (ages zero to three years from families below the federal poverty level) with necessary resources and supports. Early Head Start requires vision and hearing screenings for every infant or child enrolled in addition to developmentally appropriate early childhood care. In this partnership, to date, and using a PlusOptix vision screener and otoacoustic emission (OAE) technology, objective measurements have been completed on greater than three hundred children. This screening process is completed continually on new enrollees. On the hearing and vision screenings, the child receives a pass or referral recommendation. A refer recommendation on the hearing screening indicated abnormal nonotoscopic visual inspection of the outer ear and/or OAE results outside the pass threshold. A refer recommendation from the vision instrumentation indicates function of the eyes outside the pass threshold for ocular alignment, corneal reflex, external ocular exam, pupillary function, and red reflex.

The Human Development Center and the Louisiana Deafblind Project sought to use the 2016 Early Head Start screening data to compare “first time” referral rates of sensory screenings amongst children ages zero to three years who live under the federal poverty line to the “first time” referral rates of sensory screenings in children ages zero to three years in the general population. Upon literary review, the lack of research on hearing and vision screenings for children ages zero to three years across the general population became evident. Using the methods described above, many complications exist that prevent obtaining accurate and reliable data. For example, objective assessments are necessary for children in this age group due to their inability to provide reliable responses for a subjective assessment. However, the objective assessment’s methodology developed for these screenings require more attention and understanding from the children than they often can provide at ages zero to three years. Also, the immature nature of the developing sensory organs at ages zero to three years inhibits accurate acuity measurements, particularly for vision.

An attempt was made to analyze available local data from the Early Head Start Project. “Refer” rates for local data approximated but exceeded rates reported in the reviewed literature. However technological and procedural variations resulted in the data being unusable for comparison purposes. Our efforts present the issues with the current sensory screening methods and why the technology and methodology needs to be more sensitive to the developmental level of infants and toddlers.

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“Identification and Classification of Immune Cells in the Adrenal Gland”

Abstract

The adrenal gland is composed of two distinct parts, the adrenal cortex and the adrenal medulla, which both produce hormones used in various homeostatic processes. The outermost layer, the adrenal cortex, can be further divided into two zones in mice: the zona glomerulosa and the zona fasciculata. In the zona glomerulosa, aldosterone, a blood pressure-regulating hormone, is produced and in the zona fasciculata, glucocorticoids, which regulate blood glucose levels. Both hormones are released in response to stress. At the center of the adrenal gland is the adrenal medulla, which is part of the sympathetic nervous system. The adrenal medulla secretes the catecholamines norepinephrine and epinephrine, also in response to stress.

The function of the adrenal gland and the immune system have been thought to be independent. However, recent studies have shown that immune cells are present in the adrenal gland. The aim of this project was to determine whether B cells are present in the adrenal gland. We prepared cryosections of the mouse adrenal gland and stained them with an antibody raised against the immune cell surface marker CD45R/B220, which labels B cells [and subsets of T and NK cells]. B220-ir [immunoreactive] cells were observed in all sections. Staining was also observed with an antibody raised against CD19, another marker of B cells. To more precisely determine the location of the B220-ir cells, sections were co-stained with markers of the adrenal cortex and medulla. B220-ir cells were exclusively located in the adrenal cortex as shown by co-localization with DTAF streptavidin. No B220-ir cells were located in the adrenal medulla, which was identified using an antibody to tyrosine hydroxylase. Ongoing experiments using physiological and psychological stressors [fasting and exposure to fox urine, respectively] are being used to determine if these treatments cause a change in the distribution of the B220-ir cells.

Our results indicate that the B220-ir cells are located exclusively in the adrenal cortex and primarily in the zona fasciculata. The cell surface markers indicate that the cells are most likely B-10 cells, which produce the protein Interleukin-10. Further experiments are still being conducted to determine the function of these cells. However, the scientific literature suggests that the production of Interleukin-10 occurs in the zona fasciculata and inhibits the action of adrenocorticotrophic hormone, the primary regulatory hormone involved in stimulating glucocorticoid hormone release from the adrenal gland.

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“The Effect of Indole Consumption by *Gardnerella vaginalis* on the Survival of *Chlamydia trachomatis*”

Chlamydia trachomatis (CT) is the most commonly reported sexually transmitted bacterial infection in the United States, with 1.4 million reported cases in 2014. CT, an obligate intracellular bacterium, infects human epithelial cells. To survive intracellularly, it depends on host-derived nutrients such as tryptophan. This dependence renders CT susceptible to a host immune response that induces the cytokine IFN- γ , because IFN- γ triggers production of the enzyme indoleamine 2,3-dioxygenase that degrades tryptophan, thereby starving CT of this essential nutrient.

The efficacy of the host immune response against CT is modulated by the vaginal microbiome, which varies in composition between individuals. Individuals with Bacterial Vaginosis (BV), a dysbiosis of the normal vaginal microbiome, have high levels of anaerobic bacteria (such as *Prevotella* sp.) that produce the metabolite indole. CT can express an enzyme called tryptophan synthase that salvages indole to produce tryptophan, thus escaping the effect of IFN- γ . Therefore, it is not surprising that the majority of women who mount a successful IFN- γ -dependent immune response to clear CT do not have BV. Nevertheless, we have observed a small subset of women with BV who successfully clear their CT infection. Previous studies evaluating the microbiome in BV+ women who successfully cleared CT infections revealed they generally had high levels of *Gardnerella vaginalis* (GV), another bacterium strongly associated with BV. Although all GV strains cannot produce indole, some GV strains can convert indole to tryptophan for GV's use by expressing tryptophan synthase.

Therefore, we hypothesized that some women with BV clear their CT infection because GV consumes the indole in the infection micro-environment, thereby permitting IFN- γ -imposed tryptophan depletion to starve CT. To test this hypothesis, I studied the indole-dependent growth of CT in the presence of a GV strain that cannot consume indole (GV-A), or a strain that can consume indole (GV-B). I found that in media lacking tryptophan but containing indole provided by *Prevotella*, GV-A does not reduce CT growth, but GV-B significantly reduced CT growth, which was consistent with our hypothesis.

Our results indicate that a successful host IFN- γ -dependent immune response against CT is dependent on the composition of the vaginal microbiome. While indole-producing bacteria that increase in number during BV, such as *Prevotella* sp., are deleterious to clearance, their effect can be compensated by increasing obligate indole-consuming bacteria such as GV-B.

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High School

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Mentor's Name: Dr. Liz Simon

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“Effect of Chronic Alcohol on Protein Synthesis and Degradation After Hind Limb Immobilization in Rats”

Aging increases the incidence of fractures, resulting in periods of immobilization, leading to atrophy of skeletal muscle. Atrophy can be due to an imbalance of protein synthesis and degradation. Protein degradation pathways include: the ubiquitin-proteasome pathway, where cells marked with ubiquitin—a polypeptide that attaches to the amino group of a lysine residue side chain—are rapidly degraded; autophagy, by which damaged parts of a cell are engulfed in a lysosome; or apoptosis, programmed death of cells. Chronic alcohol consumption has been shown to decrease the rate of protein synthesis while simultaneously increasing the rate of protein degradation and leading to alcohol-induced muscle atrophy. We hypothesized that chronic alcohol consumption would decrease protein synthesis and increase degradation after immobilization, thus increasing recovery time. Three-month-old male Fischer-344 rats (n=12) were given either a Lieber-DeCarli control or alcohol liquid diet (average blood ethanol concentration of 0.1g/dl). After 10 weeks of alcohol feeding, the hip to knee joint of one hind limb was plaster-casted for one week to achieve atrophy of the quadriceps muscle. The cast was removed and the rats were allowed to recover for a week before the quadriceps muscle was collected at necropsy. qRT-PCR of ubiquitin protein ligases—MURF1 (Muscle RING-finger protein-1) and Atrogin; autophagy pathway genes - Atg5 and 7 (Autophagy protein 5 and 7) and Beclin1—were determined. The SUnSET (Surface Sensing of Translation) technique followed by western blots was used to determine protein synthesis. Myosin heavy chain immunostaining was performed to determine fiber size and type. The body weights were not different between alcohol treated and control animals. There was a main effect of alcohol and casting to decrease the quadriceps muscle weight. Our results indicate that there were no statistically significant differences in the expression of MURF1, Atrogin, and Atg5 between the alcohol casted, alcohol non-casted, control casted, and control non-casted groups. There was a trend for increased Beclin1 expression in both casted groups, while ATG7 was not expressed. Western blot analysis revealed no statistically significant changes of the protein expression among the groups. Our results indicate that there is impaired recovery following immobilization due to alcohol. However, it does not appear that protein synthesis or degradation pathways are altered after one week of recovery. Future studies will determine mechanisms of the decreased muscle weight, and whether alcohol affects protein homeostasis at an earlier time point.

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Valued-Based Reimbursement Options

Rising health care costs make it essential that progress is made in regards to how health care is paid for so that more can be done with the money available and to ensure that no part of the budget is unnecessarily spent. One of the ways to make progress is to change the way healthcare is paid for, which may mean switching for the current fee-for-service model to a more sustainable version that incorporates value-based reimbursement (VBR). Value-based reimbursement is payments or reimbursements that reward outcomes and are based on the economic value rather than on the short-run marginal cost of supplying a good or service.

To inform health policy makers about the potential healthcare cost-cutting benefits of the VBR payment model, a literature review was conducted on the current methods, concerns, and solutions of this reimbursement model. Through the search engine Google Scholar and using key words such as 'value based payment' and 'value based reimbursement', a total of twenty-five articles were found. After reading over this literature and comparing the results, a paper was written that compiled and synthesized the data.

After conducting this literature review, it was found that VBR has already been used by both private and state insurers throughout the country. Though many of the projects that implement VBR are still underway, there is evidence that VBR does, in fact, change the way services are provided for the better. For example, in cases where VBR is in effect, there are less instances of overutilization of services that are not needed because this policy does more to penalize that sort of practice. BlueCross BlueShield of Louisiana is currently implementing their own version of VBR, so seeing that this model does come with some risks and does leave some questions unanswered, it is safe to say Louisiana should wait until their project has commenced in 2018 and analyze the effects before completely switching over to the VBR model.

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Dr. Patricia Molina:
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Effect of Traumatic Brain Injury and Alcohol on Neuroinflammation and Oxidative Stress in the Hippocampus

Traumatic brain injury (TBI) affects an estimated 1.7 million individuals annually, many of whom are otherwise healthy individuals (e.g., contact-sport athletes, motorists, and military personnel). The post-TBI period is characterized by impaired neurobehavioral function and is frequently associated with escalated alcohol consumption. Previous studies from our laboratory examined the effects of alcohol consumption post-TBI on hippocampal memory-associated proteins, demonstrating that alcohol significantly decreased the expression of neuronal PAS domain 4 protein (NPAS4) and phosphorylated cAMP response element binding protein (pCREB). The present study aimed to determine potential mechanisms that may contribute to this decrease in hippocampal protein expression. Specifically, this study tested the hypothesis that alcohol consumption during the post TBI period would increase the expression of neuroinflammatory and oxidative stress markers in the hippocampus. Adult male Wistar rats were trained to self-administer alcohol in an operant setting (EtOH groups) or remained alcohol-naïve prior to being randomized to sham or TBI groups ($n=3-5/\text{group}$). TBI was produced by lateral fluid percussion (~ 2 atm; ~ 20 ms impulse) over the left sensorimotor cortex. Animals in EtOH groups continued to self-administer alcohol in the post-TBI period. Brains were excised 14-days post-TBI in order to determine the expression of neuroinflammatory and oxidative stress markers in the hippocampus by Western blots. Results showed a modest ($p<0.08$) main effect of alcohol to upregulate the expression of a neuroinflammatory protein caspase-1 compared to alcohol-naïve groups. There were no significant differences in the expression of neuroinflammatory proteins toll-like receptor 4 (TLR4) or NACHT, LRR and PYD domains-containing protein 3 (NLRP3). A similar modest ($p<0.08$) main effect of alcohol was observed on expression of the oxidative stress marker 4-hydroxynonenal (4HNE). There were no significant differences in the expression of the oxidative stress marker malondialdehyde (MDA). Taken together these findings suggest alcohol associated suppression in neurotrophic factor expression (NPAS4 and pCREB) in the hippocampus. The results do not support a significant role for neuroinflammation and/or oxidative stress in their modulation. However, the trend for increases in caspase-1 and 4HNE warrants further investigation to confirm whether these mechanisms contribute to the alcohol-induced decreases in hippocampal memory-associated proteins NPAS4 and pCREB.

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“Epithelial deletion of Sulf2 alters alveolar structure and gene expression in the lung”

RATIONALE: Heparan sulfate (HS) 6-O-endosulfatase 2 (Sulf2) is one of two HS 6-O-endosulfatases which selectively removes 6-O sulfate groups from HS. The removal of 6-O-sulfates modulates the effects of HS by altering binding sites for signaling molecules such as Wnt, fibroblast growth factors and transforming growth factor- β 1. In a comparison of lung tissues between wild type (WT) and Sulf2 conditional epithelial-specific knockout (CKO) mice, it was shown that the phenotypic appearance of the Sulf2 CKO mice resembles that of an emphysematous lung with enlarged air spaces. In this study, we aim to identify the molecular changes in Sulf2 CKO mice that result in the above observed phenotype.

METHODS: A proteomic approach was undertaken to identify differentially expressed proteins between WT and Sulf2 CKO mice. Quantitative real time PCR (qRT-PCR) and western blotting were then performed to confirm the genes identified by the proteomic approach. In addition, lung tissues from mice that have been exposed to nicotine vapor or air (control) were also analyzed by qRT-PCR.

RESULTS: Proteomics data revealed sixty-eight upregulated and eight downregulated proteins in Sulf2 CKO mice compared to WT. After categorizing the proteins by function, six genes (Parathyrosin, Prothymosin alpha, BPI fold-containing family B member 1, Glutathione S-transferase Mu 5 and 7, and Laminin subunit alpha-5) were chosen to be further tested based on their linkage in literature to emphysema. Glutathione S-transferase Mu 5 (Gstm5) mRNA showed a significant increase in the Sulf2 CKO compared to WT. Interestingly, Gstm5 mRNA was also increased in lung tissues from nicotine treated mice compared to air control mice.

CONCLUSION: Our study identified Gstm5 as an upregulated gene in Sulf2 CKO mice. As Gstm5 is expressed to detoxify electrophilic compounds including reactive oxygen species, we propose that Sulf2 CKO mice have increased susceptibility to environmental insults, and the overexpression of Gstm5 constitutes a compensatory response. Future work is needed to identify the role of Sulf2 in regulating the redox status of the lung.

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“QKI may suppress breast tumor cell metastasis by induction of E-cadherin and reduction of β -catenin”

Quaking (QKI) is an RNA binding protein belonging to a family of signal transduction and activation of RNA (STAR). QKI regulates RNA transportation, nuclear retention, mRNA, and miRNA stability and translation by directly binding the untranslated regions (UTRs) of target RNA. Recently, QKI was identified as a tumor suppressor of colon carcinogenesis. However, its role in breast tumor metastasis has not been reported. **β -catenin** is a transcriptional co-activator in the Wnt signaling pathway, which plays an important role in the tumor cell proliferation and metastasis by influencing transcriptional activity when forming complexes with the transcription factors of the LEF/TCF family in the nucleus and affecting tumor cell adhesion via formation of E-cadherin- β -catenin complexes in the membrane. **Our previous** results suggested that QKI could bind the putative binding sites in the 3' UTR of E-cadherin and increase the relative luciferase activity by dual-luciferase assay. **In this study**, we proposed that increased E-cadherin expression induced by QKI can anchor β -catenin to the cell membrane by forming a E-cadherin- β -catenin complex, thereby reducing β -catenin localization to the nucleus leading to suppression of breast tumor cell invasion and metastasis. **In order to test this hypothesis**, we are employing the immunofluorescent assay to confirm whether or not QKI stably expressed MDA-MB-231 cells have more co-localization of E-cadherin and β -catenin in the cell membrane compared with GFP control cells. Moreover, we measured the expression of E-cadherin and β -catenin using subcellular fragments from QKI stably expressed metastatic MDA-MB-231 and MCF10CAD1 α cells with respective control. Our results indicated that QKI overexpression could induce the expression of E-cadherin in the cell membrane and whole cell and reduce the expression of β -catenin in every subcellular fragment. We also measured LEF/TCF transcriptional activity in MDA-MB-231 cells using the TOP-flash luciferase reporter assay. The results showed a significant decrease in the LEF/TCF transcriptional activity in the QKI stably expressed MDA-MB-231 cells compared with GFP control cells. **In summary**, our current data shows that QKI may suppress breast tumor cell metastasis by induction of E-cadherin and reduction of β -catenin.

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LSU Health Sciences Center: School of Public Health, Tobacco Control Initiative

“Awareness and Utilization of Tobacco Cessation Services among Disadvantaged, Minority Female Smokers.”

Tobacco use has decreased greatly since the first Surgeon General's Report 50 years ago; however, smoking remains prevalent. In 2015, 36.5 million adults in the US reported current smoking, 17.7 million of which were women. Studies find that disadvantaged and minority groups such as African Americans are more likely to smoke, and while they are more likely to make a quit attempt, compared to whites they are less likely to quit. Tobacco treatment guidelines exist for health care providers to help patients quit in clinical care settings, but provider adherence often falls short, leading to disparities in treatment awareness and use among low SES, minority, and disadvantaged groups. More studies analyzing awareness and utilization of cessation treatment among disadvantaged, minority women are needed to design culturally appropriate smoking interventions. The Louisiana Tobacco Control Initiative (TCI) implements evidence-based cessation interventions for low SES patients in the state's public hospital system. TCI evaluates service delivery by administering a patient survey to obtain self-reported tobacco use and treatment.

The purpose of this study is to examine the awareness and utilization of tobacco cessation services among disadvantaged, minority women using TCI patient survey data. We hypothesize a correlation exists between awareness and utilization of tobacco services, and race, gender, and income levels.

We conducted a cross-sectional data analysis using retrospective data collected from a 2013 TCI Patient Survey administered to all patients ≥ 18 seen in primary care clinics operated by the seven LSU Health Care Services Division hospitals. Minority was defined by race (African American), and disadvantaged was defined by insurance (free, Medicaid, or Medicare), as a proxy for income. For this analysis, predictor variables included gender, race, awareness of cessation services, assistance used (class/counseling or medication), and insurance status; the outcome variable was quit attempt. We report descriptive statistics for the sample, and performed chi-square analyses to determine differences between expected and observed frequencies related to predictor and outcome variables.

The sample was predominantly disadvantaged white female smokers. In awareness, disadvantaged white females (D-W-F) were the most likely to be aware while advantaged African American females (A-AA-F) were the least aware of cessation services. For quit attempts, D-W-F made the most attempts and A-AA-F made the least. Disadvantaged African American females (D-AA-F) used classes/counseling the most and A-AA-F the least. A-AA-F used medication the most and D-AA-F the least. Chi-square (χ^2) analysis showed a significant ($P < 0.05$) difference between D-AA-F and D-W-F in awareness and a significant difference between African American female smokers and white female smokers in awareness.

Findings suggest there are variations in awareness, quit attempts, and assistance among those of different race or income levels.

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“Predator Odor Stress increases neuronal activation in the Amygdala”

Post-traumatic stress disorder (PTSD) is a very prevalent health issue in the world today. PTSD affects 7.7 million Americans, and approximately 30% of combat veterans alone. This disorder is characterized by avoidance behavior, hyperarousal, and an enhanced fear response after a traumatic situation. As a whole, the amygdala is hyperactive in humans with PTSD, but it is not known which sub-nucleus of the amygdala is contributing to this. The central amygdala (CeA) and basolateral amygdala (BLA) are sub-nuclei of the amygdala that both play a key role in stimulating a fear response and emotional arousal. The BLA helps to integrate sensory input for a stress response, and the CeA is the major output nucleus of the amygdala. The aim of this study was to assess neuronal activation in the sub-nuclei of the amygdala. The hypothesis of this study is that predator odor stress will increase neuronal activation in both the BLA and CeA of the high stress-reactive rats (avoiders). Rats were stressed using a 4 day conditioned place aversion (CPA) procedure to divide the rats into subgroups based on their avoidance of a predator odor-paired context. On Day 1, rats were allowed 5 minutes to explore two conditioning chambers with distinct tactile and visual cues. Sessions were videotaped and scored for amount of time spent in each chamber. Day 2 rats are placed in one of the chambers for 15 minutes with no odor. On Day 3, rats are placed into the opposite chamber from day 2 for 15 minutes with the urine-soaked sponge placed under the floor of the chamber, or no odor for control animals. On the fourth day, rats were again allowed to explore the two conditioning chambers in a 5 min video-recorded post-test. Avoidance was calculated as a difference score between Day 4 and Day 1. Avoiders displayed >10 s decrease in time spent in the odor-paired chamber on day 4 as compared to day 1, whereas Non-Avoiders had a <10 s decrease in time spent in odor-paired chamber. Seven days post-stress, half of the control rats and all previously stressed rats were (re-)exposed to the stress for 15 minutes. Brains were sliced and stained for c-fos using immunohistochemistry. Results from the avoidance graph showed that Avoiders show a significant difference in change in time spent in odor-paired chamber as compared to Non-Avoiders and unstressed Controls. The c-fos expression in the CeA graph showed that predator odor stress increases c-fos cell numbers in all stressed rats compared to unstressed Controls. The c-fos expression in the BLA graph showed that the predator stress odor does not increase BLA c-fos. In conclusion, predator odor stress causes avoidance in a subset of high stress-reactive rats, increases c-fos expression in the CeA of the rats (re-)exposed to it, and does not change c-fos expression in the BLA.

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“Identifying Barriers to Breast and Cervical Cancer Screenings among Undocumented Hispanic Women”

The purpose of this study is to identify barriers for breast and cervical cancer screenings among undocumented, Hispanic women and what factors are needed to increase screenings.

Low utilization rates in breast and cervical cancer screenings in Hispanic women creates high incidence and mortality rates. Breast cancer is the leading cause of cancer death in Hispanic women with approximately 2,800 Hispanic deaths and 19,800 incident cases in the U.S. in 2015 (Komen). Additionally, Hispanic women are two to three times more likely to develop cervical cancer than non-Hispanic White women (Daley). There were approximately 600 Hispanic deaths and 2,000 incident cases for cervical cancer in the U.S in 2015. High cancer incidence and mortality rates in Hispanic women demonstrate health disparities within our healthcare system that need to be addressed. Those who are underinsured/uninsured, have low-income and education levels, and are immigrants face limitations to seeking quality healthcare. This in turn creates many barriers to accessing healthcare for the underrepresented population such as undocumented Hispanic women. Barriers to seeking healthcare creates delayed screenings and treatment which often leads to high incidence and mortality rates in undocumented Hispanics and other underrepresented women.

Studies have shown that programs that provide language-specific educational sessions, ongoing mentorship and guidance, community outreach, and facilitated access to screening such as providing transportation increase screenings among undocumented Hispanic women. Other factors such as receiving a doctor recommendation, having support from a female relative/friend, and integrating cultural factors such as religion are considered to be particularly helpful as well. Future studies are focusing on cultural variations in Latina subgroups in order to further increase cancer screenings among undocumented Hispanic women.

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“Characteristics of Patients Who Test False Positive on HIV Screening in the Emergency Department”

Background: A false positive HIV test result occurs when a patient who is actually not infected with the virus tests positive on an HIV screening assay. Reporting a false positive HIV result can be psychologically traumatic to the patient. And patients who have a history of a false positive reaction may ignore a true positive result in the future. HIV testing needs to become more efficient to reduce the rate of false positive results. Rational decision making about appropriate public health policies has been impeded by a lack of validated data on the rate of false positive diagnoses in HIV screening programs (Burke DS, et al). There are many factors that have an effect on the accuracy of HIV diagnosis. For example, false-positive results can occur due to inaccurate algorithms in HIV screening programs (Shanks L, et al). Fourth generation tests have a much lower likelihood of a false positive result.

Methods: This study is a retrospective review of records of patients who tested false positive on HIV screening in an urban academic Emergency Department between the months of August 2015-May 2017. We correlated gender, age, race/ethnicity, sexual orientation, drug use, alcohol use, homeless status, comorbidities, and location of testing (main Emergency Department vs. Urgent Care setting) with the likelihood of a false positive result. We hypothesized that there are correlations between certain demographic characteristics and false positive HIV test results.

Results: 62.5% of the 16 false-positives were male, 68.8% were black, 62.5% were diagnosed in the ED, and 50% were in the 25-34 age group. We noted no correlation between false-positive HIV Ag/Ab results and the demographic variables, with the exception of age. Patients between 25-34 years has a higher likelihood of testing false positive ($p= 0.33$). Although Blacks were almost four times more likely and males were twice as likely to have a false positive result, due to the predominance of blacks and males in the testing cohort, race and gender were not statistically significant variables ($p= 0.5$; $p=0.093$)

Conclusion: While certain correlations between false-positive HIV Ag/Ab results and the demographic variables studied demonstrated a trend, these were not statistically significant (p set at 0.05 for significance). Our small sample size may be a factor; however, the small number of false positive reactions occurring since the testing program began in March 2013 (86,977 TN/ 86,977 + 49 FP) supports the high specificity of the fourth generation HIV1/HIV2- Ag/Ab used in the UMC ED (specificity= 99.9%). Future studies should include a larger sample size in order to better determine the relationship between false positivity and demographic variables. This may be accomplished through collaborative multicenter studies.

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“Assessing Skill and Scrupulousness - A Retrospective Quality Improvement Analysis of Anal Diagnostics”

Background:

The prevalence of anal cancer and anal intraepithelial lesions are 5-50 fold higher in HIV positive populations. Unlike most cancers, the detection, prevention, and expunction of anal cancer require more complex screening. Typically, the screening includes anal cytology proceeded by high-resolution anoscopy (HRA). However, the reliability of anal pap smears having the ability to predict anal disease is still unknown. This cross-sectional study will ultimately audit the correlation of anal cytology and biopsies from HRA in a cohort of HIV positive patients.

Methods:

Demographic data was collected from the Epic Systems Corporation (EPIC) to analyze the results of HIV positive men and women visiting the Infectious Disease Center at University Medical Center in New Orleans. The results from HIV positive men and women receiving anal cytology and biopsies in the same visit were recorded. The clinical information included CD4 counts and viral loads, while the anal cytology was compared to the worst biopsy result from that day's visit.

Results:

The cytology and histology results for the 42 HIV positive patients taken during the same visit were recorded. The cohort was 62% male, 52% African American, with a mean CD4 count of 402 and a median HIV viral load of 371. Of the 109 visits the patients had at the ID clinic, the results reveal that 88% of patients had anal dysplasia with 46% having AIN 1 (low-grade). Eighty-five percent (6/7) of patients with a high grade Pap smear (HSIL) had high-grade anal biopsies as compared to 20% (1/5) of patients with a normal pap smear having high-grade anal biopsies ($p = .02$). The significance of atypical squamous cells of undetermined significance (ASCUS) pap smears is unknown; however, almost half of the ASCUS diagnosed patients had high-grade lesions on biopsies. There was one discrepant subject in the study with a normal Pap smear result and an AIN3 high-grade lesion.

Conclusion:

Overall, there was a good correlation between the cytology and histology results. Most patients diagnosed with an abnormal anal pap were also diagnosed with significant anal disease through biopsies. The results also indicate that ASCUS pap smears should be followed with histology to detect high-grade lesions. Finally, the rate of false-negative anal Pap smears needs to be better evaluated in longitudinal studies.

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Characterization of a Mesoamygdala Circuit

Alcohol use disorder (AUD) is a chronic disease characterized by the uncontrolled drinking of alcohol. In the United States alone, 16 million Americans are affected, and only 3% of people are currently receiving treatment, highlighting the need for more effective therapeutic options. The neural adaptations that occur during the development of AUD are not entirely understood. Our lab focuses on brain stress and reward systems, which may be sensitive to alcohol exposure. Specifically, we focus on the ventral tegmental area (VTA), a source of dopamine (DA) neurons in the mesolimbic reward system, and the central amygdala (CeA), implicated in stress and dependence. There is a direct connection between the VTA and CeA, but this circuit is largely uncharacterized. Our overarching hypothesis is that this circuit is critical in the development of dependence-induced alcohol self-administration. Here we utilized retrograde tracing and immunohistochemistry (IHC) to characterize this circuit.

To identify CeA projecting neurons, we delivered stereotaxic injections of green fluorescent retrobeads into the CeA of adult male and female Long Evans rats. Immunohistochemistry and fluorescent microscopy were then used to locate retrobead-containing (CeA projecting) neurons in the VTA. To determine whether these cells are dopaminergic we stained for tyrosine hydroxylase (TH), the enzyme that catalyzes rate-limiting step of DA synthesis. Our data indicate that approximately 60% of CeA projecting VTA and substantia nigra *pars compacta* neurons co-express TH. No significant difference in males and females was observed. Future research will focus on this circuit in alcohol dependent rats to determine whether this circuit is activated in the development of dependence and could potentially be a target for intervention.

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“Reduction of Alcohol Use and a Comparison Between Phosphatidylethanol and Self Reported Alcohol Use in the WELL Program ”

There are currently over 35.7 million people living with HIV in the world, and 1.2 million of these people live in the US. The state of Louisiana is one of the leaders of the HIV epidemic; as well as one of the leading states in alcohol drinking disorders. When people living with HIV/AIDS (PLWHA) abuse alcohol there is an increased risk of sexual risky behavior, a further deprived immune system, as well as biomedical consequences including lower rates of adherence to antiretroviral therapies. The WELL (Wellness through Empowerment, Learning, and Living) evidence-based intervention has been designed to reduce alcohol use and risky sexual behavior, and improve overall health in PLWHA. The objective of the current analysis was to evaluate the change in alcohol use among PLWHA after participation in the WELL intervention study comparing measures of self-reported alcohol use and the biological marker phosphatidylethanol (PEth). Participants were recruited within the greater New Orleans area, predominantly from the University Medical Center-New Orleans HIV Outpatient (HOP) Clinic. Potential participants were screened for eligibility which includes being over the age of 18, a diagnoses of HIV, and having used alcohol in the last 30 days. Eligible participants were randomized into an intervention group (the WELL group), which received five educational sessions or into a treatment as usual (TAU) group. The demographics were recorded, along with multiple assessments, and a PEth blood test was taken at baseline, a three-, six-, and at 12-month follow up visits. A repeated measures preliminary analysis with unstructured covariance was conducted using SAS version 9.4. A total of 143 participants have been currently enrolled with 72 participants in the TAU group, and 71 participants in the WELL group. Based on preliminary results from these participants, intervention education seems to decrease abusive alcohol behavior as well as other associated risky behaviors. Alcohol Use Disorders Identification Test (AUDIT) scores and self-reported drinking in the past 30 days decreased for both the TAU and WELL groups, but the decrease was not statistically significant. There is a correlation between PEth values when compared to the time line followback ($r=0.540$; $p<0.001$), but not the AUDIT ($r=0.20$; $p=0.04$), both self-reported measures of alcohol consumption.

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“RNAseq Analysis of BPA Effected Human Adipocytes Grown in SWAT Culture”

Introduction:

Bisphenol-A (BPA) is a common chemical component of plastics and epoxy resins. Humans are exposed to BPA through dietary and environmental means because BPA is found in the lining of polycarbonate plastic products, disposable water bottles, plastic food containers, and baby bottles. Preliminary data suggest that BPA can act as an endocrine disruptor and promote obesity. Using a novel, microphysiologic model of human primary white adipose tissue (WAT), we sought to investigate BPA's obesogenic effects through transcriptional profiling.

Methods:

Under an IRB-approved protocol, primary fresh human WAT was collected from discard specimens produced during elective surgery. The WAT was cultured in our novel sandwiched white adipose tissue (SWAT) system. These specimens were treated with 1nM BPA, 10nM BPA, 1nM BPA + ICI, and 10nM BPA + ICI (ICI is an estrogen receptor disruptor). Controls were cultured with no BPA. At 1, 3, and 5 days, the adipocytes were reisolated by incubating with .5mg/ml collagenase in PBS for 1 hour and then pouring digested mix through a tissue strainer. Total RNA was collected from the adipocytes by using a standard RNA isolation protocol with trizol.

RNASeq was performed using Illumina Next Generation Sequencing.

Results/Conclusions:

Due to the lengthy process of cell cultivation and RNAseq and the timeframe of the summer program, no data could be obtained in time for this presentation. We expect that certain adipocyte genes will be upregulated by BPA, but the genes isolated from cells treated with ICI will not be effected.

If the expected results occur, our study confirms preliminary studies that BPA acts as an endocrine disruptor and offers a novel model to study BPA obesogenicity.

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“Cardioprotective Effects of Lysyl Oxidase Inhibition”

Abstract

According to the American Heart Association, more than 6 million Americans are living with heart failure, and every minute at least one person is diagnosed with heart failure. Adverse extracellular matrix (ECM) remodeling and fibrosis contribute to the development of heart failure. Fibrosis occurs when there is an excess deposition of collagen. The deposition of collagen is dependent on the collagen cross linking enzyme lysyl oxidase (LOX), which is produced by fibroblasts. The production of both LOX and collagen are upregulated in response to left ventricular wall stress. In this study, we use a rat model of heart failure to study the potential cardioprotective effects of a LOX inhibitor to prevent adverse ECM cardiac remodeling, dysfunction, and heart failure. We hypothesize that LOX inhibition will reverse volume overload (VO) cardiac ECM remodeling and dysfunction in rats with established disease. Male Sprague-Dawley rats were given volume overload (VO) stress by surgically creating a shunt between the aorta and vena cava, which is called an aortocaval fistula (ACF). After 8 weeks, a LOX inhibitor, beta-aminopropionitrile (BAPN: 100mg/kg/day), was continuously delivered with an osmotic minipump. At 14 weeks (i.e., 6 weeks of BAPN), echocardiography was used to assess progressive alterations in cardiac ventricular structure and function. Left ventricular (LV) catheterization was also used to assess alterations in contractility, dysfunction along with histological staining of collagen and western blot analysis to determine protein expression. Our data demonstrate that LOX inhibition attenuated the VO-induced increases in cardiac stress, and LV collagen. Echocardiography and catheterization measurements both indicated improved cardiac function post-VO in rats treated with the LOX inhibitor as opposed to the SHAM (control). Western Blot analysis indicated increases in protein expression of Focal Adhesion Kinase (FAK) and $\alpha 6$ -integrin, markers of cell adhesion to the ECM. Our findings lead us to conclude that LOX inhibition is cardioprotective in the volume overloaded heart.

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“Postinhibitory Rebound Gamma in Real Scale Interneural Inhibitory Networks of Rat Hippocampal CA1 region, using High Performance Computing”

Oscillations with higher frequency bands have been linked to cognition. In particular, gamma oscillations, with a band frequency of 25-100 Hz, have been associated with cognitive functions such as information processing, encoding, and retrieval. Abnormal gamma oscillations have also been correlated with pathological conditions, with less power of gamma oscillations linked to schizophrenia and less gamma connectivity linked to bipolar disorder. In general, understanding of gamma networks can greatly improve present knowledge of brain processes and can help solve conditions associated with it. There are several mechanisms of gamma oscillations related to activity just inhibitory interneurons (ING) or interplay between interneurons and pyramidal cells (PING). This study focuses on Interneuronal Network Gamma (ING) oscillations. PostInhibitory rebound, a mechanism which includes the tendency for voltage to rebound after an inhibitory event potentially causing a spike, is associated with ING oscillations in the presence of resonator cells and is a key factor within the simulation demonstrated in this experiment to produce gamma frequencies (Tikidji-Hamburyan et al. 2015).

Previously, a simulated network of 300 Hodgkin-Huxley type cells (Tikidzhi-Hamburyan and Canavier in preparation) was used to demonstrate that different spiking patterns, including global synchrony, two clusters in antiphase, a transitional region, and asynchrony, were exhibited in different parameter regimes of the model network. The current study scaled the network up to of 6000 cells in order to test whether the inherently larger amount of variance in a larger network. 6000 cells is on the order of the number of parvalbumin positive basket cells (PV+) in the hippocampal CA1 region of rats (Bezair et al., 2013). Due to the large computational requirements of running a 6000 cell simulation (36000 differential equations in total), the usage of high performance computing (HPC) through the Louisiana Optical Network Infrastructure, one of the most advanced fiber-optics network that provides supercomputer resources to academics around the state, was required along with the use of a message passing interface (MPI). Two different simulations of 6000 cells were run for all four different oscillation patterns, one simulation with binomial variation in the number of connections per cell and the other without. The resulting data was compiled into a raster plot along with the voltage traces of 10% of the simulated neurons. In conclusion, after analysis of results, the real-scale size of the 6000 population hippocampal CA-1 simulation of rats produced results consistent with that of 300 neuron model used in previous studies, even with binomial variation in connections per cell.

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Prof. Carmen C. Canavier, PhD, Ruben A. Tikidji-Hamburyan, PhD
LSUHSC**“Using phase resetting theory to understand the effect of Propofol on a population of cortical LTS interneurons”**

Propofol is a GABA_A-potentiating general anesthetic drug that increases the maximal conductance and time constant of decay of the synaptic GABA_A current. Like most drugs in this category, at low doses propofol is capable of “paradoxical excitation”, characterized by an increase in EEG power in the beta band (12.5-25 Hz) and a decrease in power in the lower frequency band (3.5-12.5 Hz), which includes the alpha band. Previous simulations of cortical networks (McCarthy et al., 2008) suggest that this paradoxical excitation is in part due to the emergence of antiphase firing of two clusters of low threshold spike (LTS) interneurons. At doses of propofol sufficient to induce anesthesia, power in the beta band should decrease and that in the lower frequency bands should be elevated. However, in the previous simulations, the antiphase clusters contributing to beta band power persisted at high doses. We found that if an axonal conduction delay is added to the interneuronal network, the clusters collapse into global synchrony, resulting in a population oscillation frequency in the alpha band instead.

We were able to explain our results using phase resetting theory. We conceptualized the network as consisting of two clusters; then we applied phase resetting theory to a single cluster with a self-connection that represents the other neurons in the cluster. The phase resetting curve (PRC) is measured by applying a single input, corresponding to that received from the other cluster, at different points during the oscillatory cycle. The input from the partner cluster causes the timing of the next action potential to be delayed; the amount of this delay depends on the point in the oscillatory cycle at which the input is received. The phase resetting curve can then be used to predict the existence and stability of both the two cluster antiphase mode and global synchrony. These simulations and their analysis may provide a partial explanation for the changes in relative power in the alpha and beta frequency bands for the EEG at high versus low doses of propofol and potentially further our understanding of the mechanisms of general anesthesia.

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“MYC Expression is Preferentially Targeted by miR-3189-3p in Triple Negative Breast Cancer Cells over Normal Breast Epithelial Cells”

Breast cancer is one of the most frequently diagnosed and leading causes of cancer death in women, with 1.3 million cases being diagnosed annually worldwide. Though mortality has been declining due to improved treatments like hormone therapy and drugs that target receptors of hormone epidermal growth factor 2, estrogen, and progesterone (common markers of breast cancer), there exists a distinct subtype of breast cancer that lack these molecular markers - the Triple Negative Breast Cancer (TNBC). Representing approximately 10-20% of all diagnosed breast cancers, patients with TNBC have a poorer outcome compared to the other subtypes of breast cancer as TNBC is more aggressive and difficult to be treated. Due to its lack of the common receptors used for many cancer treatments, there exists a well-defined need to identify different biomarkers and treatments for TNBC. MYC, a regulatory gene involved in cell cycle progression, apoptosis and cellular transformation, is constitutively expressed in TNBC and has been shown to be an effective biomarker and treatment target for the cancer.

MicroRNAs, small non-coding RNA molecules that target approximately 60% of the genes of humans and other mammals, have been established to play critical roles in cancer pathogenesis. Our lab has previously identified and analyzed a specific miRNA, 3189-3p, which is located in an intron of the growth differentiation factor 15 (GDF15). This specific miRNA has been demonstrated to possess anti-tumor effects and inhibit the proliferation and migration of glioblastoma cells, the most aggressive brain cancer, and function as a tumor suppressor.

Although MYC mRNA is not a predicted target of miR-3189-3p, we found that overexpression of miR-3189-3p represses MYC protein expression; therefore, to better understand the molecular mechanisms of miR-3189-3p in TNBC and its potential for TNBC treatment, here we report our studies to demonstrate the specific effects of miR-3189-3p on MYC levels in breast cancer cells in comparison to normal cells. The two cell lines used in the study were MDA-MB-231, a TNBC cell line, and MCF10A, an immortalized human mammary epithelial, non-tumorigenic cell line commonly used as an effective *in vitro* model to study normal breast cell function and transformation. As hypothesized, overexpression of miR-3189-3p resulted in the down regulation of MYC expression in the TNBC cells (MDA-MB-231); however, interestingly, we found that 3189-3p did not affect MYC expression in the MCF10A cells, suggesting that the anti-cancer, MYC-targeting properties of the miRNA in TNBC is not as effective in normal cells. In addition, overexpression of miR-3189-3p has less effect on the cell proliferation and morphology of MCF10A cells. In comparison, significant and apparent morphological changes in MDA-MB-231 cells were observed upon transfection of the miRNA, which further supports the aforementioned conclusion.

We conclude that miR-3189-3p exhibits significant and preferential inhibitory effects on c-MYC in MDA-MB-231 cancer cell proliferation but not in normal MCF10A cells. Further experimentation will be needed and implemented to holistically understand the role of MYC in this process; however overall, miR-3189-3p possesses the potential to be used as an effective cancer therapeutic agent, especially for the difficult-to-treat TNBC.

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“Utility of PCR and Sanger Sequencing for HPV Genotyping in Clinical Specimens”

Background and Objective: Human Papillomavirus (HPV) is the main etiological agent in dysplasia and cancer of the uterine cervix, with the primary oncogenic genotypes being HPV 16 and HPV 18. Infection with these and other classical high risk HPVs are currently being prevented through the expansion of vaccination efforts. In contrast, little focus is given to other high and low risk types and the role they play in contributing to cervical abnormalities. Given that there are at least 160 different HPV genotypes, it is necessary to investigate those that will not be impacted by vaccination as they have the potential to become predominant catalysts of cervical dysplasia and cancer in the future. Additionally, genotype prevalence may differ in developing countries with high burdens of HPV related disease. Current clinical and research screening tests focus on a limited subset of HPV genotypes. Future surveillance measures in vaccinated and high-risk populations will require more comprehensive HPV genotyping methods. The goal of this project is to determine if consensus PCR with Sanger sequencing can provide adequate HPV genotyping information compared to commercially available PCR and hybridization based genotyping.

Methods: This project examines genotyping modalities across two different cohorts, women attending a Colposcopy clinic in New Orleans and pregnant, HIV positive women from Kenya. Cervical samples from both cohorts were collected and DNA was extracted (Qiagen). Extracts were PCR amplified using degenerate consensus PCR primers (MY09/11) and a proprietary enhanced PCR primer mix (PGMY09/11, Roche Molecular Systems), both of which amplify the L1 gene of HPV. Amplicons were then separated by agarose gel electrophoresis and hybridized to Roche Linear Array strips containing probes for 37 HPV genotypes. Amplicons generated by MY09/11 PCR were also subjected to Sanger sequencing. HPV genotype concordance was examined across the different testing modalities.

Results: In the Kenya HIV+ pregnant women cohort, 30 samples were tested using MY09/11 PCR plus Linear Array. Four samples were HPV positive based on presence of the 450bp amplicon on agarose gel, but tested negative for all 37 HPV genotypes on Linear Array. These samples were submitted for Sanger sequencing. Of the 4 samples, 3 samples had HPVs that were not present on the Linear Array, 1 presented a missed HPV 16 infection, and 1 sample aligned to human chromosomal DNA. In the New Orleans cohort, 24 samples were tested by both MY09/11 and PGMY09/11 PCR. There were discrepancies in 14 of the samples and 9 samples chosen as the most inaccurate between the assays were submitted for Sanger sequencing.

Conclusion: The comparison of MY09/11 and PGMY09/11 Linear Array approaches have shown there is frequently discrepant results that can be clarified with sequencing technology. The MY09/11 PCR assay is more affordable, but is not as accurate as the commercially available, enhanced PGMY primer PCR assay. The commercial Linear Array assay also suffers in that it only tests for 37 types of HPV and is subjected to cross reactive hybridization amongst several high-risk HPVs.

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“Tri, Tri Again: A Trinucleotide Repeat Expansion Mouse Model”

Friedreich ataxia (FRDA) is an inherited autosomal recessive neurodegenerative disorder caused by an expanded GAA•TTC repeat within the first intron of the Frataxin (*FXN*) gene. The expanded GAA•TTC trinucleotide repeat causes decreased *FXN* expression and ultimately cell death in affected tissues. There are no treatments available for this disease. Ten to twenty years after symptoms develop, patients are usually confined to a wheelchair and they often die from cardiomyopathy. How the GAA•TTC expansion decreases *FXN* transcription, and the downstream pathways that lead to FRDA are still not completely understood. Our lab focuses on the mechanisms that cause somatic expansion of the GAA•TTC repeat.

We have recently measured somatic expansion in a mouse model. We bred male “YG22” FRDA mice that are heterozygous for the knockout of the mouse *Fxn* (*mFxn*) and hemizygous for the Tg(*FXN*)YG22Pook/human Frataxin transgene (*hFXN* FRDA) with female wild type C57BL/6J mice. This allowed for observation of repeat expansion without the limitation of lowered Frataxin expression, whether it was mouse or human. Genotyping was used to determine if the offspring were carriers of the *hFXN* FRDA transgene. If a mouse tested positive for the transgene, brain and cerebellum tissue were analyzed for their GAA•TTC expansion after X amount of time and compared to the inherited GAA•TTC repeat size of non-expanding tissues. Repeats expanded most rapidly in the cerebellum which is consistent with an ataxic phenotype.

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“RBP2 and Notch Signalling Crosstalk in ER+ Breast Cancer Cells”

Approximately 80% of breast cancers are estrogen receptor positive (ER+), for which antiestrogens such as tamoxifen are the primary method of treatment. A substantial proportion of patients with localized disease and almost all patients with advanced disease who initially respond to tamoxifen develop resistance, which is a major cause of mortality. Interestingly, patients who relapse on tamoxifen often respond to other hormone-based therapies such as aromatase inhibitors, which inhibit the production of estrogen, implying that estrogen signaling continues to play a major role in breast cancer progression. The mechanisms of breast cancer resistance to tamoxifen are poorly understood, however it is known that the Notch signaling pathway is involved in this process. Canonical Notch signaling, which is inhibited by estrogen in the breast, is critical for cellular proliferation and stem cell maintenance. Conversely, treatment with tamoxifen aberrantly *activates* Notch signaling in the breast, resulting in the Notch-dependent expression of estrogen-responsive genes leading to chemoresistance. Understanding how tamoxifen promotes the aberrant, Notch-dependent activation of ER signaling is critical for the development of new therapies to treat tamoxifen-resistant breast cancers.

One possible way Notch overrides tamoxifen inhibition of ER signaling involves the biological crosstalk between the Notch and ER signaling pathways. This crosstalk is may be mediated by RBP2 (retinoblastoma binding protein 2), a transcription factor and histone demethylase that regulates cellular proliferation, migration and invasion during cancer progression. RBP2 binds CSL, a member of the Notch transcription complex, as a *corepressor* to inhibit Notch-dependent transcription. Upon activation, Notch displaces RBP2 to activate gene expression. In contrast, RBP2 binds ERα as a *coactivator*, enhancing estrogen-dependent transcription. Therefore, we hypothesize that RBP2 facilitates crosstalk between the Notch and ER signaling pathways to contribute to ER+ breast cancer tamoxifen chemoresistance. We propose that RBP2 is a key molecule in chemoresistance through a mechanism where Notch cooperates with RBP2-bound ER to activate estrogen-responsive genes.

The goal of this summer research was to understand the effect of RBP2 and Notch signaling. RBP2 was modulated in two ways: 1) RBP2 was knocked down by siRNAs to affect overall availability of RBP2, and 2) RBP2 was inactivated by CPI-455, a small molecule inhibitor of RBP2's demethylase activity. Using these mechanisms to modulate RBP2 function, we studied the effect of RBP2 loss on proliferation and estrogen-dependent genes.

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Expression and Purification of pGEX-4T3-6A-OptParkin

Components of the ubiquitin proteasome system make up nearly 10% of the human genome, while prokaryotes have no analogous pathway for targeted degradation of proteins and cell regulation. These processes function through the covalent conjugation of ubiquitin to cell proteins through a multi-enzyme pathway. In this pathway, the ubiquitin ligases recruit a charged E2~ubiquitin carrier protein and catalyzes the transfer of the activated ubiquitin from the E2 to a protein substrate.

This project is centered on the ubiquitin ligase Parkin. Parkin is encoded by the PARK2 gene in humans and mutations in this gene are known to cause a familial form of Parkinson's disease that disrupts regulation of mitophagy. Parkin is of particular interest as an example of a hybrid ligase. The RING (Really Interesting New Gene)-Between-RING family of E3 ligases functions with a canonical RING domain and a catalytic cysteine residue formerly restricted to HECT (Homologous to the E6AP Carboxyl Terminus) E3 ligases; therefore, it is identified as a RING/HECT hybrid. To better understand the enzyme, we expressed and purified Parkin with the long term goal of high resolution structural and mechanistic studies.

Prior to these experiments, a synthetic gene was optimized for bacterial codon usage in order to reduce potential cleavage products during expression and purification. The synthetic Parkin coding region was subcloned into a pGEX-4T3-6A expression plasmid; a synthetic gene was optimized for bacterial codon usage in order to reduce potential cleavage products during expression and purification. Our studies showed that yield and stability were enhanced by inserting a -Ala₆- spacer between the GST and Parkin coding regions that mimics a previously inserted Flag epitope. Protein expression was conducted in *Escherichia coli* BL21 (DE3) cells. Cells were induced with 0.4mM IPTG for 17 hours, the protein was purified by affinity chromatography using glutathione Sepharose. The molecular weight of the resulting GST-Parkin by SDS PAGE was 76 kDa, in good agreement with the expected value of 77 kDa. Once purified, biochemically defined assays demonstrate that pGEX-4T3-6A-OptParkin is active in forming a thioester intermediate with ¹²⁵I-ubiquitin using Ubc5B. We then cleaved GST from the fusion protein and when the GST is cleaved the enzyme loses activity. These results are consistent with other HECT ligases, for which the GST moiety promotes activity of the enzyme by stabilizing an oligomeric state. This work establishes reporter functions for pGEX-4T3-6A-OptParkin, which can be used for future mechanistic studies.

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“Intrarenal Kallikrein-Kinin System (KKS) in Male Mice with Prorenin Receptor Deficiency in the Collecting Ducts of the kidneys”

The renin-angiotensin system (RAS) is essential for the regulation of blood pressure and electrolytes balance. Within this system, renin catalyzes the conversion of angiotensinogen into angiotensin I (ANG I). ANG I is then converted into angiotensin II (ANG II) by angiotensin converting enzyme (ACE). The prorenin receptor (PRR) is a new described RAS component that activates prorenin and increases renin activity, therefore it might be responsible for the augmentation of the major effector hormone ANG II. ANG II stimulates vasoconstriction, increase of sodium reabsorption, inflammation, and cell proliferation.

In the kidney, in addition to RAS, there is another system called the kallikrein-kinin System (KKS). In the KKS, kininogen is converted into bradykinin by the action of kallikrein. Unlike ANG II, bradykinin exerts effects such as vasodilation, decreased sodium reabsorption, anti-inflammation, and decreased cell proliferation. Importantly, there is a commonality between the two pathways. ACE acts differently in the RAS and KKS. In the RAS, ACE leads to ANG II production, whereas in the KKS, it degrades bradykinin. Therefore, when ANG II levels are decreased, there should be less ACE available to degrade bradykinin.

Dr. Prieto has recently generated a transgenic mouse model with specific cell type deletion of the PRR (*Atp6ap2*) gene in the collecting duct of the kidney (_{CD}PRR-KO). These mice exhibit an attenuated blood pressure response during chronic ANG II infusion due to decreased formation of intratubular ANG II along with alterations of sodium reabsorption and excessive diuresis. The status of intrarenal KKS in this mouse model is unknown. The present study aims to examine the intrarenal KKS in _{CD}PRR-KO male mice. B157-J mice (N=9) were randomly divided into 2 groups: 1) Controls (sham operation) and 2) Treated with Icatibant® (B2 receptor blocker, 250 ng/kg during 48h via SC minipump). After 7 consecutive days of training, mice were subjected to blood pressure measurements by tail-cuff method before and after treatment. In addition, metabolic caging was accomplished for the collection of body weight, food intake, urine and blood samples. After treatment was completed, mice were euthanized by conscious decapitation and final harvesting of blood and kidneys. Intrarenal augmentation of the KKS may help to partially explain the blood pressure and electrolytes alterations in this mouse model. The results will be discussed in detail during the poster presentation.

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“*Candida albicans* interactions with host stressors in the GI tract”

Candida albicans is the most common opportunistic fungal pathogen in humans, it usually is a commensal fungi found in most healthy individuals. *C. albicans* causes localized infections such as mouth and vaginal thrush, along with invasive disseminated Candidiasis that has a mortality rate of ~40%. The risk for invasive Candidiasis increases in immunocompromised patients such as: the elderly, young, people undergoing HIV or cancer treatment, and patients in the hospital. In hospitals *C. albicans* is the 4th most common acquired infection in the ICU (intensive care unit). Patients often contract Candidiasis by formation of biofilms around catheters placed in the intestinal lining. Intestinal cell encounters can therefore influence body's first response to infection, controlling pathways such as pathogen binding, IgA processing, chemokine and cytokine production, a physical barrier for the microorganisms, and activation of the adaptive and innate immune system. In previous studies in this laboratory it has been shown that the immune response in epithelial cell lines are not consistent, which is why we focused on the interaction between *C. albicans* and a physiologically relevant human intestinal epithelial cell line, Caco-2.

In this project we looked at immune response in Caco-2 cells to the presence of *C. albicans*. This was done by using a luciferase assay to determine NFκB transcriptional activation and ELISA test for IL-8 production. We also examined the expression of PAMPs (pathogen associated molecular patterns) by immunofluorescent microscopy. Additionally, the reaction of *C. albicans* to common environmental stresses in the GI tract such as hypoxia and low iron growth was assessed. Further research could be done by looking at adhesion by *C. albicans* to Caco-2 cells at different time points and MOI (multiplicity of infection). Finally cell interactions could be performed under hypoxic and low iron concentrations and with other *Candida* species. The regulation of growth and expression of cell wall components by *C. albicans* when in the GI tract may aid in development of both diagnostic and antifungal strategies.

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“Association of Dopamine Signaling with Pain Avoidance Behavior in Alcohol Dependence and Withdrawal”

In this project, we focused on identifying behavioral and molecular markers of alcohol dependence that may lead to the discovery of new or improved treatments for alcohol use disorder. To model alcohol use disorder, rats were exposed to chronic intermittent ethanol vapor for 14 hours/day to induce alcohol dependence.

Our research measured escalated drinking and increased pain-like behavior in alcohol-dependent rats. Behavioral testing was completed when the rats were in acute alcohol withdrawal (8 hours post-vapor exposure). To measure escalated drinking, animals were trained to self-administer alcohol in operant chambers. We found that alcohol-dependent animals consumed approximately twice as much alcohol as non-dependent animals.

To measure pain-like avoidance, we used the mechanical conflict-avoidance system, which measures the latency for a rat placed in an aversive light box to walk across probes of adjustable heights (0.5mm to 5 mm) to reach a rewarding dark goal box. A longer latency to exit onto the probes indicates an increased motivation to avoid pain. During the mechanical conflict-avoidance test, alcohol-dependent rats demonstrated a longer latency to exit onto the probes at 0.5 mm than non-dependent rats during acute alcohol withdrawal. Alcohol-dependent rats demonstrate increased pain-like avoidance to normally non-painful stimuli, indicating the emergence of allodynia during acute withdrawal.

The striatum is a critical component of the brain's reward and pleasure circuits, and it has been shown to play a major role in the development of addiction. Previous research has shown that acute alcohol exposure increases dopamine levels in the striatum. In contrast, dopamine levels are decreased during acute withdrawal, which inhibits brain reward systems and promotes the development of negative emotional states. Tyrosine hydroxylase (TH) is the rate-limiting enzyme involved in dopamine biosynthesis, activated via serine 40 phosphorylation. Thus, quantifying phosphorylated tyrosine hydroxylase at serine 40 (pTH40) is an effective measure of dopamine signaling.

We measured phosphorylated TH40 in the striatum of alcohol-dependent and non-dependent animals. We found no group differences in TH40 phosphorylation in the ventral striatum ($p > 0.05$). Pain-like avoidance was negatively correlated with phosphorylation of TH40 in the ventral striatum of non-dependent animals ($r = -0.7758$; $p < 0.05$), but not in alcohol-dependent animals ($r = -0.0091$; $p > 0.05$). Our findings indicate that regulation of striatal dopamine signaling is closely associated with pain avoidance-like behavior in non-dependent animals, but not in alcohol-dependent animals.

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“CRISPR-Cas9 Gene Editing for Cystic Fibrosis Genetic Therapy”

Cystic fibrosis (CF), an autosomal recessive genetic disorder, affects ~37,000 patients in the United States and ~70,000 patients worldwide. The disease-responsible gene is CF transmembrane conductance regulator (CFTR), which normally encodes a cAMP-activated chloride channel. Clinically, CF lung disease is responsible for most of the disorder's morbidity and mortality. $\Delta F508$, the most-common CF- causing mutation, is a deletion of phenylalanine at position 508 in the CFTR protein. The $\Delta F508$ mutant CFTR fails to reach the cell membrane to transport chloride ions, which causes a thick, sticky buildup of mucus in the lungs, persistent bacterial infection, and eventually pulmonary failure.

Genome editing, using the emerging Clustered Regularly Interspaced Short Palindromic Repeats-associated protein 9 (CRISPR-Cas9) technology, offers a novel platform for gene repair. Cas9, a DNA endonuclease, has the ability to create a double strand break (DSB) at a specific site in the genome, directed by a single guide RNA (sgRNA). This DSB can then be repaired by the cell using homology-directed repair (HDR). To explore the CRISPR-Cas9 approach to correct the CF $\Delta F508$ mutation, we constructed a single plasmid that expresses the Cas9 protein, the sgRNA that targets the $\Delta F508$ CFTR mutation, and the transfection reporter enhanced green fluorescent protein (eGFP). For the HDR template, we synthesized and annealed a double-strand DNA oligo that carries the wild-type CFTR sequence and an artificial SspI restriction enzyme site. We transfected the Cas9-sgRNA-eGFP plasmid and the HDR template together into a $\Delta F508$ airway epithelial cell line using the Neon electroporation system. 24-48 hours post-transfection, fluorescence activated cell sorting (FACS) was used to collect the eGFP-positive cells. The cell DNA was extracted and polymerase chain reaction (PCR) was used to amplify the gene-repaired region of the CFTR gene. The PCR product was then cloned into ZeroBlunt vector, transformed into DH5 α *E. coli*, and plated on selective agar plates. Bacterial colonies were identified by PCR amplification and analytical digestion with SspI. Of the initial 7 PCR-positive clones, 4 expressed the HDR mutation, accounting for a 57% correction rate. More clones are currently under screening, and further data will be presented on the poster. These data proved the principle that CRISPR-Cas9-mediated gene repair is feasible to correct the CF defect.

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Disparities in the Presumptive Treatment of Cervicitis

Background: Cervicitis, the inflammation of the uterine cervix, is often associated with sexually transmitted disease (Efosa, *et al*). Chlamydia trachomatis (CT) and Neisseria gonorrhea (GC) are among the most prevalent infections (Berntsson, *et al*). Common clinical symptoms for diagnosing cervicitis include vaginal discharge and cervical inflammation. If left untreated, cervicitis can lead to further complications such as pelvic inflammatory disease (PID), thus illustrating the need for quick presumptive diagnosis (Gita, *et al*). But the overtreatment of patients who ultimately do not have positive bacterial cultures can increase the antibiotic resistance in a given patient and community, resulting in the virulent and increasingly resistant infections we see in patient populations today (Brooks, *et al*). The aim of this study was to examine the disparities in the presumptive treatment of cervicitis.

Methods: We conducted a retrospective chart review of 1271 female patients presenting to the UMCNO Emergency Department during a 22-month period (August 2015 – May 2017) with a chief complaint of vaginal discharge. Medical records were reviewed for age and parity at presentation, health insurance, cervical motion tenderness, adnexal tenderness, previous STD treatment, microscopy of discharge, and whether bacterial cultures were performed.

Results: Our results demonstrated that 71.2% of subjects with complaint of vaginal discharge were black, 14.9% of subjects were white, and 13.1% did not report their race. While 6% of subjects were positive for Chlamydia trachomatis and 3.5% were positive for Neisseria gonorrhea, 64.4% were treated with antibiotics in the ED. Of the patients treated with antibiotics (with the exclusion of those who did not report their race), 16.4% (117/712) were white and 82.7% (589/712) were black [OR = 1.19]. Of the total patients, 21.8% were given prescriptions at discharge. Of these patients, 19.2% (48/250) were white and 79.2% (198/250) were black [OR = 1.212]. In all cases, women of color were about 20% more likely to be treated presumptively for cervicitis and STD than white women. Of the patients who received presumptive therapy, 12% had had prior treatment for STDs. Among these patients, 18.9% (28/148) were white and 79.2% (118/148) were black. Out of all the age categories, the 25-34 year-old females had the highest percentage of presumptive antibiotic treatment (35.3%) [p = 0.006, LR = 0.009].

Conclusion: We hypothesized that women of color were more likely to be presumptively treated for PID or STDs. Our results showed that there was a significant correlation between race and presumptive treatment of cervicitis or STDs. There was also a significant correlation between age and treatment with antibiotics. One limitation of this study was that some patients presented to the ED with additional presenting problems, which may explain why they received treatment with antibiotics or prescriptions. Additionally, we noted that a majority of patients were given antibiotic treatment despite the generally low prevalence of CT and GC in this patient population. Ultimately, this study shows significant implicit bias in the treatment of women of color for cervicitis and an over-prescription of unnecessary antibiotics in general, underscoring the need for more efficient screening and antibiotic stewardship.

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“Therapeutic Development of Novel Non-Peptide Nociceptin/Orphanin FQ Receptor Compounds for Treatment of Hypertension and Heart Failure”

Heart failure is a debilitating disease that currently affects 6.5 million Americans. Often, patients with heart failure have hypertension, increased sympathetic nerve activity and edema, all of which contribute to the progression and severity of the disease. Despite current therapeutics, heart failure is associated with a high rate of mortality. Thus, there remains a need for drugs that can work by a novel mechanism(s) to treat patients with hypertension and heart failure. In previous studies, we have shown that nociceptin/orphanin FQ, an opioid like peptide, produces marked changes in cardiovascular and renal function. However, therapeutic delivery of peptides to humans has significant limitations for treatment of chronic disease states.

Aims: The purpose of these studies was to investigate the cardiovascular and renal responses produced by non-peptide ligands that bind to the nociceptin/orphanin FQ peptide (NOP) receptor and identify a lead compound that can be further tested in rodent models of hypertension and heart failure.

Methods: Sprague-Dawley rats were instrumented for measurement of blood pressure, heart rate, and urine output, and infused intravenously (i.v.) with isotonic saline. After equilibration, changes in cardiovascular and renal excretory function was measured in conscious rats before (control) and after (experimental) i.v. bolus injection of vehicle or AT-039, AT-090, AT-127, AT-403 (non-peptide NOP receptor compounds).

Results: IV bolus injection of all AT compounds (30, 100, and 300 nm/kg) decreased blood pressure and produced diuresis; e.g., at 100 nmol/kg, AT-039 significantly decreased blood pressure ($\Delta -10 \pm 2$ mmHg) and increased urine output ($\Delta 49 \pm 14$ μ l/min). With the exception of AT-039, all AT compounds also caused sedation and hyperphagia. Based on these observations and the apparent lack of adverse central nervous system effects (sleeping, eating), AT-039 was selected as the lead compound for further investigation. In other studies, i.v. infusion of AT-039 at low doses produced marked water diuresis (increase in urine output and decrease in urinary sodium) without altering cardiovascular function, whereas higher i.v. infusion doses of AT-039 produced diuresis and hypotension without altering heart rate.

Conclusions: We have shown that AT-039 is a non-peptide NOP receptor compound that has clinically important water diuretic and hypotensive effects. Importantly, the failure of AT-039 to produce sedation or hyperphagia indicates that this compound is peripherally restricted. Further studies are ongoing to identify the novel neural, humoral, and vascular mechanisms by which AT-039 evokes its cardiovascular and renal responses. Further studies with AT-039 will also be conducted in rats with hypertension and heart failure to show that this compound is orally active and has novel therapeutic benefits in these disease states.

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“Detection of Human Papillomavirus Infection in Pregnant HIV-Infected Kenyan Women”

Background and Objective: Human Papillomavirus (HPV) is a sexually transmitted infection, affecting nearly all sexually active persons at least once in their lifetime. HPV is known to be the direct agent for most cervical intraepithelial lesions, a stepping stone in the development of cervical cancer. In terms of the relationship between HPV and pregnancy outcomes, it is suggested that there is no significant effect of infection in the absence of cervical abnormalities; however, the presence of disrupted cervical tissue and downstream inflammatory events caused by squamous cell dysplasia have been identified in potential adverse outcomes for both mother and child. This effect can be compounded with coinfection by both other sexually transmitted pathogens and human immunodeficiency virus (HIV), a common feature in locales without access to medical infrastructure and screening opportunities. Kenya, Africa has long been known for its high cervical cancer mortality burden. It is the goal of this project to determine the prevalence of specific HPV genotypes through a cross-sectional cohort, specifically pregnant, HIV-positive women seeking medical care in Nairobi, Kenya. It is also a priority to determine the relationship between HPV infection and pregnancy outcomes in this population.

Methods: Cervical samples were collected from 125 HIV positive pregnant Kenyan women who sought medical care for HIV treatment in the urbanized setting of Nairobi. The samples were extracted for nucleic acid (Qiagen) and tested for HPV by PCR targeting a consensus region of the L1 gene. A positive result was defined by the presence of a 450bp band on agarose gel electrophoresis. Samples were considered HPV negative if they tested positive for human beta-globin, indicating the sample was adequate for HPV PCR analysis. HPV genotypes were then determined through a combination of the Roche Linear Array Assay and Sanger sequencing.

Results: The prevalence of HPV infection in pregnant HIV-infected Kenyan women was 47.2%. Preliminary data show that the most common HPV genotype was HPV 83, with 20% of women testing positive. The predominant genotypes seen in cervical cancer, HPV-16 and -18, were positive in 16% and 4% of women, respectively. Multiple HPV infections were evident in 30% of this cohort with HPV 83 being the most involved in coinfection.

Conclusion: Interestingly, the predominant HPV genotypes detected in this Kenyan cohort differ from the predominant genotypes seen in Western populations, where HPV 16 and 18 carry the largest role in cervical susceptibility. Other studies have shown a similar variability in both low and high risk HPV genotype distribution across the globe, calling into question the relevance of current HPV vaccine formulations for high-risk populations in developing countries.

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“A Saturated Fat Diet Promoting Hepatic Cancer in a Mouse Model Is Associated with Increased Hepatic Expression of the Proto-Oncogene Agap2.”

Liver cancer is the second most common cause of cancer death globally according to the World Health Organization. Death caused by liver cancer is more prevalent in human males than it is in females. Obesity is a risk factor for development of liver cancer including obesity, but the dietary effects of different fat types on mechanisms driving the progression of hepatic tumors is poorly understood. Our model of fat-driven liver tumorigenesis consist of C57BL/6 mice with tumor initiation induced by 10 mg/kg i.p. injection of the carcinogen diethylnitrosamine (DEN). The mice were subsequently fed at low fat (10% kcal) control diet or various high fat (35% kcal) diets. The fats in the high fat diets were of four different types: corn oil (omega-6 fat), cocoa butter (saturated fat), olive oil (monounsaturated fat), and corn oil + DHA (omega-3 and omega-6 fats). We previously observed that feeding the saturated fat diet with cocoa butter for 30 weeks promoted tumorigenesis specifically in males but not females. We subsequently hypothesized that the cocoa butter diet alters expression of genes promoting tumorigenesis in the liver. From RNA-Sequence (RNA-Seq) analysis of a subset of the mice livers, the expression of 7 genes, Agap2, Vldlr, Ildr2, Cyp4a14, Cyp3a16, Cyp3a11, and Chrna4 were altered significantly and at least 2-fold in cocoa butter fed males compared to males fed other high-fat diets. We performed qRT-PCR analysis of these genes from all the mice (n = 96) in the study, which confirmed the altered expression as indicated by RNA-Seq. Agap2 (ArfGAP with GTPase domain, ankyrin repeat and PH domain 2) seemed the most interesting gene, as it is a proto-oncogene, i.e. a gene that promotes cancer if overexpressed. It encodes a GTP-binding protein that is a phosphoinositide 3-kinase enhancer (PIKE). Furthermore, Agap2 had highest expression in males fed the cocoa butter diet and showed the highest correlation among the 7 genes ($R = 0.494$, $p < 0.001$) with the number of visible liver tumors. We also extracted liver RNA from mice fed high-fat diets for just 15 weeks before visible tumors appear. The highest expression of Agap2 at 15 weeks was likewise observed in males fed the cocoa butter diet, indicating that increased Agap2 expression is a consequence of the dietary regime and not the cancer phenotype. We conclude that Agap2 expression is stimulated by the cocoa butter diet in male mice. We speculate that increased Agap2 expression by saturated fats contribute to tumor promotion. Further studies are required to explore such a role for Agap2 in liver cancer. This research was supported by grant #R25AA021304 from the National Institute of Alcohol Abuse and Alcoholism at the National Institutes of Health.

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Mentor: Martin Ronis, PhD

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“EtOH Inhibition of Osteoblastogenesis is Mediated in Part by Mitochondrial-Derived Reactive Oxygen Species”

Decreased bone mass, increased risk of bone fracture and osteoporosis are all linked to binge drinking and alcoholism. With 20% of women from ages 18-30 indulging in binge drinking, it is invaluable to understand the effects of alcohol on bone remodeling.

Bone remodeling is primarily regulated by osteoblasts and osteoclasts which form and reabsorb bone tissue respectively. The process is naturally in equilibrium, maintaining mineralization and bone density. Bone formation is directly related to the differentiation of mesenchymal stem cells (MSC) into osteoblasts and osteocytes. The two major pathways producing the differentiation are the Wnt- β -catenin signaling pathway and the bone morphogenic protein signaling pathway (BMP). NADPH Oxidases (NOXs) are partly responsible for producing reactive oxygen species (ROS), which are believed to play a role in MSC differentiation, possibly through the previously mentioned pathways. Either excessive or low amounts of ROS will have detrimental effects on the balance of bone turnover. The imbalanced ROS quantities will affect the signaling for MSC to differentiate into osteoblasts or adipocytes. Bone formation has been proven to decrease due to inhibition of osteoblastogenesis by EtOH as well as increased lineage commitment of MSC to adipocytes over osteoblasts, especially at higher EtOH blood concentrations associated with alcoholism. Previous research has shown that EtOH increases activation of NADPH Oxidases (NOX) and creates excessive amounts of ROS. EtOH effects on bone turnover can be blocked by dietary antioxidants. Additionally, further previous research has shown that when using p47^{PHOX} (a NOX2 subunit) knockout mice, no protective effect against EtOH inhibition of osteoblastogenesis was observed. These data suggest potential alternate sources of oxidative stress play a role in EtOH inhibition of osteoblastogenesis. One such potential pathway might be oxidative stress from ROS generated by the mitochondria.

The experiment design was to test if EtOH-mediated ROS produced by the mitochondria will influence bone formation. Mitotempo (MT) is a mitochondria-targeted superoxide dismutase mimetic which scavenges for mitochondrial ROS and was tested to see if it can rescue the effects of EtOH. MSC were harvested from six-week-old, wild-type mice of the sexes. Four treatment groups were used: a control group without either EtOH or Mitotempo, a 25 nM Mitotempo only group, a 50 mM EtOH only group, and a 50 mM EtOH and 25 nM Mitotempo group. After 15 days of growth including differentiation, data was collected through ALP staining proving the suppression of osteoblast differentiation was induced by EtOH and that MT rescues the effect ($p < .001$). Further RT-PCR and Western Blots analysis are in progress.

This experiment explored an alternate source of EtOH-induced ROS derived from the mitochondria and tested if ROS influences bone formation. This study has proven that the mitochondrial ROS does affect osteoblast differentiation. In addition, the ROS's effect on bone formation can be rescued using a mitochondrial ROS scavenger (MT). The specific underlying molecular mechanism underlying EtOH-induced inhibition of osteoblastogenesis due to mitochondrial derived is under further study.

This research was supported through the Baptist Communitive Ministries, Science Youth Initiation Project.

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“Potential Health Impacts of New Orleans Drinking Water Lead Levels and Effectiveness of Prevailing Interventions”

Introduction: Lead is a toxic metal for which there is no safe level of exposure. Blood lead levels (BLLs) as low as 1 µg/dL have been linked to adverse birth outcomes in children. Likewise, BLLs as low as 2 µg/dL have been found to cause permanent cognitive and behavioral deficits. The main source of lead in drinking water is lead service lines (LSLs). It is estimated that 65-80% of New Orleans’ (NOLA) water service lines are lead. The goals of this study are to evaluate the potential health impacts of New Orleans water lead levels (WLLs) and evaluate the effectiveness of flushing as a means of reducing WLLs.

Methods: NOLA WLLs were compared to health-based standards; and associated childhood BLLs were derived using the U.S. Environmental Protection Agency’s (EPA) Integrated Exposure Uptake Biokinetic (IEUBK) model. A Mood’s Median test was used to evaluate the potential impact of flushing on WLLs.

Results: NOLA WLLs met regulatory compliance; yet 73% of sites sampled had maximum WLLs that exceeded the California EPA’s health-based standard for fetuses, infants, and children. It is estimated that 38% of children would have BLLs >1 µg/dL and 4% of children would have BLLs > 2 µg/dL from water lead exposure only. NOLA median WLLs increased significantly after flushing water for 30 seconds and 3 minutes (the prevailing flush guidelines promoted by the NOLA Sewage and Water Board and public health officials). WLLs did decrease significantly after 6 minutes of flushing.

Conclusions: Levels of lead that are found in NOLA drinking water may have an impact on childhood BLLs. Prevailing public health guidelines to flush water for 30 second to 2 minutes are inconsistently effective and may inadvertently increase exposures. More effective low-cost solutions are needed for reducing waterborne lead exposures.

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“The Effect of the Promising PLK1 Inhibitor *BI-2536* and *Chac1* on the Cancer Initiating Notch Pathway Genes”

Polo like kinase 1 (PLK1) inhibitors are being investigated in clinical trials for cancer therapy. BI-2536 is a selective polo like kinase 1 (PLK1) inhibitor, which in clinical trials had promising results. PLK1, also known as serine/threonine protein kinase (STPK13), is a key regulator of cell division. Inhibition of PLK1 by BI-2536 results in mitotic arrest, disruption of cytokinesis and apoptosis in tumor cells. PLK1 inhibitors such as BI-2536 have high as novel treatments for cancer, a devastating disease. Previous experiments revealed that BI-2536 inhibits the Notch Pathway, which is also a molecular target for cancer therapy. The Notch pathway is critical for the cell-cell communication, cell proliferation, regulation, and homeostasis. Notch dependent dysfunction is thought to be a primary cause to several diseases in addition to cancer. Chac1 is a mammalian protein induced during endoplasmic reticulum (ER) stress. Like BI-2536, Chac1 has been implicated to inhibit Notch signaling. We sought to examine whether Chac1 gene function, as a Notch inhibitor, might explain effects of BI-2536 on the Notch pathway.

The MDA-MB-231 human breast cancer cell line was used as a model to identify changes in Chac1 and Notch gene expression by treatments with BI-2536 versus control. We hypothesized that inhibitory actions of BI-2536 on Notch signaling might be mediated by Chac1. In this study, we examined MDA-MB-231 cells treated with 25nM, 50nM or 100nM BI-2536 for 24-hours. I scored changes in mRNA expression of 31 genes involved in ER stress and Notch Pathway including Chac1 and Notch1-4, using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) assay. DMSO treated cells were used as vehicle control and 100nM Thapsigargin treated cells as a positive control for ER stress. Comparing BI-2536 treated MDA-MB-231 cells to control, we expected activation of Chac1 and reductions in Notch Pathway genes.

In MDA-MB-231 cancer cells, mRNA expression for some genes in the Notch Pathway have a trend of upregulation, such as Jag2, while others, like Jag1, seem to downregulate with increasing doses of BI-2536, compared to controls. However, there was no significant change in Chac1 mRNA expression in BI-2536 treated MDA-MB-231 cells. These data suggest that Chac1 does not mediate BI-2536 dependent inhibition of Notch1 signaling and tumor cell growth. Whether Chac1 actions on Notch are involved in other types of cancer remain to be established.

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Effect of Chronic Alcohol on Skeletal Muscle Regeneration after Unilateral Hindlimb Immobilization in Rats

Abstract

Immobilization or disuse causes a number of detrimental changes in skeletal muscle including atrophy and decreased functional capacity. Skeletal muscle regeneration is integral to recovery and is tightly regulated by a number of muscle regulatory factors including genes and microRNAs. Hazardous alcohol consumption significantly increases the amount of time that it takes to recover from a soft tissue or muscle injury. According to the 2015 National Study on Drug Use and Health (NSDUH), 86.4% of people ages 18 and older reported that they drank alcohol at some point in their lifetime. Our lab has also previously demonstrated that chronic alcohol significantly decreases the regenerative potential of satellite cells, muscle stem cells. Hence the objective of the study was to determine the effects of chronic alcohol on skeletal muscle regeneration after a brief period of immobilization of the hindlimb in rats. We hypothesized that chronic alcohol will decrease expression of factors that regulate skeletal muscle regeneration in immobilized rats. A total of 12 (3-month old) male Fischer 344 rats were used for the study. Six were fed an alcohol Lieber DiCarli diet (average blood ethanol concentration 0.1 g/dL), and six were fed a control diet for 10 weeks. A plaster cast was placed to immobilize between the hip and knee joint for seven days. The animals were euthanized after seven days of recovery and the quadriceps muscle was collected. The gene expression for the myogenic genes: Paired Box 7 (Pax7), Myogenic Differentiation 1 (Myod1), Myosin Heavy Chain 1 (Myh1), and Myocyte Enhancer Factor 2C (Mef2c) using the qPCR method was determined. MicroRNAs implicated in muscle regeneration including miR-1, miR-206, miR-133a, and miR-133b were determined using qPCR. Collagen expression was examined by staining for picrosirius red and the number of satellite cells was determined using PAX7 immunohistochemistry. The body weights were not different between the alcohol and the control fed animals. There was a significant decrease in the quadriceps muscle weight due to casting and alcohol compared to non-casted control fed animals. There were no significant differences in gene expression in the muscle between cast control, non-cast control, cast alcohol, and non-cast alcohol animals after 7 days of recovery. Similarly there were no changes in the expression of miRNAs.

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Mentor's Name: Chindo Hicks, PhD
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“A novel integrative bioinformatics approach for mapping the genomic landscape of pediatric Glioblastoma Multiforme”

Objectives: (1) Discover and functionally characterize the genetic markers and elucidate the molecular trajectories of disease progression in pediatric Glioblastoma Multiforme (pGBM), (2) Define the landscape of recurrent somatic mutation interactions with gene expression, (3) Regress expression values of the best candidate marker genes on Overall Survival (OS).

Introduction: Discovery and functional characterization of genomic alterations that drive pGBM initiation and progression could lead to more effective therapeutics. pGBM has a five-year survival rate of less than 20%. Recent studies at Children's Cancer centers across the world have catalogued single nucleotide polymorphisms (SNPs), gene expression, and clinical characteristics found in pGBM, but few studies have collectively analyzed all the available data.

Methods: Publicly available datasets meeting the following inclusion criteria were obtained from GEO: < 21 years of age, Grade IV, reported OS, measured on U133 Plus 2.0 Platform. The original CEL files containing raw probe intensity values were downloaded. The CEL files were screened for high quality, and then preprocessed and normalized by RMA. Probes were nonspecifically filtered by fluorescence units and the interquartile range (IQR) across the samples, and then specifically filtered for Affymetrix Control probes. Differential expression analysis was conducted on the Pomello II, t-test, limma module. Probes were divided into two classes, mutants and non-mutants, by cross-referencing a landscape study conducted at St. Jude Children's Research Hospital. Probes meeting the following thresholds were uploaded to Ingenuity for Core Analysis: mutants, $p < 1.0 \times 10^{-7}$ and non-mutants, $p < 1.0 \times 10^{-10}$. Missense mutations were analyzed for deleterious effect using PROVEAN Score. Activated and Inactivated Upstream regulators were regressed on OS in SAS 9.4 by stepwise regression.

Results: Of the 52 subjects meeting study criteria, the mean age was 10.9 years ($\sigma = 4.4$) and the mean survival was 11.7 months ($\sigma = 10.3$). Ingenuity Core Analysis returned 96 upstream regulators. Of these 96 regulators, 51 were activated or inactivated. These 51 altered regulators along with age and death status were regressed on survival. The final model had an $R^2 = 0.96$ and included the following genes, with mutation status in the reference population listed in parentheses: KDM5B (Nonsense), HTT (Neutral Missense), MECP2 (UTR_3), PHF21A (UTR_3), TCF4, POU3F2 (UTR_3), NEUROG1, PDX1, BRCA1, ESR1 (Deleterious Missense), PPARGC1A, SERTAD1, TWIST2, MYCN, E2F2, PAX6, SATB1 (Frameshift).

Conclusion: The following formula was generated for predicting survival from gene expression:

$$\text{OS (months)} = 58.6 - 4.73 \cdot \text{KDM5B} + 2.16 \cdot \text{HTT} - 11.5 \cdot \text{MECP2} + 8.67 \cdot \text{PHF21A} + 15.26 \cdot \text{TCF4} - 4.14 \cdot \text{POU3F2} - 8.85 \cdot \text{NEUROG1} + 8.47 \cdot \text{PDX1} + 5.81 \cdot \text{BRCA1} - 21.12 \cdot \text{ESR1} - 1.46 \cdot \text{PPARGC1A} + 1.58 \cdot \text{SERTAD1} + 5.58 \cdot \text{TWIST2} - 34.23 \cdot \text{MYCN} + 9.12 \cdot \text{E2F2} + 1.19 \cdot \text{PAX6} - 4.17 \cdot \text{SATB1}$$

14/17 of these interact with each other and could yield a strategy for therapeutic intervention. Limitations are the disjoint SNP and gene expression populations and the small sample size.

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“Effect of chronic binge alcohol and antiretroviral therapy on insulin signaling in the mesenteric adipose tissue of SIV-infected male rhesus macaques.”

Abstract

Alcohol use disorders (AUDs) are prevalent among persons living with HIV/AIDS (PLWH). Chronic alcohol consumption, HIV infection, and anti-retroviral therapy (ART) are independently associated with impairment in glucose-insulin dynamics. Our laboratory used a non-human primate (NHP) model of simian immunodeficiency virus (SIV) infection to examine the separate and combined effects of chronic binge alcohol (CBA) administration and ART treatment and has previously demonstrated that CBA administration reduces acute insulin secretion in response to a glucose challenge. It was also demonstrated that there were no significant differences in the expression of insulin signaling molecules due to CBA or ART in the skeletal muscle and liver. The three types of insulin responsive peripheral tissues are skeletal muscle tissue, liver tissue, and adipose tissue. Thus, the aim of this study was to investigate the effects of CBA administration and ART treatment on the expression of proteins in the insulin signaling cascade in the visceral adipose tissue during the asymptomatic phase of SIV-infection. Daily CBA or sucrose (SUC) administration was initiated 3 months prior to intra-rectal SIVmac251 inoculation and was continued for the duration of the study, which ended 11.5 months post-SIV infection. ART or placebo treatment was initiated 2.5 months post SIV infection and was continued for the duration of the study. Accordingly, four treatment groups were studied: SUC/SIV/ART, CBA/SIV/ART-, SUC/SIV/ART+, and CBA/SIV/ART+. For this study, mesenteric adipose tissue obtained at necropsy was examined. Using western blotting techniques, seven proteins involved in the insulin signaling cascade (p-Akt (Ser473 and Thr308), p-mTOR (Ser2448), pAS160 (Thr642), pAMPK (Thr172), PP2A-C, PTEN, and PTP1B) were tested. Neither CBA nor ART altered insulin signaling as determined by the ratio of phosphorylated/total protein expression for Akt(Ser 473), Akt(Thr473), and mTOR(Ser2448). There was a main effect of CBA to increase total mTOR expression irrespective of ART treatment. Neither CBA administration nor ART treatment altered protein expression of protein phosphatase 2 (PP2A) and phosphatase and tensin homolog (PTEN). These findings demonstrate that neither CBA nor ART impair the expression of proteins in the insulin signaling cascade in mesenteric adipose tissue in SIV-infected male rhesus macaques.

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Mentor: Angela Amedee, Ph.D
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“The Characterization of SIV Genotypic Diversity in Reservoir Tissues.”

Human Immunodeficiency Virus (HIV) affects nearly 36.9 million people around the world (CDC). The virus can be controlled by combination antiretroviral therapy (ART), which can keep plasma viral levels below detection. However, a lack of plasma virus does not mean the virus is eradicated from the patient. Proviral HIV DNA persists in tissue reservoirs infected by the RNA retrovirus. What occurs in these reservoir tissues is relatively unknown. Simian Immunodeficiency Virus (SIV)-infected rhesus macaques provide a good model to investigate viral reservoirs, as they are a relevant model of HIV disease. These model animals can be inoculated with a defined virus stock, and reservoir tissues can be obtained to investigate viral composition. We hypothesized that the abundance of specific viral genotypes viral in mesenteric lymph node reservoirs would closely match those genotypes that first established infection, known as the founder virus, in animals infected via a rectal route. We further hypothesized that the levels and diversity of virus in this reservoir would be predictive of disease progression.

In this pilot study, we examined the viral levels and viral genetic diversity in mesenteric lymph nodes collected at necropsy from ten macaques one year after rectal infection with a diverse SIV stock. Half of the animals were exposed to chronic binge alcohol consumption. Two of the animals did not receive ART throughout the study course. Among the eight that received ART for 40 weeks, six of the animals had undetectable plasma viral levels, while two of the animals had detectable plasma viral loads despite ART. SIV levels in mesenteric lymph nodes were determined by quantitative PCR and the viral genotypes were characterized by sequencing a region of the SIV envelope gene. Viral genotypes identified in this reservoir were compared to founder virus and those found at set-point (10 weeks post-infection).

The relative abundance of viral genotypes found in mesenteric lymph nodes of each animal closely matched the founder viral genotypes expressed in plasma early after infection. The diversity of viral populations at necropsy was higher than populations at founder in all but two animals. Viral loads and viral diversity were also higher in animals with high ART viremia and animals that did not receive ART. This is expected as the viral loads in these animals are not well controlled, leading to more replication and also more mutations. Diversity in this reservoir was not correlated with plasma viral load, however, the divergence from the founder virus population was significantly correlated with RNA viral loads of the mesenteric lymph node. These observations indicate compartmentalized replication occurring in the mesenteric lymph node. Further studies should be conducted in more reservoir tissues to demonstrate the exact relationship between compartmentalized replication and disease progression as well as the role that alcohol consumption plays on accelerating disease.

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“Detection Strategy to Simultaneously Evaluate Antibacterial Activity and Cytotoxicity of Berberine on Vero-GFP Cells Infected with *Chlamydia trachomatis*”

The prevalence of the human pathogen *Chlamydia trachomatis*, a unique obligate intracellular bacterium, along with the infectious diseases it causes has been a prominent global health burden due to its high contagion and detrimental sequel. Efforts to improve treatment regimes by advancing cell-based assays such as detection technologies for simply scanning cytotoxicity and efficacy of probable antimicrobials against *C. trachomatis* have garnered much attention. A natural herb-derived alkaloid known as berberine (Ber) has been studied to exhibit therapeutic and synergistic benefits as an antibacterial agent against a broad spectrum of pathogenic microorganisms. The bioactive compound has been acknowledged as a traditional Chinese medicine for treating diarrhea and can be extracted from an abundance of medicinal plants. Due to berberine's exhibition of antibacterial properties, we hypothesized that it could conceivably impede the development of *C. trachomatis* in induced Vero-GFP cells through a dose-dependent manner. Proving this hypothesis could highlight more mechanistic insights regarding berberine's inhibitory effects on intracellular bacteria and other microbes.

To test this hypothesis, it is highly desirable to determine and distinguish the authentic bacterial inhibitory effects of Ber or other drugs independent from the potential cytotoxicity in real time. Therefore, we applied a dual reporter system containing a recently developed green fluorescent protein (GFP) reporter Vero-GFP cells with infection of an engineered *C. trachomatis* strain, L2/incDmCherry, constitutively expressing red fluorescent protein mCherry. Vero-GFP cells express GFP under the control of a cyclomegalovirus promoter; thus, by simply measuring GFP levels we can monitor the dynamic changes in host ribosome activity induced by various drugs. Simultaneously, by monitoring mCherry signals, we can track the effect of Ber on *C. trachomatis* intracellular development. Vero-GFP cells were plated on a 96-well microplate infected with *C. trachomatis* overnight and later treated with Ber at different concentrations (5, 20 and 50 μ M) starting from 0 hour post infection (h pi). Cycloheximide (CHX), an inhibitor of eukaryotic protein synthesis, and dimethyl sulfoxide (DMSO) were used as positive and negative controls. Chlamydial inclusions and the expression of GFP and mCherry were monitored using fluorescence microscopy, spectrophotometry, western blot, and endpoint inclusion-forming unit (IFU) assays at different time points with a total duration of 68h post infection (h pi).

We observed that elevated concentrations of berberine elicited a direct decrease in *C. trachomatis* infection based on inclusion size, proliferation, and the progeny of elementary body (EB) production. Simultaneously GFP expression in the Vero-GFP cells was constant or slightly increased over time, suggesting Ber, at the concentrations tested, did not impair host ribosome activity.

Overall, the dual reporter system exhibited a potent fluorescent display of *C. trachomatis* development in Vero-GFP cells in the presence or absence of Ber without observable cytotoxicity. Ber could efficiently act as a prospective antibacterial or synergist against *C. trachomatis* and multiple pathogenic microbes with facilitated development.

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Mentors: Jennifer Lentz, Ph.D. and Marianne Hathaway

Protocol Development for Single-Cell Isolation and Transcriptome Profiling of Cells from the Cochlea of Usher Mice

Usher syndrome (Usher) is a recessive genetic disorder that is the most common cause of hearing loss in conjunction with retinitis pigmentosa. Three clinical types (USH1, 2, 3) and 15 genes are associated with Usher. We study type 1C Usher (USH1C which is caused by a splicing mutation (c. 216G>A) in the *USH1C* gene. Our lab has produced a knock-in mouse model with this mutation. The Ush1c mice also have a splicing defect, and hearing and vision loss similar to patients. The c.216G>A mutation is a single base pair change that introduces a cryptic splice site that results in a 35 base pair deletion and a truncated protein, Harmonin, that is important in hair cell structure and function. Treatment early in the development of hearing with antisense oligonucleotide (ASO) targeting the c.216G>A mutation corrects Ush1c splicing and rescues mid to low frequency, but not high frequency, hearing. ASO treatment of adult mice does not rescue hearing, despite correcting splicing. This data led us to hypothesize that the Ush1c c.216G>A mutation significantly affects gene expression during the development of hearing, and that ASO treatment results in a more wild-type like expression pattern.

This project aims to develop single cell isolation techniques for transcriptome profiling of cells at different ages of development from different regions of the cochlea that are responsible for low, mid and high frequency hearing. Fluorescence activated cell sorting (FACS) is used to isolate inner and outer hair cells and surrounding supporting cells from the apex, middle turn, and base of the organ of Corti in the cochlea of ASO-treated and untreated Usher mice and their wild type littermates. RNA-Seq analysis is used for transcriptome profiling. Immunohistochemistry is used to validate changes in gene expression at the protein level. This project also aims to clone the truncated Harmonin protein in *E. coli* for protein production and purification for monoclonal antibody production. The methods developed here will provide the tools to investigate the transcriptome during the development of hearing in a mouse model of congenital deafness that has been rescued by ASO treatment.

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**“Unexpected Results from Hereditary Cancer Panel Genetic Testing:
Do Duplications of MMR Genes Matter?”**

Two patients were referred to the Genetic Counseling Clinic at University Medical Center due to their personal and/or family history of breast and other cancers. Both patients met criteria for *BRCA1/2* testing. However, panel genetic testing revealed unexpected results: a whole gene duplication of *MSH2* in Patient #1 and a whole gene duplication of *PMS2* in Patient #2. Mutations in *MSH2* and *PMS2* (in addition to *MLH1*, *MSH6* and *EPCAM*) cause Lynch syndrome (LS), which is the most common form of hereditary colorectal cancer. LS is not known to be associated with an increased risk of breast cancer, but recent data shows that it may be part of the LS tumor spectrum. In a study of 528 probands who underwent panel genetic testing and were positive for a mutation in one of the genes that cause LS, breast cancer was nearly as common as colorectal and endometrial cancer, especially in individuals who had mutations in *MSH6* and *PMS2*. These findings are interesting given the presentation of cancer in both our patients and their families. However, neither patient meets Amsterdam II criteria for LS, and both duplications are currently classified as variants of unknown significance (VUS).

While various types of mutations in the mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*) are known to be pathogenic, including missense, nonsense, deletions and partial duplications, it is unknown whether whole gene duplications are pathogenic or benign. Therefore, a literature search was performed, but to our knowledge, there are no reports of individuals/families who meet clinical criteria for LS and have a whole MMR gene duplication. Whole gene duplications are known to cause other forms of hereditary colorectal cancer/polyposis (i.e. *GREM1*), however the function of *GREM1* and the MMR genes are significantly different. Follow-up testing, including chromosomal microarray may be beneficial for our patients in order to further evaluate the size and location of the duplications. Additionally, further studies, including family studies, are needed to clarify whether whole MMR gene duplications are pathogenic, as this information could drastically affect the management of the patients and family members presented in this report.

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“CRISPR/Cas9 Deletion of a Neuroendocrine Tumor Marker, *INSM1*, in Human Neuroblastoma”

INSM1 is an intron-less gene encoding a neuroendocrine transcription factor (active site, 148/1680 bp) that is critical for early embryonic neurogenesis. The protein structure of this gene product contains five zinc-finger DNA-binding motifs and is highly conserved throughout species from humans, chimpanzees, mouse, *Xenopus*, zebrafish, *Drosophila*, to *C. elegans*. *INSM1* gene expression is highly elevated in most neuroendocrine tumors. The high expression level of the *INSM1* gene promotes neuroblastoma tumor cell growth and transformation. The aim of this project is to generate an *INSM1* knockout model using the CRISPR/Cas9 system and to further study the impact of an *INSM1* knockout on neuroblastoma oncogenesis.

We designed two pairs of oligos located at the active sites (193/215 bp and 1005/1027 bp) of the *INSM1* gene that were cloned into the CRISPR/Cas9 vector (PX458). These oligos targeted the *INSM1* gene for effective CRISPR-Cas9 directed gene knockout. The oligos were first annealed and ligated to the BbsI digested PX458 plasmids, which contained enhanced green fluorescence proteins (EGFP). The recombinant plasmids were isolated and subjected to DNA sequencing analysis to confirm the proper oligo sequence cloning. Each oligo clone from either active site was transfected into a neuroblastoma cell line, BE (2)-M17 through the Neon Transfection Electroporation System. An approximately 1% transfection efficiency was achieved. The transfected BE (2)-M17 cells (~20,000 cells) were further sorted using a FACS cell sorting system based off the EGFP fluorescence 48 hours after the culture transfection. The fluorescence-positive cells were singly cloned through a limiting dilution method. The clonal cells were tested for *INSM1* protein expression using an immune-dot-blot assay with actin as an internal control. *INSM1* knockout clones were isolated for further Western blot and phenotypic analyses as compared with the parental BE (2)-M17 cells.

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“Notch 4 Signaling in NET Cellular Proliferation”

Neuroendocrine tumors (NETs) are a heterogeneous group of malignancies all derived from neuroendocrine cell lineage and affecting many different organs including the gastrointestinal (GI) tract, the endocrine pancreas, the thyroid, the skin and the respiratory tract. The curative method is surgical removal of the NET, but the high metastatic potential of these tumors results in a 5-year survival of some types of NETs to be as low as 4%. The pathogenesis of the different subtypes of NETs is not well understood, and recent studies suggest the Notch signaling pathway may be dysregulated in these tumors either by under or overexpression of Notch receptors and/or ligands, or by disruption of pathway functionality. There is a vital need for more therapeutic targets for this diverse set of tumors and the Notch signaling pathway may be one possible point of intervention.

The goal of this NSF REU fellowship was to understand the role of Notch signaling in NET cellular proliferation. Notch signaling impacts many cellular processes including proliferation, stem cell maintenance, and differentiation. Notch signaling is evolutionarily conserved across species and relies on the presence of activated Notch receptors and ligands for activity. Notch receptors are activated by sequential cleavage by ADAM10 and γ -secretase, and activated receptors can either stimulate or suppress proliferation, depending on the cellular context. To study this dual functionality, we focused on two cell lines – one cancer and one normal – and measured endpoints of Notch signaling in enteropancreatic NETs.

The cell lines β lox5 and QGP-1 were chosen for study. QGP-1 is derived from a human pancreatic neuroendocrine tumor and β lox5 is normal human pancreatic beta cells that have been immortalized with SV40-large T antigen. We learned from past data that the Notch4 protein is upregulated in our cell lines, so to assess the impact of Notch4 on cell proliferation, we used γ -secretase inhibitors (GSIs) - one developed by Pfizer and one by Roche. Recent reports confirm that not all GSIs behave the same, and the Roche and Pfizer GSIs were chosen because they demonstrate a preference for Notch4 in other cell types. We treated each cell line with these GSIs and measured proliferation using a modified MTT assay. Interestingly, we found that the Roche GSI has little to no impact on cell proliferation. However, we did see an impact with the Pfizer GSI on both cell lines, suggesting that the Pfizer GSI may be more clinically relevant as a therapy for NETs.

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“ Δ^9 -THC Disrupts Schedule-Controlled Behavior in Rats”

The legalization of marijuana for medicinal and recreational purposes provides good reason to enhance our knowledge on the effects of the cannabinoids on behavior. Δ^9 -Tetrahydrocannabinol (Δ^9 -THC) is the major psychoactive component found in marijuana that binds to cannabinoid receptors (CB), CB1 and CB2. The CB1 receptor is primarily located in the brain, which mediates Δ^9 -THC's psychoactive effects. In this study, rats were trained to press a lever for food under a fixed-ratio 20 schedule of food presentation. Test sessions were comprised of three to five 15-minute components, with a 20-minute timeout period preceding each component that allowed for a cumulative-dosing procedure. During timeout periods, responding was not reinforced. Increasing cumulative doses of Δ^9 -THC (1-18 mg/kg) were administered intraperitoneally at the start of each timeout period. Cumulative doses of Δ^9 -THC were also tested in combination with 0.56 and 1.8 mg/kg of Rimonabant (RMBT) a non-selective CB receptor antagonist. Run rate, overall rate, and the pre-ratio pause (PRP) were recorded during each test. Δ^9 -THC dose-dependently decreased run rate and overall rate, and increased PRP at a dose of 10 mg/kg. RMBT produced a rightward shift to the dose-effect curve for component rate and run rate and antagonized the effect of 10 mg/kg Δ^9 -THC on PRP. These results indicate that THC disrupts fixed-ratio responding and these disruptive effects are cannabinoid receptor mediated due to RMBT's capacity to antagonize these effects.

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Education of HPV Vaccination to Increase HPV Vaccine Administration

Background: Human papillomavirus (HPV) is the most common sexually transmitted disease. In fact, almost all sexually active individuals will contract at least one type of HPV within their lifetimes. Most people, however, do not show symptoms or signs of illness. HPV can lead to life-threatening conditions such as cervical, vaginal, vulvar, or anal cancer. Vaccination offers protection against HPV, but despite this, only 41.9% of girls and 28.1% of boys all across the United States have been vaccinated. The vaccination offers a preventable measure to decrease rates of cervical cancer. It is vital that obstetricians and gynecologists (OB/GYNs) educate their patients on the utility of this vaccine as the American Congress of Obstetricians and Gynecologists (ACOG) recommends vaccination starting at the age of 11 for boys and girls.

Methods: This project will investigate the use of educational tools to increase the number of vaccinated patients in the University Medical Center-New Orleans OB/GYN clinic. The investigators chose to create a handout and a brochure to educate patients on the vaccine. Prior to administering the handout and the brochure, the investigators will record the current number of patients that have received the HPV vaccine by referring to the University Medical Center's pharmacy records. After ten months of distributing handouts and brochures, the investigators will again refer to the University Medical Center's pharmacy to record the total number of patients that have received the vaccine over the ten-month period. Incidence of vaccine administration will be compared from before and after handouts and brochures were distributed to clinic patients.

Expected Results: The number of patients who received the vaccine over the ten-month period will determine whether the current method of education was effective in increasing HPV vaccination. The investigators expect an increased rate of approximately 10-15% of vaccine administration after handouts are distributed to patients.

Conclusion: HPV is a common sexually transmitted disease that can lead to severe consequences such as cervical, vulvar, vaginal, and anal cancer. Current ACOG recommendations have stressed administration of the vaccine to all patients for the prevention of these diseases. The educational tools created in the present study will be available to all gynecological patients at the UMCNO OB-GYN clinic; with an expected increase in vaccine administration. If the educational tools are successful, future work would include expansion of the handouts to other primary care clinics

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“Sulindac sulfide mediates inhibition of tumor cell transformation through COX-2-independent signaling”

Experimental studies have suggested that non-steroidal anti-inflammatory drugs (NSAIDs) can reduce the risk of developing cancer via antineoplastic properties. Studies have indicated the involvement of cyclooxygenase (COX)-2 in tumorigenesis and attributed chemopreventive efficacy of NSAIDs to COX-2 inhibition. However, long-term COX-2 inhibition can result in toxicities and inconsistent drug efficacy. There is some evidence that NSAIDs can inhibit tumorigenesis through COX-2-independent actions. Further research is needed to determine specifically how NSAIDs retain chemopreventive properties through COX-2-independent mechanisms. This project aims to support evidence that COX-2 inhibition is not necessary for observing NSAID-mediated antitumorigenesis in cells.

Sulindac sulfide, a nonselective COX inhibitor and NSAID, has been shown to have antineoplastic properties *in vitro* and *in vivo*. In this study, we observed the anti-transformative activity of Sulindac sulfide in *NIH/3T3* cells by using a two-stage transformation assay. We hypothesize that sulindac sulfide is involved in COX-2-independent signaling of tumor cell transformation inhibition. To test this hypothesis, we previously utilized CRISPR/Cas9 technology to achieve COX-2 gene silencing in *NIH/3T3* cells. Genome targeting efficiency was confirmed using a T7 Endonuclease I assay which showed successful COX-2 DNA cleavage. Western blotting further confirmed COX-2 protein knockdown in cells, showing significantly decreased COX-2 protein levels in CRISPR-transfected cells.

The COX-2 knockdown cells were used in a tumor cell transformation experiment that compared tumor cell transformation in untreated vs. sulindac sulfide-treated cells. During the 21-day cell transformation experiment, some cells were treated with 3-methylcholanthrene (MCA) and 12-O-tetradecanoylphorbol-13-acetate (TPA) tumor promoting agents. COX-2 knockdown cells treated with tumor promoting agents and sulindac sulfide together displayed a dramatic decrease in resulting number of tumorigenic foci cells.

The observed antitumorigenic effects of sulindac sulfide in cells lacking COX-2 expression support the role of NSAIDs in COX-2-independent chemoprevention. Further studies are required to determine sulindac sulfide's specific COX-2-independent targets and signaling pathways. An improved understanding of COX-2-independent signaling can provide a basis for the development of safer, novel cancer therapeutics that bypass the side-effects of COX-2 inhibition.

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“Genetic hearing loss in two founder populations”

Congenital and childhood non-syndromic recessive deafness accounts for approximately 80 percent of cases of hereditary deafness. It is inherited in an autosomal recessive manner (the heterozygous individual who is the carrier of the disease-causing mutation does not display any phenotypic features). The chances that the disease will appear in the offspring increase when the carrier selects a partner from the same isolated founder population due to the increased likelihood of the partner also being a carrier for the mutation. A genetic diagnosis is especially important for individuals who come from ethnic backgrounds with high rates of consanguinity.

Founder populations are those which have a common ancestry that began by a few members of the original population. The colony may have reduced genetic variation because of the small starting population size which may make the population more susceptible to certain diseases. This study focuses on genetic hearing loss in two founder populations: the Louisiana Acadians and the Mexican Mayans from the state of Tabasco, México.

The Louisiana Acadian population and Mexican Mayans from Tabasco have a higher incidence rate for certain types of hereditary deafness-causing genetic mutations when compared to the general population. Experiments performed in this laboratory have shown that there are an increased number of individuals with genetic hearing loss in these two groups. For this study, we obtained data from the Mexican census Instituto Nacional de Estadística y Geografía (INEGI, National Institute of Statistics and Geography) and US Census Bureau which provides information on individuals with pediatric hearing loss and ethnicity/race for each state. Saliva samples will be obtained for genetic analysis which include DNA extraction, PCR, targeted sequencing, exome sequencing and bioinformatics analysis. The objective is to characterize the causes of hereditary genetic hearing loss for these specific founder populations. This study will lead to the identification of deafness-causing specific mutations and genes characteristic of these founder populations. The long-term goal of this project is to provide better care of individuals in these rural underserved communities.

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Title: A novel awake head fixed preparation for testing hearing deficits in mice after acoustic trauma.

Hearing loss and tinnitus affect millions of Americans (hearing loss: ~50mil; tinnitus: ~25mil) and often result in reduced quality of life and significant emotional distress for sufferers. Tinnitus research using animal models employs subtle acoustic discrimination tests as a proxy for self-reporting of tinnitus percepts. The most common test is the gap-in-noise prepulse inhibition of acoustic startle response (ASR). During this test mice are presented acoustic stimuli while inside small holding chambers that limit, but not fully constrain movement. The holding chamber is placed on a pressure-sensing platform that measures startle responses (i.e. downward force) in response to loud acoustic startle stimuli. During test sessions acoustic stimuli consist of low intensity (65 dB) background narrowband noise with randomly timed startle sound pulses (105 dB noise; 20 ms pulse; 17-25 ms intervals). Startle pulses are either preceded by a short gap in the background noise (50 ms gap) or no gap. The ability to detect the fine temporal features of a short gap in the background noise is quantified by the ratio of ASR amplitude in gap trials to that in no-gap trials (gap/no-gap ASR ratio). In this sense the gaps acts as “inverse” prepulses that inhibit the ASR (i.e. decreased amplitude) such that a gap/no-gap ASR ratio less than 1 indicates normal hearing. While this method is widely used, there are some issues with variability of sound source direction (i.e. mice are free to move their head relative to the speakers) and central neural inhibition of sensory responses by motor movement that confound ASR measurement reliability. We employed a novel awake, head-fixed preparation for testing gap in noise pre-pulse inhibition of ASRs in mice. The behavioral chamber consisted of a freely rotating turntable platform mounted on a base with a pressure sensing load cell. Mice had custom machined head plates cemented to their skull with high strength dental cement. The mice were fixed in place by fastening the head plate to a post positioned above the platform. Mice were free to remain sitting quiescent or run at will throughout the experiment. A high-frequency speaker was positioned 12 cm away from the right ear of the mice. ASRs were measured by the load cell and responses were separated into gap and no-gap trials. Additionally we employed a rejection criteria whereby we excluded trials (both gap and no-gap) if the mouse was engaged in locomotive activity or other motor activity (e.g. whisking, grooming) that resulted in significant signal on the load cell, or rotation of the turntable platform. We found that by excluding motor activity contaminated trials there was a small but significant reduction of the gap/no-gap ASR ratio. However, there was a large increase in separability of ASR amplitudes on gap and no-gap trials (Cohen’s D effect size) when motor activity contaminated trials were excluded. Furthermore, after exposing these mice to acoustic trauma we found that our behavioral preparation reliability measured changes in the gap/no-gap ratio. This experimental preparation will be useful in future studies incorporating *in vivo* electrophysiology to further elucidate the neural mechanisms underlying tinnitus and hearing loss.