Thirty-Fourth

*Daniel T. Watts*

Research Poster Symposium

A Scientific Forum for the VCU Community

Thursday, October 26, 2017

Hermes A. Kontos
Medical Sciences Building
Virginia Commonwealth University
In Memory of

Daniel T. Watts
(1917-1994)

The Daniel T. Watts Poster Research Symposium is named in honor of Daniel T. Watts, former Dean of the School of Basic Health Sciences and Graduate Studies who passed away in 1994 at the age of 77. Dean Watts was a nationally recognized pharmacologist. In 1946 and 1947, he worked on projects to determine human tolerance to the acceleration forces experienced in aviators’ ejection seats. From 1947 to 1953, he taught pharmacology at the University of Virginia. He served as chair of Pharmacology at West Virginia University from 1953 to 1966 before coming to the Medical College of Virginia in 1966, continuing to serve as Dean as the institution was incorporated into Virginia Commonwealth University in 1968. Dean Watts held interests in intercollegiate athletics as well as biomedical research and graduate education and represented the University in that capacity. He retired as Dean, Basic Health Sciences in 1982.

During his tenure at this institution he established the foundation of the research enterprise in basic health sciences that continues today. His legacy continues both in the breadth of research and educational programs particularly the development of Ph.D. training. The growth of research and graduate training build on the pioneering efforts of John C. Forbes and C.C. Clayton, establishing the traditions which continue today. Shortly after his retirement, the Poster Symposium was initiated as a tribute to Dr. Watts and his effort, serving as an illustration of the research and development for continuing generations of life/health science researchers.
Daniel T. Watts
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Biochemistry and Molecular Biology
Abstracts
The Genetic and Environmental Relationship Between Childhood Behavioral Inhibition and Pre-Adolescent Anxiety

Jessica L. Bourdon, Jeanne E. Savage, Brad Verhulst, Dever M. Carney, Melissa A. Brotman, Daniel S. Pine, Ellen Leibenluft, Roxann Roberson-Nay, & John M. Hettema

Virginia Institute for Psychiatric and Behavioral Genetics

Background. Behavioral inhibition (BI) confers risk primarily for social anxiety yet is associated with various other anxiety disorders. This study examines genetic and environmental factors underlying the relationships between BI and symptoms of several pre-adolescent anxiety disorders. Methods. 352 twin pairs ages 9-13 and their mothers completed the Screen for Child Anxiety Related Disorders (SCARED); the mothers also completed a retrospective version of the Behavioral Inhibition Questionnaire (BIQ). Phenotypic associations were estimated between retrospectively reported childhood BI and recent anxiety symptom clusters (social, generalized, panic, and separation). Biometrical twin modeling was used to examine the unique and shared genetic and environmental factors underlying these phenotypes. Results. Significant correlations were found between childhood BI and each anxiety symptom cluster (r = .10 - .56). Heritability estimates were high for childhood BI (h² = .83) and moderate for the anxiety symptoms (h² = .51 - .68). Social anxiety symptoms shared a greater proportion of genetic and environmental variance with BI (15% each) than any other anxiety domain. Conclusion. This is the first study to explore the shared heritability between BI and pre-adolescent anxiety symptoms. Findings replicate a strong association between childhood BI and symptoms of social anxiety while also clarifying etiologic contributions to this association.

Key Words: behavioral inhibition, social anxiety, generalized anxiety, panic disorder, separation anxiety, childhood, pre-adolescence, heritability, anxiety symptoms
02. The Role of Sphingosine Kinase 2 in Alcohol-Induced Hepatic Inflammation and Injury

Eric K. Kwong, Liping Tao, Xiaojiaoyang Li, Runping Liu, Phillip B. Hylemon and Huiping Zhou.

Microbiology and Immunology

Introduction: Alcoholic liver disease (ALD) is one of the most common liver diseases worldwide. Inflammation has been implicated in the progression of ALD and liver fibrogenesis. Previous studies have identified that the activation of immune cells such as Kupffer cells and infiltrating macrophages contribute to the pathology of ALD in patients. In addition, evidence suggests that macrophage subtypes play a critical role in ALD. However, the role of sphingosine kinase 2 (SphK2) in immune cell-mediated alcoholic liver injury is poorly understood and is the focus of this study. Methods: C57BL/6J wild type and SphK2 knockout (KO) mice (10-14 week old) were fed a 5% Lieber-deCarli alcohol diet for 10 d followed by gavage of a single dose of alcohol (5g/kg). RAW264.7 macrophages were treated with alcohol or LPS. Mouse primary hepatocytes (MPH) derived from wild type (WT) or SphK2 KO mice were treated with 0-200 mM alcohol for 0-24 hours. The expression levels of target genes were determined by qRT-PCR and Western blot analysis. Liver injury was assessed by histological analysis. Results: Both alcohol and LPS upregulated SphK2, ATF4 and inflammatory mediators (MCP-1, TNFα and IL-1β) in mouse macrophages. Alcohol increased expression of SphK2 in MPH. Alcohol induced higher expressions of MCP-1 in SphK2 KO MPH and was associated with higher lipid accumulation compared to WT. Macrophage M1 subtype inflammatory mediators IL-1β, IL-6, MCP-1, iNOS, TNFα, CCR2, CD68 and F4/80 were upregulated while M2 subtype mediators Fizz1, CCL17, CCL22 and TGFβ remain unchanged in SphK2 KO mice fed an alcohol diet compared to WT. Conclusion: Alcohol induced the expression of SphK2 in macrophages and hepatocytes. In the absence of SphK2, alcohol induced more inflammatory response and immune cell infiltration in the liver. Our results suggest that SphK2 plays an important role in modulating the immune response in alcohol-induced liver inflammation.

Key Words: liver disease, alcohol, inflammation
03. The Timing of Geographic Power

Anny-Claude Joseph, Catherine A. Calder David C. Wheeler

Biostatistics

In many studies on the spatial risk of disease, investigators search for a spatial signal of elevated risk using geographic locations at the time of disease diagnosis in hopes of identifying new risk factors. However, studies often fail to find a significant spatial signal. This is likely due in part to not looking in the right place at the appropriate time. Environmental exposures related to cancer risk are intrinsically temporal and many cancers have a long latency. When these factors are considered in conjunction with a mobile population, it is likely that the spatial signal related to relevant historic environmental exposures is obscured. To investigate this hypothesis, we conducted simulation studies to characterize the effect of population mobility on the ability of generalized additive models to detect areas of significantly elevated historic environmental exposure. We generated data based on the residential histories of participants in the National Cancer Institute Surveillance, Epidemiology, and End Results non-Hodgkin lymphoma study, and varied the duration and intensity of the environmental exposure. Results showed that the probability of detection, mean spatial sensitivity, and mean spatial specificity of models decreased steadily as the time prior to study enrollment increased. This suggests that spatial areas of high-intensity exposure of relatively short duration will be difficult to detect over time when using residential locations at the time of diagnosis in mobile study populations.

Key Words: spatial epidemiology, geographic power, NHL
There remains a need for more effective therapeutics in the treatment of traumatic brain injury (TBI). In this study, we tested whether an effective TBI intervention could be developed around a post-translational target occurring within an opportune treatment window as guided by temporal proteomics. Of interest were delayed-onset processes best managed after patient stabilization on the intensive care unit, focusing on events initiated a day or more after insult, out to two weeks following controlled cortical impact injury. We identified a subset of protein changes with the temporal profile of interest utilizing a self-organizing map approach. Enriched in this map were post-translational processes tied with ionic dysregulation, among which was a highly dynamic processing of neuron-specific K+Cl- cotransporter 2 (KCC2), an essential component for maintaining chloride homeostasis that is critical to inhibitory neurotransmission. We identified a potential therapeutic window of opportunity starting on day 1 preceding unique acetylation, phosphorylation and ubiquitination events guiding the functional loss of KCC2. To test this window, we administered the KCC2-targeting compound CLP290 daily (50 mg/kg, p.o.) before, at, and after the identified 1-day point of KCC2 post-translational processing. The therapy was most effective at 1-day, preserving plasmalemmal KCC2 within perilesional somatosensory neocortex needed to maintain chloride homeostasis and effective inhibitory neurotransmission. Furthermore, TBI-impacted sensorimotor integration improved significantly with the 1-day intervention on rotarod and whisker adhesive removal task assessments. Together, our findings demonstrate the therapeutic targeting of post-translational processes revealed with temporal proteomics to effectively preserve KCC2-mediated chloride homeostasis with improved functional recovery. Furthermore, the approach defines an effective administration window that can be extended for the intervention of other post-translational events. Lastly, KCC2-targeted therapy may be extended to other neurological insults known to involve chloride dysregulation such as epilepsy and stroke.

**Key Words:** KCC2; Proteomics; Inhibition; CCI; Chloride; Post-translational
05. Estimating the Causal Effects of Air Pollution on Non-Hodgkin’s Lymphoma in Selected U.S. Cancer Registries

Keith W. Zirkle; Marie-Abèle Bind; Elizabeth Cahoon; David C. Wheeler

Biostatistics

Non-Hodgkin lymphoma (NHL) is the seventh most common cancer in the U.S. Several studies have found evidence of an association between NHL and air pollution. Particulate matter (PM) are the microscopic solid and liquid materials found in air and are considered Group 1 carcinogens. In 2005, the U.S. Environmental Protection Agency (EPA) designated certain U.S. counties and tribes as 'nonattainment' based on standards set for PM with aerodynamic diameter < 2.5μm (PM2.5). In our study, we estimate the causal effects of nonattainment designation on NHL incidence in U.S. counties in California, Kentucky, and Georgia using Surveillance, Epidemiology, and End Results (SEER) data. Traditional causal inference assumes no interference, or a subject's outcome is not affected by other subjects' treatments. In air pollution studies, regulation at one location will affect downwind locations. We introduce a new assumption that allows causal inference under interference by identifying covariates necessary to model the treatment assignment and spillover mechanism. Finally, we propose propensity score-based methods to estimate causal effects in a hierarchical Bayesian framework.

Key Words: causal inference; interference; air pollution; spatial epidemiology
06. The Role of Maternal Low Birthweight on Fetal Growth in Virginia

Sylvia Rozario, MPH, MBBS; Saba Masho, MD, MPH, Dr.PH; Derek Chapman, PhD

Family Medicine and Population Health

Background: The US has a relatively high infant mortality rate when compared to other developed countries. Small for gestational age (SGA) is an important indicator of poor fetal growth and a major cause of infant mortality and morbidity. Existing literature suggests that intergenerational risk factors influence the occurrence of adverse birth outcomes; but there is paucity of evidences to back up this suggestion. Objective(s): This study examines the effect of maternal low birthweight (LBW) on infants' fetal growth in Virginia. In order to explore the possible pathways by which maternal birthweight could be associated with infants' fetal growth, the current study also assesses the relationship of maternal medical complications with maternal LBW and infant SGA. Material/Methods: Data from a multigenerational birth dataset, created by linking 2005-2009 Virginia resident live birth data to 1960-1997 Virginia maternal live birth data, were analyzed (n=159,210). The outcome variable, infant SGA, was defined as a birthweight=<10th percentile for a given gestational age. The primary exposure variable, maternal birthweight (BW), was dichotomized as LBW (<2,500g) and normal BW (2500 + g). Adjusted odds ratios (AOR) and 95% confidence intervals (CI) were generated using multiple logistic regression models. Subpopulation analysis was conducted stratified by race/ethnicity. Results: The prevalence of maternal LBW and infants' SGA in Virginia were 7.4% and 10.6%, respectively. Maternal LBW was associated with increased odds of SGA infants after adjusting for potential risk factors in the current pregnancy (AOR=1.63, 95% CI=1.52, 1.76). Maternal medical history factors, such as respiratory disease, diabetes, hypertension, eclampsia, and previous preterm or SGA infant, were significantly associated with both maternal LBW and infant SGA. Conclusions: The mother’s birthweight may be a useful indicator in addressing adverse birth outcomes for infants. Public health program and policy must focus on factors throughout the life course of women in order to fully address inequities in birth outcomes.

Key Words: Low birthweight, Small for gestational age (SGA), Intergenerational risk factor
Kappa Opioid Receptors May Mediate Paclitaxel-Induced Changes in Affect-Like Behavior in Male Mice.

Julie A. Meade, Wisam B. Toma, Yasmin Alkhaif, D.E. Selley, Ph.D., & M. I. Damaj, Ph.D.

Pharmacology and Toxicology

Longitudinal studies of cancer survivors show long-term changes in mood, such as dysphoria and emotional deficit, including anhedonia. In order to study the underlying mechanisms of long-term changes in mood induced by cancer chemotherapy, cancer-free male C57BL/6J mice were treated with one cycle of four injections of vehicle or the chemotherapeutic paclitaxel (32mg/kg cumulative) and periodically assessed for anhedonia-like behavior. Paclitaxel caused significant, time-dependent deficits in sucrose preference and morphine conditioned place preference. The selective kappa opioid receptor (KOR) antagonist norbinaltorphimine reversed paclitaxel-induced sucrose preference deficit, suggesting an important role for the KOR neuronal system in paclitaxel-induced anhedonia. Because KOR signaling in the nucleus accumbens (NAc) can cause anhedonia, we used the [35S]GTPγS assay to determine if paclitaxel modulated KOR activity in the NAc. Surprisingly, a history of paclitaxel had a trend of reducing U50,488H-stimulated KOR activity. Our behavioral and in vitro data suggest that paclitaxel-induced changes in affect-like behavior may be due to dysregulation of KOR signaling in the limbic system.

Key Words: Paclitaxel; KOR; Depression; Anhedonia; [35S]GTPγS assay
Nitric oxide (NO) is a key gaseous neurotransmitter in both vertebrate and invertebrate nervous systems. Nitric oxide synthase (NOS) produces NO by conversion of L-arginine to L-citrulline. NO signals via soluble guanylate cyclase as well as other downstream molecules. Although a few studies implicate NO in ethanol sedation behaviors in rodents, the underlying mechanism of NO-mediated changes in these ethanol-related behaviors has not been identified. We are using flies to investigate this mechanism. The fly NOS is encoded by a single gene that is flanked by two unrelated genes as well as two genes located within the Nos transcription unit. We have backcrossed and mapped three putative Nos mutants. Two single piggyBac transposons are inserted near the 5’ end of the Nos locus, while one is located near the 3’ end. Flies homozygous for each of these transposon insertions had decreased levels of total Nos mRNA expression, indicating that the insertions cause partial loss of function (LOF) in Nos. Homozygous or transheterozygous flies containing the partial LOF Nos alleles had increased ethanol sedation sensitivity compared to isogenic controls. Nos mutant flies, however, did not have altered basal locomotion or internal ethanol levels, indicating that the changes in ethanol sedation sensitivity were unrelated to disrupted ethanol uptake/metabolism or global behavioral defects. Furthermore, recovery from ethanol sedation was normal in Nos LOF flies, suggesting that NO signaling might selectively influence the progression of—but not recovery from—ethanol sedation. We are currently investigating whether decreased function of NOS is causally related to increased ethanol sensitivity. Our data are consistent with a model in which NOS and therefore NO signaling are required for normal sensitivity to, but not recovery from, ethanol sedation.
09. B1 Cell IGE protects the Helminth, Nippostrongylus Brasiliensis From B2-IGE Induced Clearance

Yolander Valentine, Rebecca K Martin, Daniel H Conrad.

Center for Clinical and Translational Research

Follicular B cells, or B2 cells are selected for production of specific IgE. This IgE is known to bind mast cells via FcεRI and crosslinking results in degranulation and an early and late-phase response that leads to augmentation of helminth clearance. B1 cells are an innate-like B cell that differs from B2 cells structurally and functionally. The antibodies that are produced by both cells play important roles in helminth infection. Our laboratory has found that B1 cells work in an opposite mechanism from B2 cells, making copious amounts of IgE that is poly-specific in response to helminth infection and thus it is unable to degranulate mast cells. In this project, we intend to prove that B1 IgE blocks the B2 IgE-mediated increased parasite clearance by diluting its interaction with mast cells, thus preventing crosslinking and degranulation. To assess this, we used a reconstitution model with RAG1-/- mice. All mice received CD4+ T-cells, and either B2 cells, B1 cells, or both B1 and B2 cells. The mice were then infected with the Th2 inducing helminth, Nippostrongylus brasiliensis, which is like the human hookworm. Eggs in the feces were then enumerated through the infection to monitor worm clearance. It was found that B2 cells alone increased parasitic clearance significantly and that when B1 cells were reconstituted together with B2 cells, this enhanced parasite clearance was removed as hypothesized. But, to further show that it was IgE from both cells types that was responsible, we utilized the same RAG1-/- reconstitution model with B1 cells from IgE deficient animals, paired with B2 cells from WT mice. These B1 cells were unable to block the increased parasitic clearance, reinforcing that B1 IgE is responsible for this blockade. Additionally, mice reconstituted with IgE deficient B2 cells we unable to induce augmented parasitic clearance. Here we show that B1 IgE protects the helminth from augmented clearance induced by B2 IgE. This represents a potential evolutionarily beneficial mechanism for the helminth that could be an explanation for the reduction in allergic symptoms that are seen in parasite-infected individuals and if harnessed, could be used as a treatment for allergic disease.
Studies in mammals are beginning to identify central nervous system (CNS) glia functional responses to alcohol administration (i.e. how glia respond to alcohol). Very few studies in any species, however, have explored the causal relationship between glial cell function and alcohol-related behaviors. To determine if glia are fundamental to the CNS response to alcohol, we are investigating how glia influence behavioral responses to alcohol by (i) determining if (presumed) global disruption of CNS glial cell function alters alcohol sedation sensitivity and (ii) exploring the influence of known molecular-genetic pathways within glia on alcohol sedation sensitivity. To investigate these phenomena, we are assessing ethanol sensitivity in flies with constitutive (via repo-Gal4) or adult-induced (via RU486 and 7293-1GS-Gal4) expression of a variety of transgenes that overexpress, block or knockdown the function of genes known to be active in CNS glia. To date, we have found that constitutive expression of RNAi against the genes axo, JhI-21, nemy and ent2 in CNS glia increases sensitivity to alcohol sedation. These RNAi results were specific to glia, not neurons, and did not change internal ethanol levels in the flies. Additionally, we have found that increasing oxidative stress (via RNAi-mediated knockdown of cytoplasmic Sod1) in CNS glia during adulthood reduces alcohol sedation sensitivity. Our data suggest that CNS glia act through specific molecular-genetic pathways to both protect against alcohol sedation and promote alcohol sedation sensitivity. We propose that CNS glia are fundamentally and dynamically involved in nervous system response to alcohol and are important for the ability of the nervous system to withstand alcohol insult.

**Key Words:** Alcohol, Glia
Despite high utilization of outpatient services, patients with substance use disorders (SUDs) are 30% more likely to have an emergency department (ED) visit than people without a SUD diagnosis. We hypothesize that continued ED use is a result of comparatively low satisfaction with outpatient providers among SUD patients. Data come from the Virginia Coordinated Care program, a community coverage program that provides healthcare to low income uninsured patients. SUD status is identified through survey self-report and claims data. Satisfaction with provider interaction is assessed using the patient satisfaction questionnaire (PSQ18). A factor analysis is performed to generate satisfaction indices, which are used in logistics regression models to estimate satisfaction with providers by SUD status. Two-part models are estimated to determine how satisfaction with providers modifies the effect of SUD status on ED utilization conditional of having at least one visit. Roughly 16% of the sample (N=1,186) is identified as having a SUD. In nearly all measures of patient experience, SUD patients and non-SUD patients reported nearly identical levels of satisfaction. However, SUD patients were more likely to report satisfaction with provider ability (-0.73, p<0.01). Regardless of satisfaction with communication, competence, convenience or overall satisfaction, having a SUD diagnosis increased the risk of having an ED visit. Patients with SUDs continue to be among the heaviest utilizers of ED services. Yet, current policies aimed at reducing ED use have had little effect on this patient population. Findings from this study suggest that improving the patient-provider interaction will do little to decrease ED rates, as SUD patients are just as satisfied, if not more so with the outpatient care they receive than patients without an SUD.

Key Words: patient experience; patient satisfactions, emergency department; substance use disorder; patient-provider communication
12. Investigating the Association Between Statin Use and the Vaginal Microbiome

Erin M. Garcia, Gregory A. Buck, Kimberly K. Jefferson

Microbiology and Immunology

Bacterial vaginosis (BV) is a dysbiotic vaginal microflora characterized by a shift from a lactobacilli-dominated population to a population dominated by anaerobic bacteria. BV is associated with several adverse outcomes including preterm delivery, infertility, and sexually transmitted diseases, which, compounded by its high prevalence make it an important subject for continued research. Studies have implicated Gardnerella vaginalis (GV) as an important etiological agent in BV, but its distinct role in the progression of the disorder is not entirely clear. It has been hypothesized that vaginolysin (VLY), a cholesterol-dependent pore forming toxin secreted by GV, plays an important role in pathogenesis. Statins, a class of drugs used to systemically reduce the levels of low-density lipoprotein cholesterol through the inhibition of HMG-CoA reductase, have recently been explored for their utility in both the treatment and prevention of infections, including infections caused by bacteria possessing cholesterol-dependent cytolysins. Our data show that statin use is associated with a lower abundance of GV and a higher abundance of Lactobacillus crispatus compared to non-statin users. We hypothesize that this association is due, in part, to the statin-mediated inhibition of the cytolytic activity of the vaginolysin produced by G. vaginalis. Our preliminary data indicate that in a vaginal epithelial cell co-culture system, simvastatin pre-treatment reduces VLY cytotoxicity, likely mediated by epithelial cell membrane cholesterol depletion, and also abrogates GV survival.
Desmosomes are cell-cell junctions present in the epithelia and heart and provide mechanical resistance to these tissues in adults. Thus, it is no surprise that people born with defects in desmosomal proteins can have numerous defects affecting the skin, hair, and heart. However, unlike other junctional complexes, the role of the desmosomes in epidermal development has largely been unexplored. Therefore, this work fills a major knowledge gap by probing the function and regulation of a critical desmosomal protein, desmoplakin (Dsp) during the development of the epidermis of Xenopus. This work shows that Dsp is required for proper epidermal morphogenesis and mechanical resistance in the embryo. Additionally, this study highlights a role for c-Jun N-terminal Kinase (JNK) in regulating desmosome dynamics in the epidermis of the developing embryo.

**Key Words:** Desmosomes, epidermis, Xenopus, embryo, JNK
14. Genetic and Environmental Influences on Cortisol Response to Trier Social Stress Test

Chelsea Sawyers, Christina Sheerin, Meridith Eastman, Ananda Amstadter & Roxann Roberson-Nay

Human and Molecular Genetics

**Background:** Cortisol is a hormone released by the hypothalamic-pituitary-adrenal axis (HPA axis) and is involved in sustained response to stressful stimuli as well as maintaining several other body processes. Salivary cortisol has previously been implicated in physiological reactions to psychosocial stress and has previously been used as an indicator of HPA dysregulation, particularly in persons with anxiety-related disorders. In this study, we sought to determine whether salivary cortisol response to a psychosocial stressor is influenced by genetic and environmental factors.

**Methods:** Participants include 112 monozygotic and 158 dizygotic Caucasian adolescent and young adult twin pairs (Mean age= 16.8, SD=1.3; 55% female) who participated in a Trier Social Stress Test. Salivary cortisol samples were collected at four time points after completion of the task: immediately after concluding the task, and every 15 minutes following. The area under the curve (AUC) approach leverages the repeated measures over time to create a cortisol response profile. AUC was calculated using these post-task time points to create a summary variable that captured the overall cortisol response and rate of change over time for each individual. A univariate biometrical model was used to estimate additive genetic, common (shared) environment, and unique (non-shared) environmental contributions to cortisol response to psychosocial stress.

**Results:** MZ correlations for salivary cortisol were significant, r= .49, and DZ twins were also significantly correlated at r= .17. Through twin modeling, we found cortisol response to be moderately heritable (h2= .47) with no significant shared environment estimated.

**Conclusions:** Cortisol response to a psychosocial stressor is moderately heritable. This study is the first to examine the heritability of cortisol response and has implications for continued use of cortisol measures in regards to psychosocial stress and psychopathology.

**Key Words:** Cortisol, Heritability, Twin, TSST
Periodontal diseases are an issue for many adults, yet they are still poorly understood. Unlike many diseases caused by a single microorganism, periodontal diseases are due to an imbalance of the oral microbiome. In the disease state, pathogenic organisms, even in low quantities, can change the transcriptome of the microbiome to increase the immune response, leading to inflammation, bleeding, and bone loss, prominent symptoms of periodontal diseases. It's important to find a way to reduce disease symptoms and lessen infection to decrease the disease burden. We are working in an in vivo model in Drosophila melanogaster to investigate the effect of periodontal pathogens on the innate immune response. Drosophila immunity is similar to mammalian innate immunity. The Toll pathway responds to gram-positive infections and the Imd pathway responds to gram-negative infections. Virulence assays were performed. The results show that Porphyromonas gingivalis and Fusobacterium nucleatum, among other bacteria, are lethal to Drosophila. We performed qPCR analysis for a variety of Drosophila genes involved in the antimicrobial peptide response. It appears that antimicrobial peptides activated by both the Toll and Imd pathways are upregulated in these infections. Toll and Imd are upregulated 2-fold to 14-fold in mono- and multispecies infections. Drosomycin and metchnikowin, anti-gram-positive antimicrobial peptides, are upregulated more than 50-fold. Diptericin, an anti-gram-negative antimicrobial peptide, is upregulated more than 300-fold in gram-negative infections. We plan to do more extensive analysis through RNA sequencing with monospecies, multispecies, and healthy and disease oral microbiome infections.

**Key Words:** innate immunity, Drosophila melanogaster, periodontal disease
16. Characterizing the Role of Vaginal Veillonellaceae Species in Women’s Reproductive Health & Pregnancy

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Microbiology and Immunology

Two vaginal phylotypes provisionally assigned to the genus Megasphaera based on 16S rRNA gene similarity have been repeatedly associated with bacterial vaginosis (BV) and negative reproductive health and pregnancy outcomes including preterm premature rupture of membranes (PPROM) and spontaneous preterm labor. We cultivated three vaginal 'Megasphaera' clones and in combination with publicly deposited genomes, performed a comparative genomic and phylogenetic analysis of 3 'Megasphaera' phylotype 1 and three 'Megasphaera' phylotype 2 genomes. Using bioinformatics and biological measures, we determined that the phylotypes represent two distinct species with differential genomic structure, predicted functional potential, and clinical associations and may be best taxonomically classified as a novel vaginal-specific genus belonging to the family Veillonellaceae. Herein, we propose the designation Veillonellaceae phylotype 1 (VLN1) and Veillonellaceae phylotype 2 (VLN2). Both phylotypes were associated with vaginal symptoms and diagnosis of BV, while VLN1 exhibited a stronger association with the condition. VLN2 was uniquely associated with trichomoniasis, another highly prevalent vaginal infection. We also observed intriguing associations with pregnancy in a cohort of 842 case matched pregnant and non-pregnant women. Given the repeated association of VLN1 with negative pregnancy outcomes and a single paper reporting that this organism is capable of invading the upper genital tract, VLN1 is of great interest in elucidating bacterial contributions to negative pregnancy outcomes. To validate our findings, we analyzed the prevalence of both phylotypes in the MOMS-PI cohort, a group of over 1,000 women sampled throughout pregnancy, to assess the prevalence of the two organisms, their dynamics throughout pregnancy and associations with clinical data and pregnancy outcome.

Key Words: vaginal microbiome, megasphaera, veillonellaceae, pregnancy, bacterial vaginosis, trichomoniasis
Changes in the 3D structure of chromosomes have recently been found to be a mechanism of gene regulation. Hi-C, a sequencing technology extended from chromatin conformation capture (3C), is a high throughput technique which captures the conformation of DNA in the cell. An all vs all matrix of interactions between the regions of the genome can be created from Hi-C data allowing for insights into the interacting regions. Few methods have been developed for detecting 3D structural differences between multiple Hi-C datasets representing different cellular conditions. We developed an R package, HiCcompare, to provide methods to jointly normalize two Hi-C datasets and detect differences between them. We present the MD (difference vs. distance) plot, a modification of the commonly used MA plot, as a visualization of the differences between Hi-C datasets. To adjust for biases within and between Hi-C datasets, we normalize the data on the MD plot using locally weighted linear regression (LOESS). Differences can be detected using a permutation-based method to find the largest fold changes as visualized on the MD plot. We tested our algorithms on a Hi-C dataset from the RWPE1 prostate epithelial cell line and a cancer derivative with overexpression of the ERG gene. We compared our method with another method for Hi-C comparison, diffHic. HiCcompare normalization was found to perform better at removing biases between Hi-C datasets compared to several other Hi-C normalization techniques. HiCcompare was able to detect several confirmed differences in the RWPE1 dataset. Overall, the differences found by HiCcompare had higher fold changes and occurred over the range of genomic distances while regions detected by diffHic had lower fold changes and were mostly detected at shorter genomic distances.

**Key Words:** Biostatistics, genomics, Hi-C, DNA
Cell cycle events are precisely timed and tightly coordinated. Cell cycle deregulation is, in turn, a hallmark of cancer. The DREAM (DP, RB-like, E2F, and, MuvB) and MMB (Myb-MuvB) complexes are important for maintaining appropriate cell cycle gene expression. DREAM represses 800 cell cycle genes during G1 or G0 (quiescence), and the MMB complex increases G2/M gene expression, required for cell division. LIN52 is part of the MuvB core of proteins, shared in both of these complexes, and is necessary for their assembly and function. Data from The Cancer Genome Atlas reveal that deletions in the LIN52 gene are associated with decreased survival in some cancers. Here, we investigated the mechanisms controlling the degradation of LIN52 and how it may be influence the formation of DREAM and MMB. DYRK1A (dual-specificity tyrosine-regulated kinase)-mediated phosphorylation of LIN52 at serine-28 (S28) is critical for LIN52 binding to p130 and, in turn, DREAM formation. We noted that LIN52 protein levels are increased when it cannot be phosphorylated due to a loss or inhibition of DYRK1A, or when S28 was replaced with alanine (S28A). Expression of either LIN52-V5 or LIN52-S28A proteins resulted in downregulation of native LIN52, suggesting that LIN52 levels are tightly regulated in the cell. Our results support degradation of LIN52 following its phosphorylation by DYRK1A. Specifically, cells treated with cycloheximide showed a decrease in LIN52 levels that was reversed by proteasome inhibition with MG-132. Furthermore, harmine inhibition of DYRK1A, loss of DYRK1A expression, or S28A mutation all resulted in increased stability of LIN52. Using RT-qPCR, we observed that LIN52 mRNA levels slightly increased upon loss of DYRK1A activity and decreased when recombinant LIN52 was overexpressed, suggesting that transcriptional regulation could play a role in control of LIN52 levels. However, the differences in protein levels between LIN52-V5 and LIN52-S28A, despite equivalent mRNA expression, could be only explained by protein-level regulation. Altogether, these results suggest DYRK1A-mediated phosphorylation of LIN52 may lead to LIN52 degradation by the proteasome. These observations place LIN52 in a position to influence the formation of the DREAM and MMB complexes and, in turn, impact cell cycle regulation.
19. Medical Mistrust in Black Breast Cancer Patients: Acknowledging the Roles of the Trustor and the Trustee

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Health Behavior and Policy

Purpose: Studies indicate that Black patients report higher medical mistrust compared to their White counterparts. However, little is known about factors associated with higher medical mistrust amongst Black breast cancer patients. We examined predictors of medical mistrust and relationships between medical mistrust and process of care factors to identify opportunities to promote positive healthcare interactions between the trustees (e.g. providers) and Black breast cancer patients, or the trustors.

Methods: A secondary analysis was conducted of survey data from 210 African American women with confirmed diagnosis of invasive breast cancer. Participants completed telephone surveys consisting of questions pertaining to sociodemographics, attitudes, and beliefs about medical care and breast cancer treatments. Multiple linear regression determined factors associated with medical mistrust. Results: Most participants (61%) were over the age of 50 and currently single (64.8%). Women with greater medical mistrust reported less satisfaction with the trustee's technical ability (p<0.0001) and greater satisfaction with their own propensity to access care (p=0.04); however, having a Bachelor's degree did not contribute to women's levels of mistrust (0.06). Additionally, women with public insurance demonstrated greater mistrust than women with private insurance (p=0.01) or Medicare and private insurance (p=0.04). Conclusion: Findings from this study may inform future endeavors to educate providers on ways to effectively interact with and treat Black breast cancer patients. Opportunities to develop interventions that address and tackle issues of mistrust as reported by Black patients may contribute to ongoing efforts to reduce health disparities.

Key Words: Breast Cancer, Medical Mistrust, Black Women
Experiencing adverse childhood experiences (ACEs) of domestic violence (DV) and verbal, emotional, or sexual (VES) abuse could be a barrier to having breast, cervical, and colorectal cancer screening as an adult per national recommendations. The purpose of this study is to determine the association between ACEs and screening adherence. This project uses 2012 Behavioral Risk Factor Surveillance System data from adults living in the 4 states (IA, NC, TN, and WI) participating in a module on ACEs (range=9,324-15,412). Ages and gender varied for each analytic sample given U.S. Preventive Services Task Force guidelines for cancer screening (breast: women, 50-75; cervical: women, 21-64; colon: men/women, 50-75). Screening adherence was coded per guidelines for each screening test as adherent, overdue, or never screened. Descriptive statistics were calculated. Adjusted multinomial logistic regression models were run to determine the associations between domestic violence and abuse ACEs and cancer screening. Women who experienced any DV or VES abuse during their childhood were respectively 1.46 (CI: 1.20-1.78) and 1.49 (CI: 1.25-1.78) times more likely to be overdue for mammography than adherent compared to women without these ACEs. Similarly, for cervical cancer screening, women who reported DV as a child were 1.48 times more likely to be overdue than adherent compared to those with no DV (CI: 1.20-1.78) and 1.49 (CI: 1.25-1.78) times more likely to be overdue compared to their counterparts (CI: 1.16-1.84). Adults who experienced verbal, emotional or sexual abuse were 1.24 times more likely to have never had a stool blood test (CI:1.05-1.48) and were 1.32 times more likely to be overdue for a colonoscopy (CI:1.01-1.73) compared to those without abuse as a child. This study illustrates a deficit in screening adherence among those who suffered from DV and/or verbal, emotional, or sexual abuse during childhood. Thus, encouraging people to remain adherent is just as important as encouraging people to be screened, and specific care is needed for people who suffered from abuse or DV.

**Key Words:** ACEs, domestic violence, abuse, cancer
21. Assessing the Uncertainty due to Chemicals below the Detection Limit in Chemical Mixture Estimation

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Biostatistics

Simultaneous exposure to many chemicals over a lifetime may increase an individual's risk of disease. Weighted quantile sum (WQS) regression was developed in order to estimate the overall health effect of exposure and to identify the important chemicals in the mixture. However, complications arise when experimental apparatus can only detect chemical concentrations to a detection limit. In WQS analyses, these values below the limit of detection (BDLs) are either imputed or placed in the first quantile of the weighted index. The impact of the two approaches, however, is unknown in chemical mixture analyses. We compared through a simulation study a univariate Bayesian single imputation approach to that of placement of values BDLs in first quantile. The true mixture consisted of 14 chemicals with weights of 0.25 given to four and 0 to the rest. Scenarios included 0% (baseline), 10%, 33%, 50%, and 80% BDL for each chemical. We examined the ability of each method to estimate the overall chemical mixture effect and to identify chemicals most strongly associated with a binary disease. The imputation algorithm estimated that the mean odds of overall chemical mixture in a one-unit increase was 3.39 (0% BDL), 3.46 (10% BDL), 3.19 (33% BDL), 2.89 (50% BDL), and 1.49 (80% BDL), while for the placement method: 3.50 (10% BDL), 3.17 (33% BDL), 3.07 (50% BDL), and 2.46 (80% BDL). Both methods correctly identified that the four chemicals up to half of the time for values BDL up to 50% and three chemicals at 80% BDL. Results show that placing the BDLs in the first quantile is slightly better to the imputation method. A possible reason is that this imputation approach treats the imputations as fixed values and ignores the correlation between the chemicals. Thus, future research would assess whether incorporating multiple draws and multiple chemicals into the imputation model would be better than the placement method in chemical mixture estimation.

Key Words: environment, missing data, multiple imputation, Bayesian inference, WQS regression
The Modulatory Role of the α7 Nicotinic Acetylcholine Receptors (nAChRs) in a Mouse Model of Chemotherapy-Induced Peripheral Neuropathy (CIPN)

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Pharmacology and Toxicology

Paclitaxel is a chemotherapeutic agent used to treat multiple types of solid tumors such as breast, lung, ovarian, and neck cancer. However, treatment with paclitaxel results in major dose-limiting side effects named chemotherapy-induced peripheral neuropathy (CIPN). CIPN can be observed in more than 80% of patients during and after treatment with paclitaxel. Most CIPN symptoms are sensory including paresthesia, numbness, allodynia, and hyperalgesia. Unfortunately, there are no effective treatments to mitigate these symptoms; therefore, there is a dire need to develop a treatment for CIPN. One of the possible significant targets to treat CIPN is α7 nicotinic acetylcholine receptors (nAChRs) subtypes. Many studies have shown that α7 nAChRs are distributed in the pain transmission pathway including neuronal and non-neuronal cells, specifically immune cells. It has also been reported that α7 nicotinic agonists induce antinociception, anti-inflammatory, neuroprotection, and an anti-allodynic effect in rodent models of pain. The objective of the present study was to investigate the modulatory role of α7 nAChRs in the development of CIPN in α7 Wild Type (WT) and Knock out (KO) mice and to test if R-47, a Selective Silent Agonist of α7 nAChRs would reverse and prevent CIPN induced by paclitaxel. α7 WT and KO mice were treated with paclitaxel (1 mg/kg, i.p.). R-47 (1, 5, 10 mg/kg, p.o.) was given to mice treated with paclitaxel (8 mg/kg, i.p.) to reverse CIPN; R-47 (10 mg/kg, p.o.) was repeatedly given to prevent CIPN. Our results demonstrate that α7 KO mice exhibit significant worsening of mechanical threshold (initiation and maintenance) compared to α7 WT mice. Additionally, R-47 both prevents and reverses paclitaxel-induced mechanical allodynia. In conclusion, our data suggest that α7 nAChRs could be a promising target to alleviate and prevent the CIPN symptoms.

Key Words: nAChRs, CIPN
Prolactin (PRL) plays a significant role in the pathogenesis and progression of breast cancer. In pathological settings, increased production of PRL and up-regulation of its cognate receptor (PRLr) leads to constitutive signaling through proximal Janus Kinase 2 (Jak2). Jak2 associates with PRLr Box 1 motif via its FERM domain, phosphorylates c-terminus of the PRLr, which leads to Stat5 recruitment and activation. Cyclophilin A (CypA) is a member of the immunophilin family of peptidyl-prolyl isomerases (PPIs), which is constitutively bound to the PRLr that catalyze the cis-trans interconversion of proline imide bonds of peptides. The PPI activity of CypA is inhibited by the immunosuppressive drug cyclosporine A (CsA), and in turn the CypA-CsA complex inhibits calcineurin-mediated NFAT activation. The lab has investigated CypA regulation of Jak2 and found a potential role for CypA which bound to the PRLr at residue 334. Treatment with CsA inhibited CypA binding to the PRLr and blocked PRLr-driven activation of Jak2/Stat5. Recently, Waters et al., utilizing Fluorescence Resonance Energy Transfer (FRET) with transfectants expressing cyan- and yellow-fluorescent protein (CFP and YFP)-tagged forms of the growth hormone receptor (GHR, an analog of the PRLr) showed that GHR activation induced a rotational movement in C-terminus of the GHR, resulting in a loss of baseline FRET signal. Given this, we hypothesized that CypA isomerase activity on proline residues within the Box 1 motif of PRLr may facilitate conformational change in the intracellular domain of the PRLr. To demonstrate that PRL can induce a conformational change in the intracellular domain of the PRLr, FRET techniques including acceptor photobleaching and sensitized emission were utilized to calculate FRET efficiency. Like the GHR, PRL stimulation of transfectants expressing CFP/YFP-tagged PRLr constructs resulted in a loss of FRET efficiency upon PRL stimulation. In contrast, treatment with NIM811 (a non-immunosuppressive form of CsA) resulted in a return of FRET signal in the presence of PRL. These studies reveal that ligand stimulation of the PRLr results in a conformational change as measured by FRET signal to the receptor that is reversed by CypA inhibition, implicating CypA as the mediator of this conformational change and ligand-induced signaling. To further assess the consequences of CypA inhibition on the PRLr/Jak2 mediated signaling/functions, analyses of phospho-tyrosine residues that are believed to be important for interactions/signaling were investigated in breast cancer cells. It was found that NIM811 inhibited prolactin-stimulated phosphorylation of Stat5 Y694, Jak2-Y1007/1008, PRLr-Y281/-Y587, and Src-Y416 in an ER+/PR+ MCF7 cell line in a time dependent manner. Like the drug inhibition, RNAi mediated knockdown of CypA also down-regulated PRLr/Jak2-associated phosphorylation following PRL stimulation. NIM811 inhibited ER+, ER-, and HER2+ breast cancer cell proliferation, survival, motility and anchorage-independent growth. Furthermore, NIM811 inhibited mRNA expression of prolactin responsive downstream genes, such as CISH and Cyclin D1, which are known regulators of breast cancer pathogenesis. Overall, these results indicate that CypA modulates conformational change of the intracellular domain of the PRLr through its PPI activity, and alters PRLr/Jak2 complex signaling/functions in breast cancer.

**Key Words:** Prolactin, Prolactin Receptor, Jak2, Breast Cancer
Thermogenic fat is a promising target for new therapies in diabetes and obesity. Understanding how thermogenic fat develops is the first step in developing rational strategies. Previously, we have shown that Tyk2 and STAT3, part of the JAK-STAT pathway, is necessary for proper development of classical brown fat. Here, we show in primary preadipocytes that STAT3 is required for differentiation and robust expression of Uncoupling Protein 1 (UCP1). STAT3 is necessary during the induction phase of differentiation and not during the terminal differentiation phase. The loss of differentiation in STAT3-/- preadipocytes can be rescued using Wnt/Beta-Catenin pathway inhibitors when applied during the induction phase, showing temporal correlation with the requirements for STAT3. These findings define a new pathway involving both STAT3 and Wnt in the regulation of thermogenic fat.

**Key Words:** STAT3, Brown Fat, Wnt, Adipogenesis
Although cigarette smoke has been implicated in a causal relationship with various types of cancers, around 62% of all cancer patients are current smokers, recent quitters, or former smoker. While most patients who are smokers are motivated to quit after cancer diagnosis, 25 -30% of these patients continue to smoke. Furthermore, most quitters relapse after 2-3 years of post-chemotherapy. This represents a major health concern since several clinical studies revealed that perpetuation of smoking in cancer populations attenuates patient's well-being and quality of life. Smoking may impair healing, attenuate the efficacy of chemotherapy, increase the disease complications and diminish survival rates. However, the factors that involved in nicotine dependence in cancer patients is poorly understood. According to human research, it was suggested that tumor site, impact of cancer therapy and disease prognosis could be responsible of continuation of tobacco smoking among cancer patients and survivors. Recently, chemotherapy was shown to cause emotional deficit in humans (anxiety, insomnia and depression) and animals. In this project, we focused on chemotherapeutic agent, paclitaxel, because it is widely used to treat solid tumors such as lung, head, and neck and breast cancer. We previously reported that paclitaxel induced general affective deficit in mice such as anhedonia, anxiety and depression-like behaviors. We therefore hypothesized that chemotherapeutic agent, paclitaxel may alter the rewarding and withdrawal properties of nicotine. We investigated the impact of paclitaxel on spontaneous nicotine withdrawal and nicotine reward in C57BL/6J mice using then conditioned place preference (CPP) test. Our findings showed that paclitaxel worsen the somatic and affective signs of nicotine withdrawal in male mice as well as attenuation of nicotine reward in the CPP assay. These behavioral changes were not due to an impact of nicotine metabolism by paclitaxel. The work was funded by the NIH- R01 CA206028.
The smoke-free laws and tobacco-awareness campaigns in the United States have induced the alternative marketing of nicotine. There has been an increase in commercial production of oral nicotine delivery systems such as strips, orbs, sticks and lozenges. Despite the shift in consumption method, individuals are still subject to the effects of nicotine consumption, including addiction and withdrawal. Varenicline, commercially sold as Chantix®, is a commonly used partial agonist at the α4β2 nicotinic acetylcholine receptor (nAChR) subunit used to aid in smoking cessation. We aimed to investigate the effects of varenicline on oral nicotine consumption in mice. Adult male and female C57Bl/6J mice (n=10/per sex) were first given a choice of water or nicotine solution (a range of concentrations of 5-240 µg/ml) using a two-bottle free choice drinking assay to determine the oral stable concentration of nicotine in animals. Our results showed that nicotine consumption varies between males and females, with female mice drinking significantly more at concentrations greater than 60 µg/ml. Using these results, adult male and female C57Bl/6J mice (n=8/per group/per sex) were given a choice of water or nicotine (60 µg/ml) solution using a two-bottle free choice drinking assay, and after 3 days injected with either saline or a 0.3 mg/kg dose of varenicline. At four and twenty-four hours post injection, there was a significant decrease in both intake and preference for nicotine in varenicline-injected mice without a considerable effect on total fluid intake. In summary, nicotine preference in mice was sex-dependent, and a 0.3 mg/kg dose of varenicline showed a significant decrease in both oral nicotine preference and intake, in agreement with smoking cessation studies. This research was supported by the National Institute on Drug Abuse of the National Institutes of Health under Award Number P50DA036105 and the Center for Tobacco Products of the U.S. Food and Drug Administration. The content is solely the responsibility of the authors and does not necessarily represent the views of the NIH or the FDA.
27. Correlates of Adjuvant Therapy Attitudes in African American Breast Cancer Patients

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Purpose: Black breast cancer patients have lower utilization of adjuvant therapies than White women with breast cancer; yet, very few studies have examined Black women's attitudes towards treatment and their acceptance of recommended therapy. This study observed the influence of self-reported sociocultural factors that are associated with Black breast cancer patients' treatment attitudes on chemotherapy hormonal, and radiation therapy. Methods: This was a secondary analysis of data from the Narrowing Gaps in Adjuvant Therapy Study. The study included newly diagnosed breast cancer patients recruited from hospitals in Detroit, MI and Washington D.C (2006-2011). For the purpose of this study we only examined black women's association between treatment attitudes on all adjuvant therapies and sociocultural factors, using bivariate analyses. Predictors associated with treatment attitudes were evaluated from logistic regression models after considering covariates and the stepwise selection procedure. Results: The sample consisted of N=210 (58.4%) black women, of which were N=150 (55.6%) Hormonal positive (HR). The majority of the black women had negative attitudes toward chemotherapy (52.4%). The odds for having a negative attitude toward chemotherapy were higher when black women reported more financial hardships (OR: 1.82 95% CI: 1.12, 2.29) and two or more comorbidities, but were highest for three or more comorbidities (OR: 4.0 95% CI: 1.6, 10.0). Regarding patient satisfaction, the odds of a positive attitude for radiation therapy were lower among Black women (OR 0.94, 95% CI: 0.90, 0.98). Black HR positive women's negative attitudes for hormonal therapy increased for every one unit increase in financial hardship (OR 0.42, 95% CI: 0.24, 0.73). Conclusions: Results from this study provide patient's attitudes regarding recommended systemic breast cancer treatment. Our findings provide consideration of Black women perspectives during comprehensive cancer treatment care with oncologist and health care providers. Increased attention to breast cancer patients' influence of negative attitudes regarding systemic treatments may further treatment education and awareness and improve adherence patterns for Black women.

Key Words: Breast cancer, Black women, adjuvant breast cancer treatment
28. Remodeling the Brain Environment: The Role of Breast Cancer Exosomes in Brain Pre-Metastatic Niche Formation

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Pathology

Brain metastasis is a devastating, late-stage event affecting 10-30% of breast cancer patients, but it is unclear how breast cancer cells can colonize the unique brain environment. Exosomes, endosomal-derived extracellular vesicles, have been reported to support pre-metastatic niche formation through recruitment of tumor-growth supporting cells, creating sites conducive to cancer development. Still, it is not known how exosomes might prime the distinctive brain environment for metastasis despite the ability of circulating exosomes to readily cross the BBB. Glial cells, namely astrocytes and microglia, are important contributors to brain metastasis, and astrocytes in particular secrete exosomes that promote tumor outgrowth in the brain. We therefore investigated the effects of breast cancer exosomes on astrocytes in promoting brain pre-metastatic niche formation. Using ultracentrifugation, we isolated exosomes from 4T1 mouse mammary carcinoma and MDA-MB-231 human triple-negative breast cancer cell conditioned media. Exosomes were characterized using Western immunoblot, dynamic light scatter analysis, and transmission electron microscopy, and labeled with a fluorescent lipid dye. Mouse and human astrocytes were then treated with either 4T1 or MDA-MB-231 exosomes, respectively, and vesicle internalization was assessed using confocal microscopy. Astrocytes were indeed found to internalize cancer exosomes in vitro. Using qPCR analysis, we explored gene expression changes in mouse astrocytes treated with 4T1 exosomes compared to untreated controls. 4T1 exosomes were found to significantly upregulate expression of various astrocyte-derived brain extracellular matrix and matrix-associated proteins such as tenascin C and Syndecans 1 and 3, suggesting the brain matrix could be remodeled prior to cancer cell arrival. Further, astrocytes treated with 4T1 exosomes exhibited significant upregulation of inflammation-associated genes, specifically COX-2 and IL-6, and genes associated with cancer and metastasis, namely Serpini1 and AEG-1. These findings suggest that breast cancer exosomes could drive transformation of the brain environment towards a pro-metastatic site.

Key Words: Exosomes, breast cancer, brain metastasis, astrocytes
Inhibitors of apoptosis (IAPs) modulate cell death and play critical role in signal transduction that promotes inflammation. Recently, Smac mimetics, which are IAP antagonists, have attracted a lot of attention and are currently under development as novel cancer therapeutics. Cellular Inhibitor of Apoptosis 2 (cIAP2), a member of IAP family, positively affects both NF-κB and MAPK activation in response to many inflammatory stimuli and controls inflammasome and ripoptosome activation. In addition to these known functions, cIAP2 also regulates activation of Interferon Regulatory Factor1 (IRF1). Since, IRF1-/- mice are resistant to experimental autoimmune encephalomyelitis (EAE), we hypothesized that cIAP2-/- mice should also be protected from the disease. Surprisingly, induction of EAE in cIAP2-/- mice resulted in rapid and exaggerated disease. We observed increased proinflammatory cytokine expression, immune cell recruitment and demyelination in cIAP2-/- mice. Surprisingly, bone marrow reconstitution experiment demonstrated that resident CNS cells but not immune cells are critical for the exacerbated disease. We propose that cIAP2 expressed in the residential cells impedes neuroinflammatory responses. Further investigation will help us understand the critical role of SMAC mimetic in multiple sclerosis.

**Key Words:** cIAP2, EAE, microglia, CNS, cell death, inflammation
Breast cancer is the second leading cause of cancer death in women. Triple negative breast cancer is aggressive with high recurrence, metastatic and mortality rates. More than 40,000 women in the US die each year of metastatic breast cancer, for which there are currently no permanent cures. The inability to effectively predict, prevent, and treat metastatic breast cancer is a major problem. Although it is well known that tumor metastasis is regulated by cancer cell-induced systemic changes in the host, it is still not clear how this occurs or how the systemic changes regulate metastasis. We have previously shown that tumor-derived sphingosine-1-phosphate (S1P) regulates numerous processes important for breast cancer progression and metastasis, yet little is known about the role of systemic S1P that regulates immune cell trafficking. In the present work, we utilized a new syngeneic mouse model of metastatic breast cancer. To this end, E0771.LMB triple negative breast cancer cells were implanted in the mammary fat pads of wild type or SphK2 knockout mice. Global deletion of SphK2 significantly reduced tumor growth and lung metastases, with corresponding major changes in immune cell infiltration into the tumor. Intriguingly administration of a potent SphK2 inhibitor also reduced numbers of metastatic foci in the lungs after tail vein injection of EO771.LMB cells with drastic changes in immune cell infiltration into the lung. Similarly, deletion of the S1P transporter Spns2 in lymphatic endothelial cells also enhanced cancer cell killing, thereby suppressing pulmonary metastatic colonization. Furthermore, interfering with the S1P gradient by inhibition of S1P lyase also significantly reduced breast cancer metastasis. Moreover, changes in lymph fluid levels of S1P were observed together with reduction of breast cancer metastasis. Further studies are needed to understand how SphK2, Spns2, and S1P lyase control levels of S1P in the circulation and lymph to regulate the S1P gradient, lymphocyte trafficking, and consequently affecting cancer cell killing. Metastasis is the leading cause of death for cancer patients and this work suggests that targeting SphK2, Spns2 and the S1P gradient are potentially promising options for suppressing breast cancer metastasis. This work was supported by National Institutes of Health Grants R01GM043880 (SS) and R01 GM121075 (KL), and DoD Award W81XWH-14-1-0086 (SS).

Key Words: Spns2, SphK2, S1P, Metasasis
Breast cancer is the most commonly diagnosed cancer in women. Estrogen receptor-α (ERα) and its ligand 17β-estradiol (E2) play critical roles in breast cancer. E2 elicits genomic effects in ERα-positive breast cancers that are important for tumor growth. Triple negative breast cancers (TNBC), lack classic ERα and don’t respond to hormonal therapies like tamoxifen. Nevertheless, TNBC cells express ERα36 splice variant on the plasma membrane that elicits rapid, non-genomic responses to E2 and has been implicated in tamoxifen resistance. E2 activates sphingosine kinase 1 (SphK1), producing the bioactive sphingolipid mediator sphingosine-1-phosphate (S1P). Extracellular S1P stimulates S1P receptors, important for cell growth, survival, motility, invasiveness of breast cancer cells. However, E2 receptor involved has not been elucidated. In this study, we utilized TNBC cells expressing only ERα36. Treatment with E2 or membrane-impermeable E2-BSA resulted in rapid increased intracellular and secreted S1P. Neutralizing or downregulating ERα36 suppressed E2-induced SphK1/S1P axis. Interestingly, spontaneous breast cancer mouse model on high fat diet caused increased ERα36 expression which was diminished by FTY720 treatment. We also found correlations between SphK1 and TNBC only expressing ERα36 and hormone resistant breast cancer. Our data suggests targeting SphK1/S1P axis may potentially be a new therapeutic option for TNBC.

**Key Words:** Triple Negative Breast Cancer, Tamoxifen Resistance, S1P