ALBANY COLLEGE OF PHARMACY AND HEALTH SCIENCES

10TH ANNUAL RESEARCH SYMPOSIUM

COLLABORATIVE RESEARCH IN THE HEALTH SCIENCES

Poster Session, Thursday, April 16, 2020

Morning Sessions

Session 1A: 11:00 - 11:45 am
Moderator: Eric Yager, Ph.D.

11:05 - 11:10 am	Introduction	
11:05 - 11:10 am	Lauren Martin	Study of Cell-to-Cell Transmission of HIV-1 After Co-infection with HTLV-1
11:10 - 11:15 am	Raniyah Speller	Opioid Epidemic in America and the Effect of Naloxone (Narcan) on the Number of Deaths from Opioid Overdose
11:15 - 11:20 am	Erin Broughel	Factors Impacting the Decision of an Individual with Lynch Syndrome to Terminate a Provider Relationship
11:20 - 11:25 am	Payal Multani	The Future of Health Care: Improving Society
11:25 - 11:30 am	Deepika Paratane	Mortality Following Vascular Access Restoration Procedures Due to Thrombosis Among End Stage Renal Disease Patients: 2011-2015
11:30 - 11:35 am	Deepika Paratane	Nosocomial Influenza Hospitalizations Among End-Stage Renal Disease Patients During the 2013-2014 Through 2015-2016 Influenza Seasons
11:35 - 11:40 am	Closing	

Morning Sessions

Session 1B: 11:00 - 11:45 am
Moderator: Christopher Cioffi, Ph.D.

11:05 - 11:10 am	**Introduction**	
11:05 - 11:10 am	Sunghwan Cho	Investigation into the Effect of Human Cytochrome P450 2C9*30 Genetic Variant on Drug Metabolism
11:10 - 11:15 am	Ivan Wang	Synthesis of Novel RPE65 Inhibitors for the Treatment of Age-Related Macular Degeneration and Stargardt's Disease
11:15 - 11:20 am	Thomas Yarbrough	Functional Basis of Single Nucleotide Polymorphisms in CYP2C9: The Role of CYP2C9*3 in Drug Metabolism
11:20 - 11:25 am	Sneha Pandithar	Crosstalk Between Stromal Components and Endocrine Resistant Breast Cancer Via Secreted Factors Enhances Tumor Growth and Metastasis
11:25 - 11:30 am	Marie Kathryn Malone	Adipsin promotes tumor progression in ESR1 mutant breast cancer
11:30 - 11:35 am	Sam Weitzen	Degradation of Cellular REDD1 by Influenza Virus NP is Partially Reduced in DDB1 Knockdown Cells
11:35 - 11:40 am	**Closing**	

Afternoon Sessions

Session 2A: 11:45 - 12:25 pm
Moderator: Kideok Jin, Ph.D.

11:45 - 11:50 am	Introduction	
11:50 - 11:55 am	Jessica Wohlfahrt	Towards Complete Proteome Coverage of Microglia in a 2-Hour, Single-Shot Analysis: Characterization of Microglial Response to Acute Alcohol Exposure
11:55 am - 12:00 pm	Nicholas Nasta	Identification and Validation of Secreted Factors in the Crosstalk Between HOXB7 Overexpressing Cells and Stromal Cells
12:00 - 12:05 pm	Sam Evans	Evaluation of VDUP1 Null Phenotypes in Drosophila Embryogenesis
12:05 - 12:10 pm	Rebekah Garfolo	The Role of STAT3 in the Regulation of Tumor Cell Growth of U87 Human Glioblastoma Cells
12:10 - 12:15 pm	Allen McClearnen	Evaluation of a Student Pharmacist Provided Oncology Education Series to Medical Residents During an Oncology APPE Rotation
12:15 - 12:20 pm	Dorothy Liu	Drug Policy and Formulary-Related Job Postings: Current Skills and Qualifications
12:20 - 12:25 pm	Closing	

Afternoon Sessions

Session 2B: 11:45 - 12:25 pm
Moderator: Timothy LaRocca, Ph.D.

11:45 - 11:50 am	Introduction	
11:50 - 11:55 am	Nafisa Arfan	Critical Roles of Nuclear Trafficking and RIP1 During the Hyperglycemic Shift from Apoptosis to Necroptosis
11:55 am - 12:00 pm	Khadija Moussadek	Characterizing the Role of Alkyl Hydroperoxide Reductase Subunits in Acinetobacter Baumannii Pathogenicity
12:00 - 12:05 pm	Cory Parker	Initiation of Bystander Cell Death by Ricin
12:05 - 12:10 pm	Dylan Davie	The Effects of Genetic Polymorphisms on Cytochrome P450 2C9 Structure & Function
12:10 - 12:15 pm	Daniel Galke	Development of Biocompatible Spin Tip Microextraction Devices with Polyacrylonitrile
12:15 - 12:20 pm	Yelena Dunikova	Determining the Vibrio Parahaemolyticus Antioxidant Genes Required for Organism Survival in Various Environments
12:20 - 12:25 pm	Closing	

A big thank you
to all the presenters for your hard work
and to all the attendees for taking the time
to attend our Research Symposium.
See you next year!

Poster Abstracts

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Study of Cell-to-Cell Transmission of HIV-1 After Co-infection with HTLV-1

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The human immunodeficiency virus-1 (HIV-1) and human T lymphotropic virus-1 (HTLV-1) are both retroviruses that cause serious human diseases. The HIV-1 spreads in human body mostly by cell-free viruses in blood circulation, while HTLV-1 transmits by cell-to-cell transmission in which viruses pass through virological synapses between cells in close contact with each other and results in a much efficient and aggressive form of infection. In this study after HTLV-1 positive MT2 cells were infected by VSV-G pseudotyped HIV-1 GFP+ virus, it was found that HIV-1 was able to convey another round of infection by cell-to-cell transmission. HIV-1 cell-to-cell transmission was analyzed by co-culture of HIV-1 GFP+ infected MT2 cells and HIV-1 indicator cell line TZM-bl. Infection was quantified by a Luciferase assay. Meanwhile coinfection with HTLV-1 by a HIV-1 virus with deletion in nuclear capsid (NC) did not permit HIV-1 cell-to-cell transmission, indicating HIV-1 could only be pseudotyped with glycoproteins from HTLV-1 rather than being cross-packaged by HTLV-1 during coinfection. It was also found heparin, low molecular weight heparin Tinzaparin and non-anticoagulant low molecular weight heparins (NACH) significantly inhibited HIV-1 cell-to-cell transmission. Moreover, the HIV-1 cell-to-cell transmission from HIV-1/HTLV-1 coinfected MT2 cells to human monocyte THP1 cells in a co-culture resulted in an aggressive form of cell death accompanied with the increased release of Lactate dehydrogenase (LDH). The pathogenesis caused by retrovirus cell-to-cell transmission has not been well-understood. Further study will provide important information to understand mechanism and consequence of retrovirus cell-to-cell transmission and to develop potential drug candidates for treatment.

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Opioid Epidemic in America and The Effect of Naloxone (Narcan) on the Number of Deaths from Opioid Overdose

Raniyah Speller and Lauren Purington
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Opioid drug use has drastically increased in our society since 1999 and millions become addicted every year. 1 Opioid drugs are narcotic analgesics that can greatly reduce pain and give relief. Although opioids are the best drugs for pain relief, they can cause adverse side effects when taken at high dosages. They can cause brain damage, coma and death if overused. Opioids can cause a feeling of wellbeing and euphoria which is why they are commonly overused or misused. Both heroin and prescription opioid use has increased since 2007.2 Heroin is an illicit opioid that has no medicinal purposes which was synthesized from morphine. Opioids active ingredients include morphine, codeine, thebaine and nacrotine. The structure of morphine led to the development of partial agonist and antagonists. 4 Antagonists such as naloxone and nalorphine are drugs that can reverse the effects of administered opioids because of their ability to occupy the receptor sites without producing any effects. Narcan (Naloxone) is a pure opioid antagonist that can reverse the effects of administered opioids. 4 It is widely used in hospitals and by emergency personal to reverse suspected opioid overdose. Opioid related overdose is a pathologic level of drug toxicity caused by a high intake of drugs that overwhelms normal physiological functioning. Major symptoms of opioid overdose are blue lips and fingertips, slow and shallow breathing, clammy skin, convulsions, loss of consciousness and respiratory arrest.6 Opioid overdose can lead to death, depending on the amount ingested and the type of opioid used. Narcan has saved many lives but can only reverse opioid overdose. Narcan has no significant adverse side effects but can cause include nasal irritation if administered using the nasal spray form or injection site pain if administered intravenously.7 It can induce withdrawal symptoms in a dependent patient. Narcan availability and distribution has direct effect on the number of deaths from opioid overdose. In most cases, increasing Narcan distribution combined with Good Samaritan Laws to protect citizens intervening in an emergency result in a decrease in overall opioid overdose death. However, Utah and Ohio, despite having naloxone disputation, have death rates that are either stagnant or increasing. Massachusetts has more distribution of Narcan to civilians and laws to protect them if they intervene while Wests Virginia has no distribution besides to medical personnel.5 The opioid overdose death rate in West Virginia is higher than Massachusetts opioid death rate.3 If more civilians were equipped with Narcan and had knowledge on how to decipher if a person is having an opioid overdose, it could save someone's life. Decreasing the number of opioids prescribed coupled with more Narcan availability and increased public awareness could have a major positive effect on the opioid epidemic in America.

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Factors Impacting the Decision of an Individual with Lynch Syndrome to Terminate a Provider Relationship

Erin Broughel and Allison Burton-Chase
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Lynch Syndrome (LS), also known as hereditary non-polyposis colorectal cancer (HNPCC), is a genetic cancer syndrome that puts those affected at a much higher risk for developing multiple cancers, including colorectal, endometrial, and ovarian. Due to the complexity of the screening and surveillance guidelines for this syndrome, individuals with LS require high quality relationships with their health care providers. This study evaluated the specific factors LS patients had for terminating their patient-provider relationships.

Study participants were recruited through social media. The data was collected through detailed surveys and in-depth semi-structured phone interviews (n=55). Data from this study was analyzed for changes in providers. Within the 55 interviews, 125 providers changes were reported with about 82% of participants reporting at least one change. The reasons for change were also recorded and indicate that most patient- provider relationships are terminated due to lack of provider LS knowledge, poor interactions, or a combination of both factors. The results of this study suggest a need for better communication between LS patients and providers and increased knowledge of LS-specific care.

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The Future of Health Care: Improving Society

Payal Multani and Wendy Parker
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The Collaboratory is a community effort led by ACPHS and Trinity Alliance to provide health care information and education within the South End of Albany. The embedded community- based services include food assistance, medication reviews, housing assistance, and health insurance navigation. ACPHS operates a Public Health Pharmacy Team onsite at the Collaboratory. This study will explore and offer guidance to our PHPT on how to improve all patient care interactions. Confronting the social determinants of health will provide us the ability to work with community members on a wide range of issues including economic instability, education, physical environment, food insecurity, employment, social networks, and limited access to healthcare. To bridge this gap, research has indicated that this divide will decline if we maximize the patient/ health care provider relationship. Identification will influence awareness of links between social group membership and illness as well as preventative and treatment practices. In other words, health care providers should be more aware that certain diseases are more common amongst the lower income community. These diseases that have been eradicated or rather have been with combated with preventive care has been found, patients with lower income are not provided these services. Rather there undermined in the health care system ultimately leading to an increase in disease and common infections that will result in death, if it is not treated (Harwood and Sparks). In this study, the staff of the Collaboratory will be interviewed to assess how the patients are responding to the services provided by the Collaboratory. Based on the staff's responses we can provide certain changes to the to the program and the services that the program offers. In this study, one will explore the different approaches that the Collaboratory takes to improve patient care. and this will be achieved by interacting with the staff in the Collaboratory.

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Mortality following vascular access restoration procedures due to thrombosis among end stage renal disease patients: 2011-2015

Deepika Paratane, Colleen C. McLaughlin, Carol-Ann Swain, Katie E. Cardone, and Margaret K. Doll
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Hemodialysis is the most common form of dialysis among patients living with end stage renal disease (ESRD) and requires the establishment of a vascular access site. Vascular access failure due to thrombosis is common among hemodialysis patients and is associated with an increased risk of mortality. However, the underlying causes for excess mortality following vascular access failure are poorly understood. One possibility is that excess mortality may be related to procedures undertaken to restore vascular access. In this study, we aim to describe the acute risk of mortality following a vascular access procedure among a cohort of ESRD patients.

Methods: This study will use data from the United States Renal Data System (USRDS), an integrated database containing Medicare billing data for US ESRD patients. ESRD patients who underwent a vascular access procedure due to thrombosis during the study period (2011-2015), and met the following eligibility criteria at the time of the procedure will be included: (i) age 18 years or older, (ii) receipt of hemodialysis for at least one year prior, and (iii) for whom Medicare was their primary payer. Vascular access procedures will be identified using administrative billing codes for vascular access restoration procedures in combination with thrombosis diagnosis codes. The acute risk of mortality following a vascular access procedure will be characterized as the mortality rate within the first 7 days following a vascular access procedure. Mortality rates will be estimated using patient-days and 95% confidence intervals. Stratified analyses will be performed to compare mortality between patients by vascular access type prior to the procedure.

Results & Conclusions: Assembly of the study cohort is currently underway. The results of this study will provide a better understanding of the relationship between the risk of mortality and vascular access restoration procedures.

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Nosocomial Influenza Hospitalizations Among End-Stage Renal Disease Patients During the 2013-2014 Through 2015-2016 Influenza Seasons

Deepika Paratane, Caroline Quach, and Margaret K. Doll
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Objectives: Influenza is a leading cause of death in many high-income countries, including the United States (US). Persons with end-stage renal disease (ESRD) are at high risk of influenza-related morbidity and mortality and have frequent healthcare system interactions that may increase their risk of nosocomial influenza (NI). This study examined the burden of NI among ESRD patients hospitalized with influenza.

Methods: We used data from the United States Renal Data System (USRDS) to estimate the proportion of influenza hospitalizations among ESRD patients attributable to NI during the 2013-14 through 2015-16 influenza seasons. USRDS is an integrated database containing Medicare billing data for US ESRD patients. Influenza hospitalizations were identified via influenza-specific ICD-9 (2013-2014) and ICD-10 (2015-2016) inpatient billing codes recorded during the influenza season. The Medicare-mandated billing variable, present on admission (POA), was used to classify influenza diagnoses as either NI (i.e. POA = no) or community-acquired (i.e. POA = yes). We compared demographic and clinical characteristics of NI and community-acquired patients.

Results: During the study period, ESRD patients experienced 16,197 influenza hospitalizations. Of these, 15,762 (97.3%) had a POA value of yes/no and were included in the study. NI accounted for 3.4% (95% CI: 3.2%, 3.7%) of influenza hospitalizations. On average, NI patients were hospitalized 19.1 (17.3, 20.9) days, or 12.5 (95% CI: 10.7, 14.2) days longer than community-acquired patients. NI patients were 2.1 (95% CI: 1.5, 2.8) times more likely to die during admission than community-acquired patients. No differences in mean age were found, however, community-acquired patients had a mean of 0.7 (95% CI: 0.2, 1.3) years longer duration of ESRD than NI patients.

Conclusion: We estimate that NI accounts for approximately 3% of ESRD patient influenza hospitalizations. Given the high risk of morbidity and mortality among this population, continued efforts should be made to reduce NI transmission.

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Investigation into the Effect of Human Cytochrome P450 2C9*30 Genetic Variant on Drug Metabolism

Sunghwan Cho, Sonia Parikh, and Manish Shah
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Cytochrome P450 (CYP) enzymes are a superfamily of heme-containing proteins found on the membrane of the endoplasmic reticulum. CYPs constitute the major enzyme family in drug metabolism with increasing importance in pharmacogenetics. The human CYP2C9 enzyme, which is responsible for the metabolism of over 20% of available clinical drugs, is highly polymorphic. More than 80 genetic variants of this enzyme have been identified, with many exhibiting significantly altered activities toward various medications compared to the wild type (WT). Despite the extensive functional and structural studies of various CYPs from different species, the fundamental basis of genetic polymorphisms is scant. The CYP2C9*30 is a rare allelic variant, which is found in Japanese population. It represents an amino acid substitution at position 477 from alanine to threonine that has resulted in significantly altered activity of the enzyme towards various substrates compared to the WT. We have expressed and purified the CYP2C9*30 variant in E.coli to the optimal quality. The in-vitro enzymatic assays revealed reduced activity and substrate turn-over by CYP2C9*30 compared to the WT. The previously solved crystal structure of the losartan complex demonstrated the reorientation of important amino acid side-chains due to the genetic variation at 477 from alanine to threonine. The goal is to investigate the activity of CYP2C9*30, using various substrates, and characterize the effect of variation to threonine on drug metabolism. The results will yield insights into the altered catalytic activity of the CYP2C9*30 and have important implications for understanding genetic variations and differences in drug metabolism.

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Synthesis of Novel RPE65 Inhibitors for the Treatment of Age-Related Macular Degeneration and Stargardt's Disease

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Age-related macular degeneration (AMD) is the leading cause of blindness for individuals aged 60 years or older. There are two forms of AMD; dry (atrophic) and wet (neovascular), with the more prevalent dry form accounting for nearly 90% of all diagnosed cases. There is no FDA-approved therapy for the most prevalent dry form of AMD. Histopathologically, dry AMD represents a slowly progressing neurodegenerative disorder in which specialized neurons (rod and cone photoreceptors) die in the central part of the retina called the macula. Age-dependent accumulation of cytotoxic lipofuscin in the RPE matches the age-dependent increase in dry ADM prevalence and thus is frequently cited as one of potential pathogenic factors contributing to the disease progression. Given that cytotoxic bisretinoids are synthesized from visual cycle retinoids as byproducts of the properly functioning visual cycle, partial pharmacological inhibition of the visual cycle was suggested as a treatment strategy for dry AMD and Stargardt's disease.

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Functional Basis of Single Nucleotide Polymorphisms in CYP2C9: The Role of CYP2C9*3 in Drug Metabolism

Thomas Yarbrough, Sonia Parikh, and Manish B. Shah
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Cytochrome P450 (CYP) enzymes are responsible for the clearance of majority of medications that are available in the clinic. A single nucleotide polymorphism (SNP) is a variation in a base pair in a DNA sequence that may lead to the variation in the amino acid sequence of the protein. SNP in drug metabolizing CYP may influence individual's response to drugs, resulting in adverse events or drug-drug interactions. More than 1000 such alleles in different CYPs have been identified, with many having significantly altered enzyme activity. Of all the drug metabolizing CYP enzymes, the CYP2C9 is responsible in the metabolism of ~20% of drugs that include antihypertensive losartan, anticoagulant warfarin, analgesic ibuprofen and antidiabetic drugs tolbutamide and glimepiride. It is a highly polymorphic enzyme with at least 80 known alleles. The *3 allele is one of the most prevalent genetic variants of CYP2C9 that is present in around 15% of Caucasians, and about 2-4% of African or Asian descent. A marked reduction of catalytic activity towards many important CYP2C9 substrates has been demonstrated by the CYP2C9*3 variant. The enzyme represents SNP that results in amino acid substitution where isoleucine is replaced for a leucine at position 359 in the protein sequence that comprises of 490 amino acids. In this study the CYP2C9*3 enzyme was expressed in E.coli and purified using affinity and ion-exchange chromatography. We have characterized the purified variant using multiple enzymatic assays for its activity towards the anti-hypertensive drug losartan. The results revealed significantly reduced activity of the enzyme compared to the wild-type. The crystal structure of CYP2C9*3 in complex with losartan had clearly demonstrated altered binding of the drug compared to the wild-type complex. Overall, the aim of our study is to elucidate the effect of genetic polymorphisms on drug binding and response that will eventually help in the development of drugs effective to broader population.

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Crosstalk Between Stromal Components and Endocrine Resistant Breast Cancer Via Secreted Factors Enhances Tumor Growth and Metastasis

Sneha Pandithar and Kideok Jin
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Various endocrine therapies have been developed such as selective estrogen receptor modulation (SERM), selective estrogen receptor downregulator (SERD) and ligand deprivation using aromatase inhibitors (AI). Tamoxifen (TAM), a SERM and AIs are the most commonly used adjuvant treatment for postmenopausal women with early-stage estrogen receptor positive breast cancer. Despite the relative safety and significant anti-neoplastic and chemopreventive activities of tamoxifen and AIs, many initially responsive breast tumors develop resistance and ultimately recur. Many underlying molecular events that confer resistance are known, but a unifying theme is yet to be revealed. While the tumor microenvironment is being increasingly recognized as a key factor in multiple stages of disease progression, particularly local resistance, immune-escaping, and distant metastasis, our previous work shows that the critical molecular and cellular players secreted from a crosstalk between breast cancer cells and stromal cells can regulate the tumor growth and process of metastasis in breast cancer.

In this report, we established a tamoxifen resistant breast cancer (TAMR) cells. We screened secretomes from normal fibroblasts in co-culture of TAMR cells using cytokine antibody arrays to target 105 human cytokines simultaneously. We identified upregulation of IL-6, CXCL5, IL-8, EMMPRIN, CXCL1, GM-CSF, GDF-15, CCL5, BDNF, BAFF, LIF, and CXCL10 in the secretomes from fibroblasts in crosstalk of ERBC cells. We ranked the expression level of each factor by real time qRT-PCR and determined that CXCL1 and IL-6 were the top candidates. We confirmed by U-PLEX (MSD) that CXCL1 and IL-6 secreted from fibroblasts treated with TAMR tumor conditioned medium (TCM) was upregulated compared to treatment with serum free media (SFM). Our data showed that the proliferation of TAMR cells co-cultured with fibroblasts was enhanced compared to monoculture. Furthermore, TAMR cell migration, a key step in tumor metastasis, was promoted by conditioned medium (CM) from TCM-induced fibroblasts. Significantly, inhibition of the CXCL1 and IL-6 signaling pathway by Reparixin, an inhibitor of the CXCL1 receptor CXCR1/2, and Tocilizumab, an inhibitor of the IL-6 receptor abrogated TAMR cell growth and migration.

These findings implicate IL-6 and CXCL1 signaling as a critical event in ERBC tumor growth and metastasis via crosstalk between cancer cells and stromal components. Further, these studies suggest that IL-6 and CXCL1 act as key regulators orchestrating ERBC. Therefore, we have provided evidence that supports the hypothesis that functional inhibition of the IL-6 and CXCL1 signaling pathway has the potential to circumvent ERBC growth and metastasis.

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Adipsin Promotes Tumor Progression in ESR1 Mutant Breast Cancer

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Abstract: ESR1 point mutations at ESR1 ligand binding domain are frequently identified in metastatic tumor, cfDNA (cell free DNA), ctDNA (circulating tumor DNA) and ptDNA (plasma tumor DNA) derived from ESR1 positive breast cancer patients treated with SERMs, SERDs and AIs. ESR1 mutations enhance ESR1 transcriptional activity in the absence of estrogen and induce estradiol independent growth as well as increase resistance to SERMs or SERDs in ESR1 mutant overexpressed cells. ESR1 mutations are found more frequently in cfDNA and tumor DNA from patients with metastatic disease compared to patients with primary tumor, Although SERM, SERD, AI and CDK4/6 inhibitor therapies have demonstrated preclinical and clinical benefits for breast cancer with ESR1 mutations, the development of resistance remains a significant challenge and the detailed mechanisms and potential therapeutic targets in metastatic breast cancer with ESR1 point mutations is yet to be revealed.

Tumor and organ microenvironments are crucial for cancer progression and metastasis. Crosstalk between multiple non-malignant cell types in the microenvironments such as blood and lymphatic endothelial cells and cancer cells promotes tumor growth and metastasis. Anti-angiogenic and anti-lymphangiogenic therapies may be combined with each other for the potential of improved outcomes for patients.

In this study, we identified the secretion of Adipsin from two different genome-edited MCF-7 ESR1 cell lines harboring Y537S and D538G when compared to wild-type cells using the Human Cytokine Array Q440 to quantitatively detect 440 human inflammatory factors, growth factors, chemokines, receptors, and cytokines simultaneously. We validated that the expression of Adipsin was highly upregulated in MCF-7-Y537S and D538G ESR1 mutant cells by real time qRT-PCR and ELISA. Interestingly, we observed that when cells were cultured in estrogen deprivation, the mRNA expression of Adipsin was significantly increased in MCF-7-ESR1 mutant cells while the treatment of tamoxifen and fulvestrant abrogated the upregulation of Adipsin. These results suggest that Adipsin is an ER target gene. Since the cleavage of factor B by Adipsin results in the conversion of C3 to C3a, which binds to its cell surface receptor (C3aR), our data showed that the C3a production was significantly increased in ESR1 mutant cells compared to WT by ELISA. In addition, we found that C3aR expression was increased in ESR1 mutant cells. To elucidate that C3aR signaling pathway promotes the proliferation of MCF-

7-ESR1 mutant cells, we examined the ESR1 mutant cell viability using SB290157, C3aR inhibitor, and found that ESR1 mutant cell growth was decreased in the treatment of SB290157. Furthermore, we found that the apoptosis was significantly induced in ESR1 mutant cells compared to WT by Annexin V assay.

These findings implicate Adipsin signaling as a critical event in ESR1 mutated breast cancer metastasis. Further, these studies suggest that Adipsin act as key regulators orchestrating breast cancer with ESR1 mutations. Therefore, we have provided evidence that supports the hypothesis that functional inhibition of the Adipsin signaling pathway has the potential to circumvent breast cancer metastasis. These findings implicate Adipsin signaling as a critical event in ESR1 mutated breast cancer metastasis.

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Degradation of Cellular REDD1 by Influenza Virus NP is Partially Reduced in DDB1 Knockdown Cells

Samuel David Weitzen and H. John Shariffi
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Mata et al. identified the cellular protein REDD1 as a host defense against IAV infection. REDD1 is initially upregulated in response to IAV infection however the levels of REDD1 decline thereafter suggesting that IAV evolved a mechanism to counter REDD1. Artificially increasing REDD1 levels above that which the virus can deplete, allows REDD1 to turn off cellular metabolism thereby impeding virus production. In uninfected cells REDD1 levels are kept low by constitutive degradation mediated by the CRL4 ubiquitin ligase complex. Our hypothesis is that the initial upregulation of REDD1 in response to IAV infection is countered by the NP protein of IAV. We further hypothesize that NP depletes REDD1 by enhancing its constitutive degradation mediated by the CRL4 complex. Our hypothesis is based on a precedent set by the HIV protein VPR which depletes the cellular protein UNG2 by enhancing its constitutive turnover (also mediated by the CRL4 ubiquitin ligase complex). Here we present our latest data, which shows that in DDB1 Knockdown Cells, REDD1 degradation by NP is reduced. While statistically this data is inconclusive, it gives credence to the hypothesis that the CRL4 ubiquitin ligase is responsible for NP-mediated degradation of REDD1. Additional experiments are needed to confirm that CRL4 specifically and exclusively is responsible for the degradation of REDD1.

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Towards Complete Proteome Coverage of Microglia in a 2-hour, Single-Shot Analysis: Characterization of Microglial Response to Acute Alcohol Exposure

Jessica Wohlfahrt, Jennifer Guergues, Owen Nadeau, Bin Liu, and Stanley Stevens
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Alcohol abuse results in deleterious effects on the central nervous system, including pathophysiological mechanisms related to the neuroimmune response. Specifically, alcohol exposure has been reported to induce an inflammatory phenotype in microglia; however, emerging evidence suggests a dynamic range of yet to be fully characterized microglial activation phenotypes. Given the global-scale molecular complexity that drives alcohol-induced microglial activation, the purpose of this study was to develop and implement an improved mass spectrometry-based proteomic approach that can provide comprehensive proteome coverage with high quantitation precision in a single shot analysis. Our methodology development using BoxCar data acquisition (developed by Mann lab) was performed on adult-derived mouse microglia (IMG cells). Offline fractionation using high pH reversedphase HPLC followed by mass spectrometric analysis of a pooled lysate from control and proinflammatory microglia (LPS treated) resulted in the identification of 135,631 peptides from 8,537 unique proteins. Control and LPS-treated IMG cells were then analyzed in triplicate using BoxCar data acquisition and the results were searched using MaxQuant with the match-between-runs feature enabled for comparison to the original dataset from the fractionation. The BoxCar approach resulted in the identification of 7,074 ± 46 microglial proteins in a 2 hr single-shot analysis, which is an approximate 50% improvement in identification rate over our conventional data acquisition approach. To the best of our knowledge, this study highlights the deepest proteome coverage to date for microglia and represents a significant methodological advancement in terms of rapid and comprehensive characterization of the neuroimmune response in alcohol exposure models.

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Identification and Validation of Secreted Factors in the crosstalk between HOXB7 overexpressing cells and stromal cells

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Estrogen Receptor (ER, ESR1) is the driving transcription factor in about 70% of all breast cancers, making it a suitable target for endocrine therapy. In a previous study, it was found that HOXB7 confers endocrine-resistance in breast cancer and HOXB7 overexpression induces angiogenesis and macrophage recruitment via TGFβ2 upregulation. These results imply that HOXB7 plays a critical role in the crosstalk between endocrine-resistant breast cancer and tumor microenvironment (TME). This study aims to identify and validate the role of secreted factors in the crosstalk between HOXB7 overexpressing cells and stromal cells that promote cell proliferation and migration. MCF7- HOXB7 cell line was transfected with a plasmid encoding GFP-HOXB7 tag and puromycin resistance using Lipofectamine 3000. Cells were cultured in 1 ug/ml of puromycin media to establish a successful recombinant HOXB7- GFP-puro clone. Positive GFP and puro-resistant clones were assessed for HOXB7 overexpression by qRT-PCR and Western Blot. Three clones with high HOXB7 overexpression were analyzed via Human Cytokine Array Q440 with tumor conditioned media to detect 10 upregulated, key secreted factors induced by HOXB7 overexpression. Currently, the lab is working to validate these significantly upregulated secreted factors from three different HOXB7 overexpressing breast tumor cells lines (MCF-7, T47D and ZR75-HOXB7) via qRT-PCR to select 3 candidates. We will confirm secreted protein levels of the selected secreted factors via ELISA analysis using tumor conditioned media and select 1 key factor for functional analysis. To identify the role of the secreted factor we will perform cellular viability assay, migration assay and apoptosis assay using recombinant proteins and anti-secreted factor neutralizing antibody. We will investigate if co-cultured HOXB7 overexpressing cells with stromal cells enhances HOXB7 overexpressing cell proliferation and migration via Cyquant proliferation assay (Life Tech) and Oris Cell migration assay (Platypus tech) using specific inhibitors of the key secreted factor. The apoptosis assay will be used to examine the inhibitory effects to HOXB7 overexpressing cells by treatment of inhibitors.

Together, this study will provide a novel secreted factor as a drug target to inhibit breast cancer cell with HOXB7 expression and ultimately enable us to identify drug regimens with activity against the endocrine-resistant breast cancer that can be used to design and conduct clinical trials.

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Evaluation of VDUP1 Null Phenotypes in Drosophila Embryogenesis

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Vitamin D Up-regulated Protein-1, or VDUP1, is an important factor in proper neurodevelopment in fruit flies. Common fruit flies, or Drosophila melanogaster, lacking VDUP1 do not develop fully as mutation to the gene which encodes VDUP1 is fatal. The specifics as to why this occurs is not known. Dr. Dearborn and I used VDUP1 null fly lines expressing GFP and four separate miranda mutant fly lines to better understand the role VDUP1 plays in the asymmetric division of neuroblasts. Neuroblasts are important progenitor cells for the central nervous system, and their asymmetric division is key to proper CNS development. We have utilized immunocytochemistry, fluorescence microscopy, and confocal microscopy to visualize the localization of VDUP1, as well as other important factors like prospero and miranda, in drosophila embryos to better elucidate how all these pieces fit together in asymmetric neuroblast division and CNS development overall. VDUP1 is an important factor in human neurodevelopment as well, so our findings many have translatable implications.

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The Role of STAT3 in the Regulation of Tumor Cell Growth of U87 Human Glioblastoma Cells

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Glioblastomas (GBMs) are among the most aggressive types of brain cancer and effect millions of people throughout the world. The STAT3 pathway is known to promote tumor growth through cytokine signaling and accordingly, my hypothesis is that inhibition of STAT3 should decrease the growth of GBM cells. This was investigated using the U87 glioblastoma cell line. Treatment of U-87 cells with IL-6 failed to increase phosphorylation of STAT3, however it was noted that a high level of pSTAT3 was present in untreated cells. This STAT3 phosphorylation was reduced by treatment with a JAK inhibitor, suggesting that pathways besides IL-6 signaling are responsible for the high level of STAT3 phosphorylation. Inhibition of STAT3 with BBI608 treatment resulted in a significant decrease in cell growth. Since Bmi-1 has been shown to regulate tumor cell growth, cells were treated with PTC-209, a BMI-1 inhibitor. This treatment also resulted in decreased cell growth, although Western blot analysis of extracts from BBI608-treated cells (24 hour treatment) indicated that there was no change in Bmi-1 expression. These results suggest that Bmi-1 may regulate U-87 cell growth through STAT3-independent pathways. Other proteins which have also been shown to control tumor cell growth and may be subject to regulation by STAT3 (Nanog, Oct-4, SOX2, C-Myc) are currently being tested to determine the downstream signaling used by STAT3 to regulate tumor growth.

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Evaluation of a Student Pharmacist Provided Oncology Education Series to Medical Residents During an Oncology APPE Rotation

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Purpose: Medical residents receive little formal education regarding pharmacology of antineoplastics and supportive care regimens in medical school. In contrast, pharmacy students receive extensive training on these topics during their training. Currently, there are no published studies that have evaluated the use of student pharmacists to assist in the education of medical residents. To meet this educational gap, an education series on common oncology topics was developed and delivered to medical residents on their inpatient oncology rotation by student pharmacists. This study aims to assess the benefit of student pharmacist provided education to medical residents and identify future educational needs.

Methods: During their oncology APPE rotation, student pharmacists, in conjunction with their oncology APPE preceptor, developed an oncology education series on the following commonly encountered topics: 1) Chemotherapy Pharmacology and Adverse Effects; 2) Tumor Lysis Syndrome; 3) Neutropenic Fever; 4) Nausea/Vomiting; 5) Hypercalcemia of Malignancy; 6) Pain Management. Education topics were created in Microsoft Word or Microsoft PowerPoint and provided as handouts to all attendees. The education series was reviewed with student pharmacists one week prior to delivering the specific topic with medical residents. A total of two oncology topics were delivered each week to medical residents for a total of three weeks. The APPE pharmacy preceptor was present at all education events but did not directly deliver any content. To evaluate the benefit of the student pharmacist provided education, medical residents who attended were asked to anonymously complete a survey with eight Likert-scale questions. The survey was scaled as follows: 1= strongly disagree; 2= disagree; 3= neither agree nor disagree; 4= agree; 5= strongly agree. An open-response question was included at the end of the survey to identify future educational needs of medical residents.

Results: Eighteen residents attended the first educational series provided by student pharmacists. All participants who attended the educational lectures responded to the survey. The lowest score on any question on the survey was a 4 (agree). A total of 83% of attendees strongly agree that the student pharmacist presentation was beneficial to their continuing education. A significant majority (78%) of medical residents strongly agreed that the education lecture they

attended improved their knowledge and confidence in prescribing the proper treatments for oncology patients. All participants strongly agreed that they would like more educational presentations provided by pharmacy students. The three most commonly requested educational needs identified by medical residents for future educational series were: 1) Antimicrobials and their spectrum of activity; 2) Constipation/Diarrhea management; and 3) Management of common chemotherapy adverse effects.

Conclusion: Student pharmacists are capable of developing and delivering effective education lectures on common oncology topics to medical providers. Furthermore, medical residents are eager to receive additional oncology education from student pharmacists.

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Drug Policy and Formulary-Related Job Postings: Current Skills and Qualifications

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To keep up with the changing needs of the managed care workforce, it is important to understand which skills and qualifications are sought by employers in the field. Researchers have performed quantitative content analyses of job postings in other disciplines to understand the current needs of employers. Our project utilized similar methods to evaluate the current managed care job market. We aimed to understand what skills and requirements are needed for today's managed care job market through inductive analysis of current job postings. This was done by identifying job postings from Indeed.com using the following search terms "formulary", "pharmacy benefit", "drug information", "pharmacy and therapeutics", "drug utilization", and "drug policy". Searches were performed monthly for three consecutive months (October through December 2019). Detailed job descriptions, including minimum education requirements, preferred prior work qualifications, physical expectations, were gathered monthly for a 5% random sample of jobs. Between weeks 2-4 of each month, the full text content from each sampled job ad was downloaded and transferred to an electronic note-storing application (Evernote, Inc) for subsequent data analysis. Articles were organized by search term and time period. SAS Text Miner was used to analyze the content of the job postings, and extract the common qualifications and skills found in the database. These common qualifications and skills were coded and organized systematically through inductive analysis. The initial data sample included 1,681 randomly selected job postings collected during the 3 month data collection period. The following search terms "drug information", "formulary" and "pharmacy benefit" accounted for the largest percentages of job postings, 45%, 27% and 13%, respectively. Lower percentages were seen for "drug utilization", "drug policy" and "pharmacy and therapeutics", which accounted for 6%, 5%, and 4% of job postings, respectively. From our preliminary data, we have noted a stable number of jobs posted on Indeed.com between months for all search terms. Further analyses are ongoing to classify relevant job articles based on skills and qualifications. Final results from the inductive analysis will be available at the time of the Research Symposium.

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Critical Roles of Nuclear Trafficking and RIP1 During the Hyperglycemic Shift from Apoptosis to Necroptosis

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Necroptosis plays a significant role in the pathogenesis of different neurological conditions like ischemia-reperfusion and neonatal hypoxia-ischemia. This programmed cell death (PCD) pathway is driven by receptor-interacting protein kinase 1 (RIP1), RIP3, and mixed lineage kinase domain-like (MLKL) protein. Unlike apoptosis, necroptosis is a caspase-independent pathway with a highly inflammatory outcome. Reactive oxygen species (ROS) are a driving factor in necroptosis and are necessary for its induction and the activation of RIP1. In addition, necrotic molecules accumulate in the nucleus driving this PCD pathway. We have previously shown that hyperglycemia causes a shift from apoptosis to necoptosis in a manner that depends on RIP1 and ROS. We wish to further analyze the mechanism of this novel cell death shift with a focus on nuclear trafficking and changes in gene expression. Using isopycnic density gradient centrifugation we will separate the cytoplasm from nuclear fractions to analyze the trafficking of RIP1, RIP3, and MLKL as well as caspases during the hyperglycemic shift to necroptosis. Using quantitative PCR (qPCR) we will determine changes in gene expression during the hyprglycemic shift to necroptosis. Key genes to be analyzed will include rip1, rip3, mlkl, caspase-3, caspase-6, caspase-7, pyruvate dehydrogenase, sod1, and sod2. Using CRISPR-Cas9 we will determine the effect of mutating RIP1 on nuclear trafficking and gene expression during the hyperglycemic shift to necroptosis. Using antioxidants as well as inducers of ROS, we will determine the effect of ROS on nuclear trafficking and gene expression during this phenomenon.

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Characterizing the Role of Alkyl Hydroperoxide Reductase Subunits in Acinetobacter Baumannii Pathogenicity

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Acinetobacter baumannii is a gram-negative, aerobic bacteria that is a member of the ESKAPE pathogen group notorious for causing antimicrobial-resistant nosocomial infections. The increased occurrence of hospital-acquired infections and antibiotic resistance warrants the need to further understanding of A. baumannii and its virulence factors that contribute to its pathogenicity. Once in the host, A. baumannii encounters a great deal of oxidative stress due to the host immune response and later as a side effect of antimicrobial action. To determine which genes may play a pivotal role in protecting A. baumannii from ROS, a transposon mutant library was screened for survival following hydrogen peroxide (H2O2) treatment. This screening identified one AhpC and two AhpF transposon mutants as genes of interest, and further studies were done to assess their virulence contributions. To alleviate oxidative stress induced by host ROS, A. baumannii employs AhpC and AhpF to cycle cysteine disulfides as a mechanism to convert hydroperoxides to alcohol and water. Our preliminary screenings done on an A. baumannii transposon mutant library lead to our hypothesis that subunits, AhpF and AhpC, of AhpR contribute to A. baumannii's virulence in that they protect A. baumannii in hyperoxidative environments by detoxifying peroxides through a cyclical redox reaction. To assess the extent of pathogenicity AhpC and AhpF affords A. baumannii, the three transposon mutants identified in preliminary screenings along with a wild-type (WT) strain were treated with various antibiotics and oxidative stressors. All Ahp mutant strains conferred increased susceptibility when treated with pyrogallol and meropenem. Upon treatment with triton, gentamicin, and sodium dodecyl sulfate, all Ahp mutants along with WT were resistant. AhpC mutant exhibited significant increased susceptibility to H2O2, slight increased susceptibility to cumene, but increased resistance to paraquat. Both AhpF mutants exhibited increased resistance to H2O2 and similar susceptibility to WT upon treatment with paraquat. When treated with cumene, one AhpF mutant showed a slight increase in susceptibility and the other exhibited increased resistance. Growth curve analysis and CFU recovery shows that all Ahp mutants exhibited varying degrees of restricted growth upon treatment with higher doses of H2O2 as compared to WT. Moving forward, we would like to further investigate the role AhpC and AhpF plays in A. baumannii's pathogenicity by screening with other oxidative stressors and antimicrobials. Further analysis and characterization of the alkyl hydroperoxide reductase subunits will help progress drug therapy development for individuals with antimicrobial-resistant A. baumannii infection.

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Initiation of Bystander Cell Death by Ricin

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The cytotoxic protein ricin is derived from the castor plant, Ricinus communis and is known to induce a large amount of human lung epithelial cell death in vivo. Ricin is a ribosome- inactivating protein which destroys target cells by protein inhibition. As the lung epithelium is heavily affected during ricin toxicosis while cultured lung epithelial cells are relatively insensitive to direct attack by ricin, we hypothesize that ricin kills monocytes/macrophages leading to bystander death of epithelial cells. Bystander cell death was determined by treating A549 lung epithelial cells with supernatants from ricin-treated human macrophages (U937 cells). Ricin-induced cell death was determined using WST-1 assay at different treatment times. Following treatment of U937 cells with ricin, Fas ligand (FasL), a TNF family member and death ligand, was released into supernatants. Death of bystander A549 cells depended on FasL and ricin released into supernatants. Inhibition of RIP1, RIP3, and MLKL prevented death of bystander A549 cells. In addition, A549 cells treated with supernatants exhibited phosphorylation of RIP1 indicating the activation of necroptosis. Interestingly, reactive oxygen species (ROS), normally required for necroptosis, appear dispensable for death of bystander A549 cells. Future research will focus on identifying other steps in this process which may lead to the development of novel therapeutics for ricin toxicosis.

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The Effects of Genetic Polymorphisms on Cytochrome P450 2C9 Structure & Function

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Cytochrome P450s (CYP) are a family of membrane associated heme-containing enzymes involved in xenobiotic metabolism with recent relevance in pharmacogenomics. The human CYP2C9 enzyme metabolizes up to 15-20% of drugs that include warfarin, losartan, ibuprofen, and tolbutamide. It is highly polymorphic with several genetic variations associated with significantly altered activity of the enzyme. The CYP2C9*11 allele occurs predominantly in African Americans but also in Caucasian and other ethnic populations with a frequency of around 0.05. The *11 genetic variation represents a substitution of amino acid residue at 335 from arginine to tryptophan (R335W) that has demonstrated decreased catalytic activity towards various substrates including S-warfarin. The R335W was created in CYP2C9 wild-type (WT) construct using site-directed mutagenesis and co-expressed in E. Coli alongside chaperone proteins (GroEL/ES). Generation of purified CYP2C9*11 protein enabled catalytic studies and crystallization in complex with the drug substrate losartan. The enzymatic assays revealed marked reduction in losartan turn-over by CYP2C9*11 compared to the WT enzyme. Additionally, comparison of the amino acid sequence revealed that R335 is conserved across the CYP2C subfamily of enzymes. The R335 is located on the distal J-J' loop and interacts with multiple amino acid side chains on the J and J' helices. The substitution with hydrophobic and bulkier tryptophan located on the surface region may result in destabilizing interactions among several helices including those located near the active site heme, affecting protein stability and function. The preliminary computational analysis suggests that the effect of variation could transduce to the active site. Overall, these observations establish the effect of distal variation prompting further evaluation by structural and biophysical studies.

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Development of Biocompatible Spin Tip Microextraction Devices with Polyacrylonitrile

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Bioanalysis within laboratories is expected to accomplish more with increasingly stringent parameters. In today's context, this includes better precision, selectivity, and sensitivity, while also maintaining low cost. This study focuses on developing new spintip solid phase microextraction devices as an effective and cost-efficient means for bioanalysis, with polyacrylonitrile (PAN) being incorporated as a means of making the devices more biocompatible with biological samples with high protein concentrations, such as serum, plasma, and whole blood. Solid phase microextraction devices were made in spin tip format at both 11.7% and 14.1% PAN concentration; the solid phase also contained lipophilic slica-C18 particles for analyte extraction. The spin tips then underwent triplicate testing with testosterone in concentrations of 10, 2.5, 0.625 and 0.156 ng/mL (expected range in biological samples), as well as a constant concentration of 13C-labeled testosterone. The extracted amounts of these hormones were then determined by LC-MS/MS analysis and the relative standard deviation (RSD) of both groups of spintips was determined. Biocompatibility was tested by measuring the amount of albumin retained on the extraction phase. The 11.7% PAN spin tips yielded an RSD range of 5.7%-20.1% with testosterone and 8.1%-18.4% with testosterone 13C. The 14.1% PAN spin tips yielded an RSD range of 3.4%-38.3% for testosterone and 2.1%-30.2% with 13C-labeled testosterone. Spintips containing PAN at a concentration of 11.7% yielded more uniform extracting phases for bioanalysis. The disparities in the extracted concentration, as evidenced by the RSD values, could be attributed to lost mass during preparation of the spin tips for analysis, in which case would necessitate pre-washing as a preemptive measure to counter this loss. It is also reasonable to suggest that a different form of lipophilic particles, such as amide-C16 which offer better compatibility with aqueous samples, may help the PAN spintip devices to measure concentrations of biomarkers and drugs more reproducibly. Optimizing biocompatibility between the biological sample and the extraction phase by making sure the silica-based particles are covered in PAN will ultimately allow for more accurate drug analysis and better therapeutic monitoring of patients.

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Determining the Vibrio Parahaemolyticus Antioxidant Genes Required for Organism Survival in Various Environments

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Vibrio parahaemolyticus serovar O3:K6, is the most common cause of bacterial seafood-related illness and is responsible for 48% of all reported Vibrio sp. infections in the United States. Limited literature is available regarding organism pathogenesis, especially relating to the host response and immunity to infection. It is known that many pathogenic bacteria produce antioxidant enzymes that allow for survival in the presence of reactive oxygen species (ROS). Here, we investigate the antioxidant genes involved in V. parahaemolyticus survival when exposed to ROS produced by H2O2 , paraquat, t-BOOH, and cumene hydroperoxide. Using OD and bacterial CFU we determined that organism survival was decreased upon exposure to H2O2 and t-BOOH but not decreased when exposed to paraquat and cumene hydroperoxide. Using RT-qPCR we determined that exposure to ROS generated by these oxidant chemicals caused an upregulation in OxyR1 and OxyR2 expression, as well as an upregulation in expression of antioxidant genes with OxyR1 binding motifs including alkyl hydroperoxide reductases (ahpC1, ahpC2, ahpF), catalases (katE1, katE2), glutaredoxin, and soxR. ROS exposure does not cause an upregulation in the expression of superoxide dismutases (FeSOD, MnSOD, CuZnSOD), catalase/ peroxidase genes (katG1 and katG2), and thiol peroxidase peroxiredoxin. We are currently looking to elucidate the antioxidant gene response and survival of V. parahaemolyticus in U937 and THP-1 cell lines. Characterization of organism survival in various environments will provide a more comprehensive understanding of V. parahaemolyticus virulence and pathogenesis. Ultimately, the goal is to formulate treatment algorithms to facilitate pathogenic organism elimination.

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