ALBANY COLLEGE OF PHARMACY AND HEALTH SCIENCES

Research Symposium

Friday, April 9, 2021

9:00-9:10 am		**Opening Remarks** **T. Gregory Dewey, PhD,** President
9:10-9:20 am		**ACPHS Research Highlights** **Martha A. Hass, PhD,** Director of Research
9:20-10:20 am Keynote Speaker		**Anton Simeonov, PhD,** Scientific Director, Division of Pre-Clinical Innovation National Center for Advancing Translational Science (NCATS) Challenges and Opportunities in Small Molecule Translation
10:20-10:35 am		**Wendy Parker, PhD** Implementing a Public Health Pharmacy Team and a Shared Decision-Making Model: Initial Work at the ACPHS Collaboratory in the South End of Albany
10:35-10:50 am		**Elisabeth Vines, PhD** (2020...) A Graphic Journal
10:50-11:05 am		**Margaret Doll, PhD, MPH** Trends in the uptake of pediatric measles-containing vaccine in the United States: A Disneyland effect?
11:05-11:20 am		**HaAin (Andy) Zheng, PhD** Endocannabinoid System of the Blood Brain Barrier – Drug Distribution Questions and Therapeutic Opportunities
11:20-11:25 am		**Break**
11:20-11:40 am		**Angelika Nelson** The Analysis of HIV-1 Cell-to-Cell Transmission in Co-Culture
11:40-11:55 am		**Binshan Shi, PhD** Study of Cell Death Induced by Retrovirus Cell-to-Cell Transmission
11:55 am-12:10 pm		**Nicole Shakerley, PhD** Thioredoxin Modulates Antimicrobial Susceptibility in Acinetobacter baumannii
12:10-12:25 pm		**Vibert Putra, Candidate for BS, Microbiology/MS, Molecular Biosciences** Thioredoxin A-Mediated Modulation of Acinetobacter baumannii Antibiotic Resistance
12:25-12:35 pm		**Break**
12:35-1:27 pm		**POSTER PRESENTATIONS** 2020 Student Summer Research Awardees (See next page)
1:27-1:35 pm		**Martha A. Hass, PhD,** Director of Research 2021 Student Summer Research Awardees 2021 Researcher of the Year
1:35-1:50 pm		**Eric Yager, PhD** Zika Virus Infectivity is Dependent on Host Glycosphingolipid Biosynthesis
1:50-2:05 pm		**Febronia Mansour, Candidate for BS, Microbiology** Sex-Specific Effects of Prediabetes in Multi-Etiology Dementia
2:05-2:20 pm		**Jacob Miller, Candidate for MS, Molecular Biosciences** OxyR of Francisella tularensis contributes to the suppression of AIM2 inflammasome
2:20-2:35 pm		**Anarv Mathur, BS, Microbiology** Characterizing Daptomycin Resistance in Staphylococcus aureus
2:35-2:50 pm		**Melissa Sher, Candidate for MS, Molecular Biosciences** Native Transcriptome Profiling of Virus-Infected Chinese Hamster Ovary Cells
2:50-3:00 pm		**Closing Remarks** **Anuja Ghorapade, PhD,** Dean and Vice President of Academic Affairs **Martha A. Hass, PhD,** Director of Research

2020 Student Summer Research Awardees

12:35 - 12:40 pm	 **Martha A. Hass, Ph.D.** Director of Research	**Introduction: SSRA Program and 2020 SSRA Awardees**
12:40 - 12:47 pm	 **Sunsik Chang**	Histone recognition by the ATAD2B bromodomain
12:48 - 12:55 pm	**Marina Juan**	Molecular mechanisms of acetyllysine recognition by PfBDP1 bromodomain
12:56 - 1:03 pm	 **Kaitlyn Strumski**	Synthesis of co-drugs derived from mycophenolic acid and aminolevulinic Acid
1:04 - 1:11 pm	 **Phillip Truong**	**Mitochondrial trafficking during the hyperglycemic shift from apoptosis to necroptosis**
1:12 - 1:19 pm	 **Thomas Yarborough**	**Structure-function studies of human cytochrome P450 2C9*3: insights into the effect of genetic polymorphism**
1:20 - 1:27 pm	**Victoria Fenton**	Assessing advance care planning in individuals with Lynch Syndrome
Presented on Thursday, April 8, 2021	 **Yelena Dunikova**	**Determining the Vibrio parahaemolyticus anti-oxidant genes required for survival in various environments**
	 **Nicholas Nasta**	**Identification and validation of secreted factors in the crosstalk between HOXB7 overexpressing cells and stromal cells**

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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Implementing a public health pharmacy team and a shared decision-making model: Initial work at the ACPHS Collaboratory in the South End of Albany

Wendy M. Parker, PhD; Colleen McLaughlin, PhD; Katie Cardone, PharmD; Allison Burton-Chase, PhD; Barry DeCoster, PhD; Stacy Pettigrew, PhD; Margaret Doll, PhD; Naomi Pickett, BS

The aim of this project was to evaluate the efficacy of deploying a Public Health Pharmacy Team (PHPT) in the South End community as an enhancement to an existing community health worker program. This initial partnership was a direct result of funding from New York State's Medicaid Delivery System Reform Incentive Payment (DSRIP) 1115 waiver and our teams are now pursuing longer term, sustainable funding streams.

The goal of the PHPT is to implement innovative pharmacist led interventions aimed at reducing avoidable emergency department visits and hospital admissions. Pharmacists have, historically, acted as public health advocates and practitioners within their communities, but a robust public health pharmacy model has not yet been developed in the United States. This can be attributed to a lack of compensation and structured programming for pharmacists in certain areas of public health action. As the landscape shifts from fee-for-service payment to payments on a value-based and per-life basis, the PHPT unearthed the need to revisit our needs assessment, assess our data collection tools and systems, and enhance our community partnerships in order to clearly articulate our team's impact and value.

We started this project prior to the height of the COVID-19 pandemic, incorporating students into the first round of data clean-up and quality improvement. In December 2020, we onboarded a consultant to assist with our higher-level data needs. Although our Collaboratory priorities have evolved and our timeline was challenged due to the pandemic, we have laid the groundwork for continued success as the Collaboratory re-opens and has staff on-site.

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(2020...) A graphic novel

Elisabeth Vines, PhD

(2020…) is a graphic novel chronicling the developing effects of COVID-19 on the world and on Albany College of Pharmacy and Health Sciences in particular, thereby illustrating both macro and micro effects of a pandemic. The title is inspired by Ben Dolnik, whose July 6 opinion essay in the New York Times described this year as one of living in parentheses. Initial journal entries in (2020…) reveal the early concern with the semester beginning in January 2020 and the news that a new disease had appeared in China at a "wet market"; page by page the virus overtakes our lives, while taking the lives of many and disrupting and destroying livelihoods, social life, economics, and businesses.

Humans struggle to give meaning to the events around us, and we do this with the arts. The progress of the virus illustrates how our understanding can be muddied by politics, ignorance, and conflicting interpretations of "truth." The graphic novel format takes advantage of visual and written information, reflecting how we receive information visually and verbally in sometimes confusing and overwhelming amounts. Even something as "clear" as science appears in conflicting messages. (2020…) is an attempt to document the twisting path we've taken in this journey to understand and control this pandemic.

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Trends in the uptake of pediatric measles-containing vaccine in the United States: A Disneyland effect?

Margaret K. Doll, PhD, Samuel D. Weitzen and Kathryn T. Morrison

Background: The measles outbreak that began in December 2014 at the California Disneyland theme park in the United States (U.S.) received a high amount of media attention. Media attention can influence health-related behaviors. We investigated the effect of the Disneyland outbreak on measles-containing vaccine (MCV) uptake among U.S. children.

Methods: We used 2012–2017 National Immunization Survey-Child (NIS-Child) data to examine MCV uptake among U.S. children by 19 months of age. We classified MCV coverage among birth cohorts as exposed based on age at the time of the outbreak. A difference-in-differences design with adjustment for categorical birth cohort was implemented in base models to estimate the exposure effect on the outcomes, 1-dose MCV coverage or age at first MCV dose, with pneumococcal conjugate vaccination as a control. Primary analyses included this model adjusted for geographic region, maternal education, race/ethnicity, household income, and insurance status, and an exposure-interaction term with maternal education. All analyses included sampling weights.

Results: The study population represented 34,471,357 children. In base models, the Disneyland outbreak was associated with a 1.0% (95% CI: 0.2%, 1.8%) increase in >=1-dose MCV coverage and a 6.6 (95% CI: 4.8, 8.5)-day decrease in MCV administration age. In primary analyses, the outbreak was associated with a 3.9% (95% CI: 3.1%, 4.8%) increase in >=1-dose MCV coverage among children of college-educated mothers, and a 3.2% (95% CI: 0.6%, 5.9%) decrease among children of mothers earning less than a high school degree. Decreases in MCV administration age ranging from 5.9 (95% CI: 3.3, 8.5) to 9.1 (95% CI: 6.8, 11.4) days were observed across maternal education categories.

Conclusions: The Disneyland outbreak was associated with differential effects on MCV coverage by maternal education and decreases in MCV administration age among U.S. children. These findings may provide useful insights to inform methods to address pediatric MCV undervaccination.

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Endocannabinoid System of the blood brain barrier– Drug distribution questions and therapeutic opportunities

HaiAn (Andy) Zheng, PhD and Kofi Hagan

Endogenous Cannabinoid System (ECS) comprises the signaling network of endocannabinoids (eCB), such as N-arachidonoylethanolamine (AEA) and 2-Arachidonylglycerol (2AG), their receptors, and enzymes that biosynthesize or metabolize eCB. Recently, we confirmed the presence of cannabinoid receptors on the human blood brain microvascular cells (HBMEC), which are building blocks of the blood-brain-barrier (BBB) at the interface between the central nervous system (CNS) and peripheral circulation. We also found that endocannabinoids can modulate BBB integrity and permeability. These findings and our ongoing research may explain the neuroprotective effects of cannabinoids, which made botanical cannabinoids promising therapeutics for brain injuries, stroke, and neurodegenerative diseases. The permeability modulation effects of cannabinoids also raised a clinical question of cannabis-drug interaction -- Can cannabinoids significantly change drug distribution and bioavailability across BBB and other barriers between physiological compartments? 12.9%, 20.0%) of schools, with no differences between public or nonpublic schools. Among nonpublic schools charging tuition, 52.0% (95% CI: 44.6%, 60.0%) reported that enrollment changes financially impacted their school, affecting a mean of 10.7% (95% CI: 7.7%, 13.8%; median: 8%, IQR: 2%, 12%) of the school's operating budget. Collectively, these results indicate a significant proportion of schools experienced changes in student enrollment, absenteeism, and medical exemptions; however, these results may not be generalizable to survey nonparticipants.

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The analysis of HIV-1 cell-to-cell transmission in co-culture

Angelika Nelson and Binshan Shi, PhD

The dissemination of HIV-1 is accomplished via two separate mechanisms, which largely differ in the influence of the pathogenesis of HIV-1 infection. This study primarily focuses on HIV-1 infection through direct cell-to-cell transmission from an infected cell, to an intact cell, by way of virological synapses, rather than via cell free virus. Cell-to-cell transmission not only allows viral evasion from the host immune system, but also significantly increases the efficiency of HIV-1 transmission. Despite this knowledge, HIV-1 cell-to-cell transmission is not thoroughly studied. This project consists of the analysis of HIV-1 cell-to-cell transmission characteristics, as well as the role of potential inhibitors. It was found that after infecting HTLV-1 positive MT2 T cells, by pseudotyped HIV-1 GFP+ virus, HIV-1 was spread predominantly by cell-to-cell transmission to an uninfected recipient cell. Several different co-cultures between HIV-1 GFP infected MT2 cells, and uninfected recipient cells, were used to examine HIV-1 cell-to-cell transmission. Fluorescence emitted was quantified by the means of a flow cytometer. The recipient cells were stained by CellTracker® Deep Red (Thermofisher), 24 hours prior to the co-culture. The outcome was a percentage of cells that were both positive for Deep Red fluorescence, and GFP green fluorescence. When compared to the trans-well control, which exclusively indicated HIV-1 infection via cell free virions, the rate of HIV-1 cell-to-cell transmission, in the coculture between HIV-1 GFP infected MT2 T cells, and human monocyte THP-1 cells, was significantly higher. Further, two retrovirus inhibitors, AZT and EFA were found to block HIV-1 cell-to-cell transmission. In addition, it was found that the use of heparin and small molecular heparin (NASH), could also inhibit HIV-1 cell-to-cell transmission. This experiment is considerably important because it provides us with a foundation that, not only studies and screens for the mechanisms of HIV-1 cell-to-cell transmission inhibitors, but also is significant in the further investigation of HIV-1 disease progression and consequence.

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Study of cell death induced by retrovirus cell-to-cell transmission

Lauren Martin, Angelika Nelson, Lalhming Zaura,
Liang Xi, Caleb Sherry and Binshan Shi, PhD

Inflammation has been found extensively associated with retrovirus infection, however studies to investigate host innate immune response towards infection by cell free retrovirus have shown elusive conclusions. HIV-1 cell-to-cell transmission significantly increases the efficiency of transmission, and therefore has significantly increased opportunity to induce host cell innate immune response. In this study, it was found that after infecting HTLV infected T cell MT2 cells HIV-1 can transmit predominantly by cell-to-cell transmission in next round of infection. By using this HIV-1 cell-to-cell transmission model in a coculture between HIV-1 infected MT2 cells and human monocyte THP1 cells, cell death pathway analysis was performed, and potential innate immune response pathway was investigated. The results showed that HIV-1 cell-to-cell transmission from MT2 cell to THP1 cells induced cell death by a WST assay, and this observed cell death was in company with increased release of LDH, suggestion a lytic form of cell death. Meanwhile this cell death could be inhibited by both pan caspase inhibitor ZVAD and caspase-1 inhibitor ZYVAD. Further analysis showed the cleavage of pore forming molecule Gasdermin D was significantly increased as a consequence of HIV-1 cell-to-cell transmission, demonstrating the inclusion of program cell death pyroptosis. In addition, heparin, and low molecular weight non-anticoagulant heparin Tinzaparin and NACH all exhibited strong blockage to HIV-1 cell-to-cell transmission using the above model. Pyroptosis is a highly inflammatory form of programmed cell death. This study has provided important information to pathogenic mechanisms caused by retrovirus cell-to-cell transmission. Continued investigation in this direction will not only increase our knowledge in retrovirus biology but also shed light on the treatment and control of retrovirus caused disease.

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Thioredoxin Modulates Antimicrobial Susceptibility in Acinetobacter baumannii

Catherine M. Phelps, Vibert Putra, M Hatch, Khadija Moussadek, Phillip Truong, BP Aulanadam, Meenakshi Malik, PhD and Nicole L. Shakerley, PhD

Due to rapidly increasing drug resistance and limited knowledge of specific contributing mechanisms, A. baumannii has been classified as a critical pathogen for the development of new therapeutics by both the World Health Organization and CDC. Bacterial antioxidant enzymes have been shown to aid intracellular survival and antibiotic resistance pathways in many microbes. These enzymes represent a largely unexamined pool of potential drug targets in Acinetobacter baumannii. We hypothesized that A. baumannii antioxidant enzymes modulate the bacterial redox-environment in response to antibiotics, thereby contributing to antimicrobial resistance. Our studies demonstrate that mutation of antioxidant genes encoding for the thioredoxin system renders Acinetobacter more susceptible to antibiotic meropenem, a standard choice of treatment for infections caused by A. baumannii, indicating that the antioxidant enzymes of A. baumannii represent an unexplored contributor to antibiotic resistance. Thioredoxin A (trxA) is an oxidoreductase produced by A. baumannii, which maintains bacterial redox homeostasis by recycling electrons and correcting damage caused by reactive oxygen species. In this study, we examined a trxA deficient strain of Acinetobacter (ΔtrxA) derived from a clinical isolate strain to ensure that our organism was similar to those seen in patients. ΔtrxA was shown to be more sensitive to several oxidizing compounds demonstrating its increased sensitivity to stress. Using a combination of in vitro and in vivo infection models, we demonstrated that ΔtrxA exhibits reduced replication in lung macrophages and attenuated virulence in a live wax worm model. Additionally, ΔtrxA demonstrated significantly greater sensitivity than wild-type to all antibiotic classes tested, suggesting that thioredoxin may be a novel treatment target. To reproduce this heightened sensitivity in the wild type strain, Ci-79 was exposed to thiol stress-inducing agent diamide alongside a conventional antibiotic, meropenem. Treatment with both agents demonstrated synergistic activity in Ci-79 as well as other clinical isolate strains of A. baumannii. Further testing with FDA approved thioredoxin inhibitors utilized in combination with front line antibiotics also showed synergistic activity against Ci-79 and two additional clinical isolates, providing proof-of-principle for a novel treatment protocol against multidrug-resistant Acinetobacter. Findings in this study support further investigation of additional antioxidant enzymes of A. baumannii as potential therapeutic avenues. By characterizing these unexplored pathways that contribute to antibiotic resistance, we can identify targets and develop novel therapeutic strategies to combat antibiotic resistance in this deadly pathogen.

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Thioredoxin A-mediated modulation of Acinetobacter baumannii antibiotic resistance

Vibert Putra, Catherine M. Phelps, Khadija Moussadek, Phillip Truong, BP Arulanadam, Meenakshi Malik, PhD and Nicole L. Shakerley, PhD

Within the last few decades, Acinetobacter baumannii (A.B.) has been declared a global threat due to this nosocomial pathogen's exceptional ability to attain or upregulate its antimicrobial resistance capabilities. A.B. has the propensity to persist within the healthcare environment and has been attributed to various forms of hospital-acquired infections: ventilator-associated pneumonia, skin and soft tissue infections, catheter-associated urinary tract infection and bacteraemia. Successful treatments have become exceedingly difficult with increasing reports of metallo-β-lactamase- and oxacillinase serine-β-lactamases-carrying multidrug-resistant strains that have inevitably eliminated our last line antibiotics. Thus, a new strategy is needed to effectively treat A.B. infections. Thioredoxin A (trxA) is an electron-recycling enzyme in the thioredoxin antioxidant system that restores protein structures damaged by oxidative stress and regulates downstream effector proteins. TrxA is activated by reduced thioredoxin reductase (TrxR). We hypothesise that the thioredoxin system in A.B. modulates bacterial resistance responses to carbapenems. To examine this hypothesis, we have employed a trxA-deletion mutant (ΔtrxA) derived from a pneumonia associated clinical isolate Ci79. Preliminary data has demonstrated that ΔtrxA does not lead to significant growth defects but is highly susceptible to thiol stress, oxidative stress and multiple classes of antibiotics. Consequently, ΔtrxA is more susceptible to macrophage killing and thus, less virulent in a Galleria mellonella in vivo model. Moreover, the increased antibiotic sensitivity of the ΔtrxA mutant can be recapitulated in Ci79 and multiple clinical isolates following treatment with TrxR inhibitors. Using broth microdilution chequerboard assay and 24-hour static concentration time kill assays, inhibition of thioredoxin system using TrxR inhibitor in clinical isolates has shown to increase their susceptibility to meropenem. We predict that the increased sensitivity to antibiotics could be attributed to the altered functions of thioredoxin system's downstream targets and/or the dysregulated redox homeostasis in A. baumannii. The long-term goal of this study is to investigate the molecular mechanism and role of the thioredoxin system in modulating A. baumannii antibiotic resistance. Our mechanistic investigations provide insights into the extent of the thioredoxin system's role during antibiotic stress. Our project will act as steppingstone to understand the role of thioredoxin A in A.B. antibiotic resistance and, in the long term, their potential as a therapeutic target.

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Zika Virus Infectivity is Dependent on Host Glycosphingolipid Biosynthesis

Eric Yager, SA Ogbamikae and KV Konan

Most viruses are dependent on host biochemical pathways and factors to replicate their genome, synthesize viral proteins, and produce viral particles. Emerging studies have revealed the importance of virus-host lipid interactions in the life cycle of several clinically important viruses. Specifically, viruses can target lipid metabolism, signaling, and trafficking to remodel host cells into an environment favorable for viral replication. Zika virus (ZIKV), a member of the Flavivirus genus, is responsible for the recent widespread epidemic of Zika fever in Central and South America that has been associated with serious birth defects and neurological illnesses. Biochemical analyses of ZIKV-infected cells have revealed significant alterations in cellular lipid metabolism. Various lipid species are enriched in membrane microdomains and the endoplasmic reticulum, sites known to be involved in viral entry, protein synthesis, and budding. Our central hypothesis is ZIKV co-opts cellular lipogenic pathways to facilitate virus production. Data from our studies have revealed that the production of infectious ZIKV virus particles is dependent on the cellular enzyme glucosylceramide synthase (GCS) and the glycosphingolipids it helps produce. Similarly, the expression of GCS and several other genes involved in lipid metabolism was increased in cells upon ZIKV infection. Further, pharmacological inhibition of an enzyme involved in lipid droplet production lead to a significant decrease in ZIKV replication, indicating that the early steps in ZIKV assembly occur on lipid droplets. Increased understanding of the role of host lipogenesis in the stages of the ZIKV life cycle may aid the development of antiviral therapies targeting this virus, as well as related Flaviviruses.

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Sex-Specific Effects of Prediabetes in Multi-Etiology Dementia

Febronia M. Mansour, Olivia J. Gannon, Lisa S. Robison, Alvira Tyagi,
Abigail E. Salinero, David Riccio, Melissa A. Thomas, and Kristen L. Zuloaga

Multi-Etiology Dementia (MED) is the existence of dementia due to multiple types of pathology, such as the overlap of Alzheimer's Disease (AD) and vascular contributions to cognitive impairment and dementia (VCID). While there has been little research specifically focused on MED, it is estimated that 60% of AD patients also have vascular pathology, making it important to examine how AD and VaD risk factors relate to MED. While there are shared risk factors for AD and VCID, women are more likely to have AD and men are more likely to have VCID. However, in people with diabetes, this sex difference in VCID reverses and women with diabetes are 19% more likely to have VCID than men with diabetes. It is unknown if this pattern would persist in the prediabetic population and how it would impact MED. We hypothesize that prediabetes will exacerbate cognitive deficits and pathology in MED, with a greater effect in females. To model AD, we used a triple transgenic (3xTg-AD) model in which mice develop amyloid and tau pathology and control wild type mice (B6129SF1/J; WT). To model MED, we performed a right common carotid artery occlusion surgery on 3xTg mice to elicit chronic cerebral hypoperfusion. We used a high fat (HF) diet to induce prediabetes. While all mice on a HF diet showed impaired glucose tolerance, weight gain, and increases in visceral adiposity, female mice in the MED and AD groups had worse metabolic consequences on a HF diet than the WT females, a trend not seen in the males. Additionally, there was a significant correlation between visceral adiposity and impairments in spatial memory, measured by the Morris Water Maze. Using immunohistochemistry, we found amyloid pathology to be most severe in MED HF females. MoorFLPI laser speckle system was used to examine deficits in cerebral blood flow. Although sex differences were not observed in cerebral blood flow (CBF), all MED groups demonstrated a significant decrease in CBF. Histological analysis was completed using a Prussian Blue iron stain kit to detect cerebral microbleeds and we are currently assessing the interaction between sex, diet, dementia, and microbleed counts. In conclusion, AD and MED females were more greatly affected by the negative metabolic consequences of a HF diet, both cognitively and pathologically, supporting that prediabetes may be a greater dementia risk factor for women.

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OxyR of Francisella tularensis contributes to the suppression of AIM2 inflammasome

Jacob Miller, Kayla Fantone, Zhuo Ma, Jasmine Uzzell, Alexander Bleau, Samantha Barasch, Chandra Shekhar Bakshi, PhD and Meenakshi Malik, PhD

Francisella tularensis (Ft) is an intracellular gram-negative coccobacillus and the causative agent of tularemia. Ft has documented usage during World War II as a bioweapon and now is considered a potential bioterror agent by the Centers for Disease Control and Prevention (CDC) due to its low infectious dose, ease of dissemination via aerosolization, and high mortality rates. If left untreated, pneumonic tularemia has a fatality rate between 30-60%. For these reasons, the CDC has deemed Ft a Category A Select Bioterror Agent. Previous studies from our lab have shown that Ft evades the innate immune response of cells, such as macrophages and dendritic cells, via suppression of specific proinflammatory pathways that result in increased cytokine production, recruitment of innate immune cells, and bacterial eradication. A critical component is the cytosolic sensor, Absent in Melanoma 2 (AIM2) that senses double-stranded DNA in the cytosol of infected cells and then assembles a multi-protein complex known as the inflammasome. The activation of inflammasome results in the secretion of IL-1β and IL-18, which are the key proinflammatory cytokines required to clear Ft infection. Studies have demonstrated that Ft suppresses the activation of AIM2 inflammasome, however, its mechanism is currently unknown. We hypothesized that F. tularensis directly suppresses the AIM2-mediated responses by inhibiting the redox dependent signaling that concomitantly leads to its priming and activation. To address this hypothesis, an Ft mutant deficient in the transcriptional regulator of oxidative stress (ΔoxyR) was used and compared to Type B Francisella tularensis spp. holarctica, or the Live Vaccine Strain (LVS) wild-type. OxyR is a master regulator of key antioxidant enzymes of Ft and is also required for maintaining the redox homeostasis in infected macrophages. C57BL/6 cells were infected at an MOI of 50 for 24 hours and then lysed. Their contents were separated through gel electrophoresis and probed through western blot analysis. Our results showed an elevated expression of IL-1β in macrophages infected with the ΔoxyR mutant as compared to its wild-type counterparts. The elevated levels of IL-1β are associated with the activation of Caspase-1 in the ΔoxyR mutant infected macrophages. The expression of IRF1 and GBP2, important signaling components upstream of the AIM2 inflammasome, are also significantly higher in macrophages infected with the ΔoxyR mutant as compared to the wild-type. These results indicate that the redox environment modulated by oxyR of Ft may have a crucial role in the suppression of key signaling components of the AIM2 inflammasome. The ongoing studies are further investigating if the activation of the AIM2 inflammasome is due to the modulation of macrophage redox environment. Collectively, the findings from these studies will aid in extending the knowledge of how Ft-encoded factors subvert the host's innate immune responses.

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Characterizing Daptomycin Resistance in Staphylococcus aureus

Anarv Mathur, Smruti Mishra, Zhuo Ma, Abdullah Al-Hashmi,
Meenakshi Malik, PhD and Pradeepa Jayachandran, PhD

Staphylococcus aureus (Sa) is an ESKAPE pathogen that has been implicated as one of the leading causes of antibiotic-resistance and nosocomial infections in the United States. The rate of development of multi-drug resistant strains of Sa due to the overuse of antibiotics has surpassed the current rate of commercial antibiotic development. Previous studies from our lab have demonstrated the rapid rate of resistance development against daptomycin, a positively charged lipopeptide, in Methicillin-Resistant Staphylococcus aureus (MRSA) strains, as well as its reversal upon exposure to oxacillin (a phenomenon termed as See-Saw Effect). Using a cytochrome c binding assay, we found that bacterial membrane charge became less negative during exposure to daptomycin and was then reversed upon exposure to oxacillin. Additionally, using transmission electron microscopy, we observed an increase in cell wall thickness after daptomycin resistance which was also reversed on exposure to oxacillin. Our study was the first to characterize these phenotypic changes, supporting the see-saw effect. We previously showed mutations in lipid synthesis pathway genes, mprF, cls2, and pgsA result in daptomycin resistance. Cardiolipin synthase (Cls) is a lipid biosynthesis pathway enzyme, and Sa possesses two variants of the gene, cls1, and cls2. However, mutations were only found in Sa cls2 gene. To characterize the differences between the various forms of cardiolipin synthase, we performed bioinformatic analysis and compared sequences from 108 bacterial species. Using a neighbor-joining tree, we found that the two sequences cluster together, indicating that they are functionally redundant. Multiple sequence alignment indicated that the mutation sites in Sa were highly conserved in other species, particularly in Enterococcus faecalis (Ef), which is an ESKAPE pathogen implicated in daptomycin-resistant infections. Comparison of Cls sequences of Sa and Ef using pairwise alignment revealed that mutations in Sa map to the transmembrane domain; while the mutations in Ef map to the active site of Cls, and that all mutation sites for both Sa and Ef are highly conserved. Additionally, 3-D structure prediction of the active site showed that the mutation sites of Ef align well with the corresponding sites of Sa. We also found that the mutation sites located in the transmembrane domain of Cls2 increased the hydrophobic character, which we believe leads to a gain of function. To conclude, this study characterized the phenotypic changes associated with daptomycin resistance in MRSA, showed that the mutation sites in Sa Cls2 were highly conserved, and laid the foundation for a novel approach to combating multi-drug resistance.

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Native Transcriptome Profiling of Virus-Infected Chinese Hamster Ovary Cells

Melissa Sher, Eric J. Yager, PhD, H. John Sharifi, PhD,
David VanHoute, PhD, Jennifer Kelliher, MSc

Chinese Hamster Ovary (CHO) cells, used extensively to produce therapeutic recombinant proteins, may become infected with viruses without apparent impact to cell metabolism or morphology. Contamination often spreads before infections are discovered, thereby squandering resources and impacting available inventory. A molecular diagnostic method quantifying differentially expressed genes during viral infection has the potential to rapidly assess the health of a bioreactor throughout the manufacturing process and facilitate early identification of viral contaminants. However, the transcriptional landscape of CHO cells during infection is not well documented. The proposed study will characterize the transcriptome of CHO cells infected with a common viral contaminant (Minute Virus of Mice, MVM) whose mechanism of infection is known to impact gene expression. The developed assay may be used to further characterize differentially expressed genes (DEGs) induced by other viruses of varying genome classes. The proposed study aims to elucidate a panel of DEGs common to viral infections which may be assessed as a rapid, non-specific method to monitor the health of CHO cell cultures.

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Histone recognition by the ATAD2B bromodomain

Sunsik Chang, Cameron Montgomery, Kate Quinn,
Margaret Philips, Karen C. Glass, PhD

The N-terminal tails of histones are a binding site of proteins involved in the recognition of histone post-translational modifications (PTMs). Multiple histone mutations have been discovered that are associated with human cancer cases. The mutated histone tails, called oncohistones, impact recognition by histone reader proteins, and are known to contribute to cancer progression. The ATPase family AAA domain-containing protein 2 (ATAD2), has a highly conserved bromodomain involved in the recognition of acetylated histones. While ATAD2 is well studied and its overexpression has been linked to a poor survival rate in breast cancers, its paralog ATAD2B may have slightly different ligand-binding properties due to non-conserved residues in its canonical acetyllysine binding pocket. Our previous research shows the ATAD2B bromodomain has a broader substrate binding affinity certain histone PTMs as compared to ATAD2A. We speculate this difference in binding activity of ATAD2B could be a key feature in understanding the progression of certain cancers and maybe serve as an important therapeutic target for future cancer research. The primary goal of this research was to investigate the impact of onco-mutations on acetyl-lysine recognition by the ATAD2B bromodomain. We used isothermal titration calorimetry (ITC) to determine the binding affinity of acetylated oncohistone peptides with the ATAD2B bromodomain. A secondary goal was to characterize the molecular mechanism of acetyllysine histone recognition by the ATAD2B bromodomain using X-ray crystallography. Our studies provide new details on the mechanisms contributing to gene regulation in the progression of cancer, which can then be utilized to work towards future cancer treatments.

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Molecular mechanisms of acetyllysine recognition by PfBDP1 bromodomain

Marina Juan, Saleh Alkrimi, Samuel P. Boyson,
Margaret Philips, and Karen C. Glass, PhD

In the most lethal form of malaria, symptoms are only associated with the red blood cell stage of infection where P. falciparum undergo repeated rounds of replication, cell lysis, and reinvasion of erythrocytes. The P. falciparum bromodomain protein 1 (PfBDP1) has been shown to play an integral role in the expression of invasion-related genes in the parasite. Knockdown of the PfBDP1 bromodomain significantly decreases the transcription of multiple invasion-related genes and hinders the ability of P. falciparum to penetrate red blood cells, making it a potential target for antimalarial therapy. In current literature, the binding affinities for the known histone ligands of PfBDP1 have not been determined, and there is no structural information available for PfBDP1 in complex with its histone ligands. We used isothermal titration calorimetry (ITC) on two different constructs reported for the PfBDP1 bromodomain to characterize its binding affinity to post-translational modifications on histones H3 and H4. We also carried out NMR titration experiments to structurally characterize the PfBDP1 bromodomain-histone ligand interaction and identify amino acid residues critical for ligand coordination.

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Synthesis of Co-Drugs Derived from Mycophenolic Acid and 5-Aminolevulinic Acid

Kaitlyn Strumski and Martha A. Hass, PhD

Mycophenolic acid (MPA) and 5-aminolevulenic acid (ALA) have been known to reduce the symptoms associated with the autoimmune skin disease, psoriasis. Our hypothesis is that the combination of these two drugs, when delivered simultaneously to the skin, will provide synergistic therapeutic benefit in suppressing the symptoms of psoriasis. This research project aims to synthesize two co-drugs derived from MPA and ALA, designed for topical delivery. The first compound, mycophenolate-aminolevulinate methylester (MPA-ALA ME) is prepared by linking the phenol group of methyl mycophenolate (MPA-ME) to the carboxylic acid of ALA to form an ester. The second targeted co-drug is mycophenolate-aminolevulinate diester (MPA-ALA DE), synthesized using a 1,3-propane diol linker to connect MPA to the ALA through their respective carboxylic acid functional groups. The parent drugs are released when the metabolically-labile co-drug esters are delivered to the viable layers of the skin and hydrolyzed in psoriatic skin cells. This co-drug strategy ensures that both parent compounds penetrate the skin at the same rate, thus allowing for synergistic activity.

For synthesis of MPA-ALA ME, the carboxylic acid of MPA was protected as a methyl ester (MPA-ME) and a tert-butyloxycarbonyl group was used to protect the amine of ALA (ALA-BOC). MPA-ME was prepared in good yield (71%) and characterized by infrared (IR) spectroscopy, melting point analysis and thin layer chromatography through comparison with a known standard. A melting point of 101^{o}C was determined for the MPA-ME, which was consistent with the known melting point of MPA-ME. ALA-BOC was prepared in modest yiled (11%) and was also characterized by IR spectroscopy, melting point analysis and thin layer chromatography through comparison of a known standard. A melting point of 79^{o}C was determined for ALA-BOC, which was consistent with that of the standard. The phenol group of MPA-ME was then coupled to the carboxylic acid of ALA-BOC to form the ester using DMAP and DCC in CH2Cl2. The product of the coupling reaction, ME-MPA-ALA-BOC, was purified by flash column chromatography (silica gel, 50:50 hexane: ethyl acetate) to give a 22% yield. Characterization of this product is underway using spectroscopic methods (IR, NMR spectroscopy; mass spectrometry). The BOC group will then be removed with TFA in CH2Cl2 or with HCl in methanol. To produce the second co-drug, MPA-ALA DE, a 1,3-propane diol linker will be used to connect the carboxylic acid of MPA with the carboxylic acid of ALA-BOC.

The parent compounds, MPA and ALA were successfully protected as MPA-ME and ALA-BOC. Synthetic methodology was developed to prepare the ester link between the two compounds. The product of the coupling reaction will be fully characterized, and the BOC group will be removed to prepare the final product, MPA-ALA ME. Once the first co-drug is successfully synthesized and characterized synthesis of the second co-drug, MPA-ALA DE, can commence.

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Mitochondrial trafficking during the hyperglycemic shift from apoptosis to necroptosis

Phillip V. Truong, William D. McCaig, Kevin R. Metz, Timothy J. LaRocca, PhD

Necroptosis is a pro-inflammatory programmed cell death (PCD) pathway. Unlike apoptosis, necroptosis is caspase-independent and is mediated by the necrosome complex, consisting of receptor-interacting protein kinase-1 and -3 (RIPK1, RIPK3), and mixed lineage kinase domain-like pseudokinase (MLKL). We have previously discovered the hyperglycemic shift from TNF-α-induced apoptosis to necroptosis and wish to further delineate its mechanism. As we noted a central role for mitochondrial reactive oxygen species (ROS) in this shift to necroptosis, we aim to analyze the cellular trafficking of critical cell death factors as part of this mechanism. In this study, we show that RIPK1, MLKL, and mitochondrial fission regulator, Drp1 traffic to the mitochondria during the hyperglycemic shift to necroptosis. We also show that this trafficking is primarily driven by ROS in experiments utilizing the superoxide dismutase inhibitor, diethyldithiocarbamate (DDC). DDC-induced ROS production led to the inactivation and loss of executioner caspases-3, -6, and -7 in the cytoplasm. Conversely, amounts of RIP1 and MLKL increased and localized to the mitochondria following DDC treatment. DDC-induced ROS also led to the increased translocation of Bax, Bak, and dephosphorylated Drp1 to the mitochondria. Finally, DDC-induced ROS promoted the oligomerization of RIP1, MLKL, Bax and Bak in the mitochondria.

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Structure-function studies of human Cytochrome P450 2C9*3: Insights into the effect of genetic polymorphism

Thomas Yarbrough, Sonia Parikh, and Manish B. Shah, PhD

Cytochrome P450 (CYP) enzymes are membrane associated heme-containing proteins involved in the metabolism of xenobiotics that include clinical drugs with increasing importance in pharmacogenomics. A single nucleotide polymorphism (SNP), a variation in a single base pair in a DNA sequence, may lead to the change in the amino acid of the protein resulting in altered enzymatic activity. SNP in drug metabolizing CYPs have the potential to influence an individual's response to different drugs, resulting in a decrease or increase in adverse events or drug-drug interactions. Greater than 700 such alleles have been discovered in different populations, many of which have significantly reduced activity of the enzyme. Two copies of wild-type allele are correlated with extensive or normal metabolism of drugs, often referred to as *1. A person with two variant copies of allele (*3/*3 or *x/*x) may have either a reduced or a rapid response to the metabolism of drugs and referred to as either a poor or rapid metabolizer based on the genotype. The human CYP2C9 enzyme is known to metabolize around 15-20% of the currently available clinical drugs that include some of the important drugs like antihypertensive losartan, anticoagulant warfarin, and antidiabetic drugs tolbutamide and glimepiride. The *3 allele of CYP2C9 is among the most prevalent genetic variants of CYP2C9 as it is present in around 15% of different populations. A marked reduction of catalytic activity towards many important drug substrates has been linked to the presence of CYP2C9*3 variation in affected patients. The *3 in CYP2C9 represents a change in amino acid from isoleucine to leucine at position 359 in the sequence of the protein. The purpose of the study is to understand the effect of genetic polymorphism on drug binding to CYP2C9 using functional, structural and/or computational methods. The CYP2C9*3 enzyme was expressed and purified from E.coli in a recombinant manner for functional and structural analysis. The activity of the enzyme was measured in the reconstituted system that included P450 reductase and losartan by the consumption of NADPH or change in absorbance at 340 nm. The *3 variant illustrated significantly reduced ability to turnover the substrate compared to the wild-type of CYP2C9. Efforts to crystallize CYP2C9*3 genetic variant in complex with different drug substrates were unsuccessful. The computational ligand docking was employed using the previously solved CYP2C9*3 structure to elucidate the differences in binding of the drug substrates that include warfarin, irbesartan, glyburide, and tolbutamide. The *3 variant clearly illustrated the altered binding of drug substrates compared to the wild-type structure in the molecular docking studies. The data suggest that the reorientation of the phenylalanine side chain at position 476 (F476) alters the binding of drugs near the access channel and in the active site of CYP2C9*3 compared to the wild-type enzyme. Overall, the results yield insights into the reduced enzymatic activity in patients carrying CYP2C9*3 allele and may help understand the differences in drug metabolism.

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Assessing advance care planning in individuals with Lynch Syndrome

Victoria Fenton, Lauren Fletcher, Jennifer Bowles,
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Lynch syndrome (LS) is a hereditary cancer syndrome characterized by an increased risk of multiple cancers, predominantly endometrial and colorectal, at a younger age (typically < 50). In prior research, high death anxiety and a lack of provider-initiated communication about advance care planning (ACP) have been shown to decrease a patient's likelihood of having advance directives. Providers often have gaps in knowledge and are uncomfortable with these conversations. We used a mixed methods approach (quantitative survey with a follow-up telephone interview) to assess knowledge, preferences, and attitudes regarding ACP in individuals with LS (n = 20). This study also assessed which ACP documents individuals already had in place and which persons (providers, family, or friends) an individual made aware of the documentation and/or preferences. These data were analyzed to determine patient preferences for who is responsible for initiating these conversations, identify motivating factors and barriers to these conversations, and determine whether the current conversations are adequate to meet the needs of this patient population. Participants recognized the importance of ACP and expressed interest in creating these documents. However, knowledge and confidence about these topics were lacking, with many participants attributing this to their young age and lack of experience. Although uncomfortable, many patients want to have ACP discussions with their providers, but frequently patients were only asked if these documents are completed with no further discussion. These findings can inform educational efforts to improve knowledge of ACP and interventional research to increase use of ACP by individuals with LS.

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Determining the Vibrio parahaemolyticus Anti-oxidant Genes Required for Survival in Various Environments

Yelena Dunikova, Brittany Vojnar, Brittney L. Maring, Chen Shu Dong, Stephanie Waters-Wezalis, Michelle A. Parent, PhD

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. At this time, limited literature is available regarding organism pathogenesis and especially the host response and immunity to infection. It is well known that pathogenic bacteria may produce enzymes such as catalase, alkyl hydroperoxide reductase, and peroxiredoxins allowing for survival in toxic environments, specifically in the presence of reactive oxygen species (ROS). Previously, we have investigated the antioxidant enzymes involved in V. parahaemolyticus survival when exposed to ROS found in oxidants such as H2O2 , paraquat, t-BOOH, and cumene hydroperoxide and those produced by the macrophage during infection. 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. RT-qPCR revealed that exposure to the ROS generated by these oxidant chemicals caused an upregulation in various OxyR1 regulated genes such as ahpC1, katE1&2, glutathione, and ahpF. These same genes were also upregulated during U937 macrophage infections suggesting that they serve an important role in organism survival against ROS found in oxidants and in the macrophage. Currently, we are investigating the macrophages response during infection, focusing on investigating macrophage phenotype (M1 versus M2) during infection through looking at surface markers and cytokine production. Characterization of the macrophage response and organism survival will provide a more comprehensive understanding of V. parahaemolyticus virulence and pathogenesis.

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Identification and validation of secreted factors in the crosstalk between HOXB7 overexpressing cells and stromal cells

Nicholas Nasta and Kideok Jin, PhD

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 Robo3 in the crosstalk between HOXB7 overexpressing cells and stromal cells that promote cell proliferation and migration. The SLIT/ROBO pathway includes three Slit glycoproteins and four Robo receptors that have been implicated in tumor development. Robo receptors belong to the immunoglobulin superfamily of cell adhesion molecules. Robo3, specifically, is a known inhibitor of the Robo2 signaling pathway and is involved in both an independent signaling pathway and in crosstalk with the MET and Wnt/-catenin pathways.

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 (A4, A6, H3) were analyzed via Human Cytokine Array Q440 with tumor conditioned media to detect 10 upregulated, key secreted factors induced by HOXB7 overexpression.

In the previous study, we screened the upregulated factors via qRT-PCR and found that Robo3, CFXIV, Follistatin, CALCA and ALCAM were significantly upregulated while Syndecan-3, AMICA, IL-11, CXCL14, and SOST have no significant expression in HOXB7 overexpressing cells compared to vector controls. We selected Robo3 which was most significantly upregulated in three different HOXB7 overexpressing breast tumor cells lines (MCF-7, T47D and MDA-MB231) in this study. Currently, we are working to validate secreted protein levels of Robo3 via ELISA analysis using tumor conditioned media. To identify the role of Robo3 we will perform functional analysis assay. 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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