ALBANY COLLEGE OF PHARMACY AND HEALTH SCIENCES

ACPHS Student Research Research Symposium

Poster Session, Thursday, April 8, 2021

Welcome and Introduction: 11:00 - 11:03 am
Martha A. Hass, PhD, Director of Research

Breakout Rooms - Morning Sessions

Group A - Session 1: 11:03 - 11:45 am
Moderator: Margaret Doll, Ph.D., M.P.H.

11:03 - 11:05 am	Introduction	
11:05 - 11:10 am Poster 1	Preeti Kumar	Impact of genetic blood disorder diagnoses on the mental health of the patient
11:10 - 11:15 am Poster 2	Saima Hannan	Environmental injustices in Ezra Prentice Homes: bringing environmental and health equity to low income communities of color
11:15 - 11:20 am Poster 3	Patrick Wood	Service learning in public health: student participation in the Collaboratory vaccine clinics
11:20 - 11:25 am Poster 4	Morgan Lathrop	Mask wearing compliance: ACPHS and South End, Albany observational study
11:25 - 11:30 am Poster 5	Matthais Caryofilles	The influenza vaccine uptake project: an evaluation of resident-reported barriers to influenza vaccination in Albany, New York
11:30 - 11:35 am Poster 6	Cameron Montgomery	Influence of combinatorial histone PTMs on ligand recognition by ATAD2B bromodomain
11:35 - 11:40 am Poster 7	Saleh Alkrimi	Histone recognition by Plasmodium falciparum bromodomain protien 1
11:40 - 11:45 am	Closing	

Breakout Rooms - Morning Sessions

Group B - Session 1: 11:03 - 11:45 am
Moderator: Christopher Cioffi, Ph.D.

11:03 - 11:05 am	**Introduction**	
11:05 - 11:10 am Poster 8	**Kofi Hagan**	**Endocannabinoids may enhance blood brain-barrier function during ischemic stroke**
11:10 - 11:15 am Poster 9	**Noelle Capriglione**	**Anti-proliferative effect of aminolevulinic acid (ALA) and mycophenolic acid (MPA) with blue light irradiation**
11:15 - 11:20 am Poster 10	**MacKenzie Quirk**	**An in vitro model to assess combination activity of 5-aminolevulinic acid and methotrexate**
11:20 - 11:25 am Poster 11	**Zaheen Uddin**	**Characterizing microemulsion formulations for topical delivery of prodrugs**
11:25 - 11:30 am Poster 12	**Rebekah Garafolo**	**The influence of axonal signals in the regulation of Schwann cell energetic metabolism**
11:30 - 11:35 am Poster 13	**Pablo Ortiz**	**Brusatol as a possible STAT3 inhibitor in U87 glioma cells**
11:35 - 11:40 am Poster 14	**Danielle Yu**	**Analysis of enactment of marijuana bylaws on opioid-related deaths in New York counties**
11:40 - 11:45 am	**Closing**	

Breakout Rooms - Afternoon Sessions

Group A - Session 2: 11:45 am - 12:30 pm
Moderator: Timothy LaRocca, Ph.D.

11:45 - 11:50 am	Introduction	
11:50 - 11:55 am Poster 15	Basmah Zahid	Identification of viral factors regulating host translation during SARS-CoV-2 infection
11:55 am - 12:00 noon Poster 16	Arthur Worrad	Investigating the mechanisms involved in HIV associated neuroinflammation
12:00 - 12:05 pm Poster 17	Yelena Dunikova	Determining the Vibrio parahaemolyticus anti-oxidant genes required for survival in various environments
12:05 - 12:10 pm Poster 18	Dakota Paine	Sulforaphane promotes activation of the antiviral protein SAMHD1 to protect macrophages from HIV infection
12:10 - 12:15 pm Poster 19	Vincent Farrazi	The antiviral protein SAMHD1 is a major driver of sulforaphane-mediated protection of macrophages from HIV
12:15 - 12:20 pm Poster 20	Alexandria Bautz/ Robert Tipple	Scanning Subclones to Identify Monocytic Cells that Maximal Produce Wild Type and Mutant SAMHD1
12:20 - 12:25 pm Poster 21	Alexis M. Parry/ Alexa R. Boni	Review: The Biosynthesis of Recombinant Human Insulin by Escherchia coli
12:25 - 12:30 pm	Closing	

Breakout Rooms - Afternoon Sessions

Group B - Session 2: 11:45 am - 12:30 pm
Moderator: Kideok Jin, Ph.D.

11:45 - 11:50 am	**Introduction**	
11:50 - 11:55 am **Poster 22**	**Nafisa Arfan**	**Nuclear trafficking of cell death molecules during the hyperglycemic shift from apoptosis to necroptosis**
11:55 am - 12:00 noon **Poster 23**	**Kevin Metz**	**Role of RIP1 in cellular trafficking during the hyperglycemic shift to necroptosis**
12:00 - 12:05 pm **Poster 24**	**Andrew Fiorica**	**The role of VDUP1 in neural stem cells: analyses of the relationships between Brat, VDUP1, and Miranda**
12:05 - 12:10 pm **Poster 25**	**Gianna Flint**	**Analysis of VDUP1 expression Patterns in Asence mutants and using hexameric GFP reporter constructs**
12:10 - 12:15 pm **Poster 26**	**Daniel Galke**	**Adipsin and its downstream components as ER target genes**
12:15 - 12:20 pm **Poster 27**	**Nicholas Nasta**	**Identification and validation of secreted factors in the cross talk between HOXB7 overexpressing cells and stromal cells**
12:20 - 12:25 pm **Poster 28**	**Sneha Pandithar**	**Crosstalk between stromal components and endocrine resistant breast cancer via secreted factors enhances tumor growth and metastasis**
12:25 - 12:30 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

Return to agenda

Impact of genetic blood disorder diagnoses on the mental health of the patient

Preeti Ramesh Kumar and Allison M. Burton-Chase, PhD

Thalassemia and sickle cell anemia are disorders of hemoglobin that affect millions of people worldwide. These disorders negatively impact the quality of life and survival of millions of individuals throughout the world. They are caused by mutations in the adult globin gene that result in the production of abnormal hemoglobin. Thalassemia is characterized by a reduced number of red blood cells resulting in diminished oxygenation throughout the body. Sickle cell anemia, in contrast, is caused by a point mutation in the beta gene on chromosome 11. This mutation changes the shape of red blood cells from a circular shape to a "sickle," which causes the cells to clump and get stuck in the bloodstream. These hereditary conditions cause a portion of the world's population to experience high medical costs as well as an increased likelihood of mental illness diagnosis. Anxiety and Depression can cause many harmful side effects to the body, including weight loss/gain, sleep disturbances, and irritability which could lead to further health issues. However, the connection between the increased stress level related to the diagnosis of an inheritable blood disorder should not be overlooked since it may cause a spike in the number of diagnoses. Clinically, the inclusion of behavioral therapy as well as regular medication may help reduce the stress level of patients, which in turn could reduce the number of cases of anxiety or depression.

Return to agenda

Return to agenda

Environmental injustices in Ezra Prentice Homes: bringing environmental and health equity to low income communities of color

Saima Hannan, Sophia Livecchi, Haja Isatu Bah,
Stacy Pettigrew PhD, MS

Our narrative case study research worked to document and better understand the historical and more contemporary environmental injustices faced by the residents of Ezra Prentice Homes, the outcomes of these inequities on residents' health, the suite of advocacy tools employed by residents and civil society, as well as (multiple) agency policy responses and the role of the media. The predominantly black residents of Ezra Prentice Homes in Albany, New York have been facing ongoing environmental injustice. They have been forced to share their backyards with crude oil trains, industrial activities at the Port of Albany, sewage treatment plants, the recycling center, and thousands of large diesel-engine trucks that drive by on a daily basis. The public housing complex is directly adjacent to existing polluting industries and transportation routes, negatively impacting various social determinants of health. In order to better understand the roles of the diversity of stakeholders, their stakes, level of engagement, and extent of their contributions to potential solutions, we triangulated both our data sources and methods. Through purposeful sampling we conducted approximately thirty interviews from a variety of stakeholders, which allowed us to compile a broad spectrum of narratives as well as provide a diversity of perspectives both within and outside of this marginalized community. Our research has shown physical health implications of being in close proximity to environmental injustices, along with mental health implications. The effects of the COVID-19 pandemic on the residents were also explored.

Return to agenda

Return to agenda

Service learning in public health: student participation in the Collaboratory vaccine clinics

Patrick Wood, Rima Kaddouh, Stacy Pettigrew PhD, MS

Objective: Advance equity in COVID-19 vaccine distribution by utilizing community and grassroots networks to prioritize appointments among medically underserved communities in Albany's South End and communities of color across the Capital District.

Background: Proportionally, Caucasians are receiving the COVID-19 vaccine at a much higher rate than the minority population, even though minorities are most at risk. In New York State, 70.4% of the population is Caucasian while 17.3% is African American. However, 78.4% of the vaccinated population is Caucasian and 9.5% is African American. In Albany County, 77.8% of the population is Caucasian and make up 87% of the vaccinated population, while 7.4% of the vaccinated population are African American despite making up 13.2% of the population.

Methods: African American and Latinx community leaders and African American churches reached out to members of their communities and collected contact information of seniors interested in receiving the COVID-19 vaccination. Students constructed a secure Redcap database to collect names. Flyers were distributed by community organizations and networks with a link to enter information into the database, along with a phone number (for the AVillage, Inc. office). Students returned calls and called people from the database to set up appointments. At the clinic site, students took patient temperatures, checked patients in, filled out state forms, disinfected surfaces and pens, and ensured that patients were counselled and felt comfortable before getting the vaccine, as many people had uncertainties. Additionally, in an effort to increase vaccination rates, we also collaborated on a social media campaign with the South End Community Collaborative. Patients were given the opportunity to make a video for their fellow community members to talk about what they experienced receiving the vaccine and why they wanted the vaccine, so that others will be less fearful of getting the shot.

Return to agenda

Return to agenda

Mask wearing compliance: ACPHS and South End, Albany observational study

Morgan Lathrop, Nicole Swiatkowski, Rima Kaddouh, and Stacy Pettigrew

Objective: As COVID-19 struck Albany, ACPHS students took the initiative to observe the campus community on how effectively COVID-19 mask wearing guidelines were followed. The observational study focused on comparing results to the South End community and their compliance of following CDC guidelines. As public health students, we believe it is important to acknowledge the effect of COVID-19 on surrounding neighborhoods and how communities are implementing safety precautions to protect their residents.

Methods: A five to six week long observational study took place in the Fall 2020 semester across various locations on the ACPHS campus and surrounding Albany areas. Data was recorded by using both excel and a REDCap database. On the ACPHS campus, data was collected at key locations such as residence halls, the Student Center, and the O'Brien building. In the South End, observations were made at corner stores, Market 32 on Delaware Avenue, and Walmart in Glenmont. These locations were selected as they are the most accessible grocery stores for the South End community. The criteria that was used to gather ACPHS campus data included: type of mask wear (i.e. mask worn properly, nose out, mask visible but not worn, and no mask visible), sex, and whether the subject was a student, faculty, or unknown. South End data collection included: type of mask wear, sex, approximate age group, day of the week, and time.

Results: Data collection on mask wearing for ACPHS campus indicated 88.1% of the population wore masks, whereas 6.3% of the population did not. Of the total no mask population, 48.0% were observed at the Student Center. Observations in the South End indicated much lower mask compliance; 69.2% of individuals wore masks and 9.8% wore no masks. The total number of subjects observed at Walmart was 775, where 84.4% subjects wore a mask and 1.14% did not. At Market 32, a total of 752 subjects were observed, where 74.6% wore a mask and 6.3% did not. The total number of subjects observed at corner stores was 777, where 48.9% of subjects wore a mask and 21.6% did not. Significantly more people wore masks at ACPHS compared to the locations observed in the South End ($p<.0001$). More people were observed wearing masks at both Walmart and Market 32 compared to corner stores in the South End ($p<0.0001$).

Conclusion: Throughout our observational study, there was little change in mask compliance on the ACPHS campus. However, specific areas on campus had higher rates of low compliance. Overall, the ACPHS campus had high rates of guideline compliance compared to the South End. Market 32 and Walmart had high mask compliance, which could be due to strictly enforced COVID-19 guidelines. On the other hand, corner stores had a lower mask compliance rate, which could be explained by the failure to enforce mask wearing guidelines.

Return to agenda

Return to agenda

The influenza vaccine uptake project: an evaluation of resident-reported barriers to influenza vaccination in Albany, New York

Matthias Caryofilles, Nicholas Nasta, Emina Krupic, Dani Schechter, Thomas Yarbrough, Katie Cardone, PharmD, Elisabeth Vines, PhD, Stacy M. Pettigrew, PhD and Margaret K. Doll, PhD

Background: Vaccine hesitancy has been significantly increasing in the United States. Specifically, perceptions regarding the influenza vaccine may have changed due to the current coronavirus (COVID-19) pandemic. Assessment is needed to plan community interventions for influenza vaccination. In this project, we assessed and evaluated barriers, misconceptions, and attitudes towards influenza vaccination with the aim of informing best practices for an influenza vaccine clinic at the Albany College of Pharmacy and Health Sciences (ACPHS) Collaboratory.

Methods: In September 2020, we collaborated with Trinity Alliance of the Capital Region in the South End Neighborhood of Albany, NY to distribute our survey examining influenza vaccine uptake and barriers to vaccination through an online sign-up system for food pantry delivery events. In January 2021, we also collaborated with the South End Community Coalition to include some of our influenza vaccine survey questions in a survey to examine Covid-19 vaccine hesitancy. REDCap, a secure survey tool, was used to develop, distribute, and analyze survey responses. Participant data was aggregated by zip codes (12202, 12208 and 12209) that comprised or were adjacent to the South End Neighborhood.

Results: Of the 101 survey participants, 57 resided in 12202, 24 resided in 12208, and 20 resided in 12209. In this analysis, 19% of respondents were not vaccinated against influenza in 2020 and did not plan to get the influenza vaccine while 8% cited they were not yet vaccinated but planned to. We found that 42% of participants were more likely to uptake the influenza vaccine because of the pandemic, while 46% of participants reported that the pandemic did not change their attitude regarding influenza vaccination. Analyses to examine the specific barriers to influenza vaccine uptake are underway.

Conclusion: A high proportion of survey respondents reported receipt of influenza vaccine. The COVID-19 pandemic may have played a role in increasing influenza vaccination. Future analyses will be beneficial to inform influenza vaccination clinics at the ACPHS Collaboratory.

Return to agenda

Return to agenda

Influence of combinatorial histone PTMs on ligand recognition by ATAD2B bromodomain

Cameron Montgomery, Kathleen Quinn, Sunsik Chang, Margaret Phillips, Samuel Boyson, Karen Glass, PhD

The “histone code” theory attributes the regulation of genetic expression to highly specific posttranslational modifications (PTMs) of key histone residues. The PTM profile of each histone, or proteoform, is essential for appropriate recruitment of histone reader proteins and the transcriptive machinery. The ATPase family, AAA domain-containing protein 2 (ATAD2, or ANCCA) is a nuclear protein that has been shown to be a co-activator of the androgen and estrogen receptors. Notably, ATAD2 is over-expressed in many cancers, including breast and prostate cancer, and its activity is likely contributing to the proliferation of cancer cells. ATAD2 contains an AAA ATPase domain and a C-terminal bromodomain. Bromodomains are a group of highly conserved histone reader modules that function to recognize acetylated lysine residues. ATAD2B is a poorly studied paralog of the ATAD2 gene, and although ATAD2 and ATAD2B are highly conserved, there is very little known about the function of ATAD2B, or its role in oncogenesis. Our previous research demonstrated that the ATAD2B bromodomain recognizes several mono-and diacetylated lysine residues on histone H4 and H2A. However, it is not well understood how the combinatorial effect of acetylation, methylation, and phosphorylation of specific histone residues contributes to the recognition of acetylated lysine by bromodomains. We carried out isothermal titration calorimetry assays and structural studies using Xray crystallography to characterize the binding affinity of the ATAD2B bromodomain with multiply modified histones, as well as to elucidate the molecular coordination of multiple PTMs within the binding pocket. These studies provided structural insights on the molecular mechanisms driving ligand coordination by the ATAD2B bromodomain. This information in tandem with novel binding data showing how the combinatorial effect of histone PTMs influences ligand recognition, provides a strong foundation to further our understanding of how bromodomains sense epigenetic signals to regulate gene expression and cellular proliferation.

Return to agenda

Return to agenda

Histone recognition by Plasmodium falciparum bromodomain protien 1

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

Bromodomains are evolutionarily conserved protein modules that specifically recognize acetylated lysine residues on histone tails of the nucleosome. During the red-blood-cell-stage of infection with Plasmodium falciparum, the parasite undergoes repeated rounds of replication, egress, and invasion. Erythrocyte invasion involves specific interactions between host cell receptors and parasite ligands and coordinated expression of genes specific to this step of the life cycle. A parasite-specific protein known as bromodomain protein 1 (PfBDP1) binds to chromatin at the transcriptional start sites of invasion-related genes and directly controls their expression. Conditional PfBDP1 knockdown causes a dramatic defect in parasite invasion and growth, and results in transcriptional down-regulation of multiple invasion-related genes at a time point critical for invasion. Conversely, PfBDP1 overexpression enhances expression of these same invasion-related genes. PfBDP1 has been shown to interact with acetylated histone H3 and a second bromodomain protein, PfBDP2, suggesting a potential mechanism for gene recognition and control. Since PfBDP1 critically coordinates expression of invasion genes, targeting PfBDP1 could be an invaluable tool in mitigating malaria infections. The domain architecture of PfBDP1 includes a single C-terminal bromodomain and several ankyrin repeats. PfBDP1 is thought to act by tethering a transcriptional activator complex to acetylated histone H3 to control genes required for parasite invasion. We hypothesized that the bromodomain is crucial for this function of PfBDP1 via recognition of acetylated lysine modifications. We carried out several histone binding assays to identify novel histone interactions the of PfBDP1 bromodomain. We also utilized a combination of structural biology and biophysical approaches to identify amino acid residues critical for ligand coordination. The result of this study will provide molecular details about the recognition of acetylated histone ligands by the PfBDP1 bromodomain and shed light on how this domain recruits the PfBDP1-PfBDP2 complex to chromatin. Importantly, these results may improve the development of new therapeutics to treat malaria.

Return to agenda

Return to agenda

Endocannabinoids may enhance blood brain-barrier function during ischemic stroke

Kofi Hagan, Marcel Musteata, PhD, Jeffrey Voigt, PhD, Alejandro Adam, HaiAn Zheng, PhD

Purpose: Endogenous Cannabinoid System (ECS) comprises the signaling network of endocannabinoids (eCB) such as N-arachidonoylethanolamine (AEA) and 2-Arachidonylglycerol (2AG), their cannabinoid receptors and enzymes of biosynthesis and metabolism. Cannabinoid receptors were recently found at the blood brain barrier (BBB), the interface between the CNS and peripheral circulation. To systematically investigate the ECS of BBB at cellular and molecular level, we characterized CB1 and CB2 receptors in a human brain microvascular endothelia cell line (HBMEC). This study is to confirm our hypotheses that eCBs may modify and regulate the BBB physical integrity, especially against pathological stress such as ischemia.

Method: The impact of AEA on endothelial membrane integrity was assessed by trans-endothelial electronic resistant (TEER) under normal and ischemic conditions. CB1 and CB2 in the HBMEC were quantified and monitored by RT-PCR gene expression and Western-blot protein analysis and their expression explored under oxygen-glucose-deprivation (OGD) stress or normal condition.

Results: The CB1 expressions level were higher than CB2 in HBMEC under normal condition. Under ischemic stresses, the expression of CB1 decreased while CB2 increased. The barrier permeabilities decreased upon addition of AEA under both normal and ischemic condition, which indicates the protective effect of eCB.

Conclusions: Our study confirmed the presence of the ECS at the BBB. The endocannabinoids may maintain and regulate the BBB integrity through the ECS, especially under stress. Further studies to discover and understand the complete ECS of the BBB and their mechanism is essential to understanding the distribution and regulation of eCB of the BBB, as well as evaluate the impact of medical cannabis on the BBB, which is a critical drug delivery barrier and therapeutic target.

Return to agenda

Return to agenda

Anti-proliferative effect of aminolevulinic acid (ALA) and mycophenolic acid (MPA) with blue light irradiation

Noelle Capriglione, Jeffrey Voigt, PhD and Martha A. Hass, PhD

Psoriasis is a chronic skin disease that is characterized by hyperproliferation of keratinocytes and inflammation. Both aminolevulinic acid (ALA; photodynamic therapy, PDT) and mycophenolic acid (MPA, chemotherapy) have been used in the treatment of psoriasis, however the combination of these two therapies has not been explored previously. ALA increases the concentration of the endogenous photosensitizer, protoporphyrin IX (PpIX) in keratinocytes. Upon irradiation of treated cells with blue light in the presence of molecular oxygen, hyperproliferation is suppressed. A major aim of this project is to establish that treatment of keratinocytes (HaCaT cells) with ALA, followed by exposure of the cells to blue light results in a decrease in cell proliferation. Once established we aim to show that suppressed cell proliferation in HaCaT cells is maintained in the presence of MPA. Finally, with the combination of equimolar concentrations of ALA and MPA, we hope to increase the amount of suppressed hyperproliferation, as compared to each drug alone.

HaCaT cells were treated with various doses of ALA and incubated for 4h. The amount of PpIX generated was measured using fluorescence spectroscopy. HaCaT cells treated with ALA induced an increase in PpIX, in a dose dependent manner, consistent with previous reports in the literature. An MTT assay was used to demonstrate that cell viability was maintained throughout the 4h incubation period for treatment with the highest concentrations of ALA, MPA and ALA + MPA (1mM). Once elevation of PpIX was established in treated cells, experiments to determine the effect of blue light on the treated cells were done. Cells, untreated (control) and treated with ALA (1mM) were incubated for 4h at 37°C and then exposed to blue light for varying periods of time. Cell proliferation was monitored using the MTT assay. Preliminary data suggests that cell proliferation is reduced in cells treated with ALA and exposed to blue light, compared with untreated cells. Experiments are continuing to determine the dose of ALA needed to maintain this effect and to explore the effect of MPA on proliferation of ALA-treated HaCaT cells.

Return to agenda

Return to agenda

An in vitro model to assess combination activity of 5-aminolevulinic acid and methotrexate

Mackenzie Quirk , Jeffrey Voigt, PhD and Martha A. Hass, PhD

Combining methotrexate (MTX) chemotherapy and 5-aminolevulinic acid (ALA) photodynamic therapy for the treatment of psoriasis has not previously been explored. Methotrexate (MTX) is a first line agent used to treat all forms of moderate to severe psoriasis. Its efficacy is attributed to the blockade of folic acid synthesis through inhibition of dihydrofolate reductase (DHFR), resulting in attenuated proliferation of rapidly dividing keratinocytes in psoriatic skin. ALA, a drug currently used in photodynamic therapy (PDT) to treat psoriasis, limits hyperproliferation of keratinocytes through a protoporphyrin IX-(PpIX) mediated mechanism. This project focuses on in vitro cell and enzyme assays intended investigate the potential therapeutic benefits of combining MTX and ALA and to lay the ground work for the development of co-drugs derived from MTX and ALA.

The specific aims of this project are twofold; 1) to determine the effect of MTX on the generation of protoporphyrin IX (PpIX) induced by ALA in HaCaT cells, and 2) to determine the effect of ALA on inhibition of DHFR by MTX using a DHFR inhibitor screening assay. HaCaT cells are treated with each individual drug and a 1:1 molar combination of the two drugs and the amount of PpIX generated is measured using fluorescence spectroscopy. HaCaT cells treated with ALA induced an increase in PpIX, in a dose dependent manner, consistent with previous reports in the literature. Experiments are underway to treat HaCaT cells with MTX alone and combination doses of ALA and MTX, and to measure the effect MTX has on the PpIX levels in the cells. Similarly, experiments to determine the effect of each individual drug and a 1:1 molar combination of the two drugs on the inhibition of DHFR is underway. To date, we have verified that MTX and the disodium salt of MTX effectively inhibit DHFR. Experiments with ALA alone and MTX with ALA are planned.

Return to agenda

Return to agenda

Characterizing microemulsion formulations for topical delivery of prodrugs

Zaheen Uddin and Martha A. Hass, PhD

Purpose: Psoriasis is a chronic auto-immune, human skin disease that is characterized by acceleration of the life cycle of skin cells (keratinocytes) resulting in inflammation and plaques. The overall goal of this project is to develop a topical formulation to deliver ester co-drugs derived from aminolevulinic acid (ALA) and mycophenolic acid (MPA) to the viable layers of psoriatic skin to control the symptoms of psoriasis. ALA is a drug used in photodynamic therapy (PDT) that controls plaque formation while MPA targets inflammatory symptoms. The specific focus of the work described here is on the development of microemulsion (ME) formulations, assessing their stability and generating phase diagrams to describe these formulations, and characterizing selected ME by particle size.

Methods: A variety of ME compositions with different ratios were explored using isopropyl myristate (IPM) as oil phase, and a variety of surfactants or surfactant blends (Tween 80, Span 80, 1,2-octanediol). Microemulsions (ME) derived from oil/surfactant/water were prepared with a range of ratios by the aqueous titration method and pseudo ternary phase diagrams for each ME composition were prepared. ME were characterized using the Winsor Physical stability of the drug formulations was monitored over 48 hours. Model ester prodrugs, aminolevulinic acid benzyl ester (ALA-BE) and mycophenolic acid methyl ester (MPA-ME) were used to assess stability and phase diagrams of loaded ME. HPLC methods were developed to measure intact and hydrolyzed prodrugs in the formulations and for future use to quantify delivery of these compounds to the viable skin layers. Selected stable ME formulations of the ester co-drugs are being characterized for particle size and viscosity.

Results: Pseudo ternary phase diagrams were generated from ratios of oil:surfactants:water formulations using the Winsor classification system. A number of stable, unloaded ME were identified using these phase diagrams. ME with oil:surfactant ratios ranging from 1:9 to 9:1 were prepared with increasing amounts of water. Among the stable ME, oil:surfactant ratios of 8:2 and 7:3 were selected to load with the ALA-BE prodrug. The average particle size of stable ME drug-loaded formulations was found to be in the range of 100-250 nm. All of the drug formulations were stable at room temperature for 48 hours. Calibration curves for ALA-BE were obtained using HPLC analysis at six different concentrations in the range of 1.0-100.0 μM. The ester prodrugs were clearly resolved in the HPLC chromatogram and their retention times were established under the conditions developed.

Conclusions: The prodrug ALA-BE was successfully formulated in stable ME formulations. Future studies are focused on formulation of MPA-ME with the same ME followed by assessment of the delivery of ALA-BE and MPA-ME to viable layers of the skin using an ex-vivo porcine skin model.

Return to agenda

Return to agenda

The influence of axonal signals in the regulation of Schwann cell energetic metabolism

Rebekah Garfolo, Yannick Poitelon, PhD and Sophie Belin, PhD

Peripheral neuropathies are a major public health problem (affecting ~ 20 million people in the U.S.) with a significant social and economic burden. Current treatments are limited to controlling symptoms and do not address the cause of nerve degeneration. Peripheral neuropathies can be caused by a lack of myelin due to injury, genetic or idiopathic causes, which interferes with the transmission of neuronal signals. Myelin is a membranous sheath that encapsulates axons to enhance the conduction of action potentials in the nervous system. Schwann cells are the main glial cells in the peripheral nervous system and are responsible for maintaining axonal health and myelin production. Myelination is metabolically a highly demanding process, requiring Schwann cells to timely and precisely increase and coordinate RNA, protein and lipid synthesis, protein targeting, and massive membrane production.

Axonal neuregulin-1 type III (NRG1TIII) is a key regulator of Schwann cell myelination, as overexpression of NRG1tIII increases myelin formation. Interestingly, overexpression of axonal NRG1tIII correlates with overexpression of peripheral myelin protein 2 (PMP2), the Schwann cell-specific fatty acid binding protein. Fatty acid binding proteins act as lipid transporters and are uniquely abundant in tissues which substantially engage in lipid metabolism. Our preliminary data suggests that treatment of Schwann cells with NRG1tIII or overexpression of PMP2, results in an upregulation of glycolytic ATP production, with minimal variation in aerobic respiration. This implies that axonal signals can induce a metabolic shift in Schwann cells to increase energy production independently of mitochondrial respiration, likely to meet the high metabolic demand required for myelination. I am proposing that axonal signals (i) induce a metabolic shift in Schwann cells by (ii) upregulating glycolysis to enhance energy production.

I will characterize the effect of axonal signaling on Schwann cell metabolism using the Seahorse live-cell metabolic platform, which measures oxygen consumption and extracellular acidification to quantify mitochondrial and glycolytic ATP production, respectively. Additionally, I will determine the dependency (how much cells need) and capacity (how much cells can use) of fatty acids, glucose, and glutamate as fuel sources for mitochondrial respiration. (i) Purified Schwann cells (alone or stimulated by neuronal membranes from control mice); (ii) co-culture of Schwann cells with neurons; and (iii) isolated sciatic nerve fibers from mouse models will be used in these assays to observe changes in the myelinating and developmental stages. I will also identify the roles of PMP2 and NRG1TIII in Schwann cell energetic metabolism, along with how varying expressions can influence these processes.

Return to agenda

Return to agenda

Brusatol as a possible STAT3 inhibitor in U87 glioma cells

Pablo Ortiz and Jeffrey Voigt, PhD

Nrf2 is responsible for the regulation of antioxidant proteins which protect the cell from chemical and oxidative stresses. STAT3 is a transcription factor responsible for the regulation of cell proliferation, inflammation, angiogenesis, and apoptosis. Constitutive activation of the Nrf2 and STAT3 signaling pathways have been linked to the development and survival of human malignancies. Brusatol, an herbal medicine-derived quassinoid, is currently being studied as an anti-cancer agent which inhibits tumor growth by inhibiting Nrf2 signaling through the enhancement of protein ubiquitination, though the full mechanism of brusatol may involve inhibition of constitutive STAT3 expression. This hypothesis is that brusatol exerts its anticancer effect through the inhibition of STAT3 signaling. MTT assays demonstrated the toxicity of brusatol on U87 cells and NRF2 appears to be expressed in U87 cells. Western blotting will be used to investigate the effect of Brusatol on STAT3 activation and function.

Return to agenda

Return to agenda

Analysis of enactment of marijuana bylaws on opioid-related deaths in New York counties

Danielle Yu and Jacqueline Cleary, PharmD, BCACP

Opioids are controlled substances that may cause respiratory depression leading to death if misused. According to the Centers for Disease Control and Prevention (CDC), opioid overdoses composed nearly 70% of all drug overdose deaths in 2018. These consequences have led to radicalized perceptions of prescription opioids for both patients and health care providers. In 2017, the U.S. Department of Health and Human Services officially declared the opioid crisis as a public health emergency.

In the midst of this opioid crisis, marijuana has been gaining popularity across the nation for both medical and recreational use. States are beginning to legalize marijuana for medicinal and/or recreational use at different rates and for different indications. In July 2014, New York State (NYS) became one of the growing number of states to legalize medical marijuana. The state has dictated use of marijuana for conditions including chronic pain or pain that degrades health and function capability. Despite lack of evidence, NYS has also included substance use disorder as an approved indication for medical marijuana.

This observational study focuses on evaluating trends in opioid-related deaths following enactment of NYS's medicinal cannabis legislation. NYS validated government websites are used to verify cannabis legislation and indications for medical cannabis use. Data on opioid-related deaths, both prescription and illicit, as well as age-adjusted rates are collected via CDC Wide-ranging Online Data for Epidemiologic Research (WONDER) database. This database is used to compile the number of opioid deaths and age-adjusted rates in each of NYS's sixty-two counties. This database is used to collect values from 2012 and 2013, the two years prior to NYS medical cannabis legislation, to years following the legislation through year 2019. Trends in deaths and age-adjusted rates are observed in regards to illicit opioids, prescription opioids, and all opioids. Illicit opioids were defined as only heroin while prescription opioids were inclusive of opium, other opioids, methadone, other synthetic narcotics, and unspecified narcotics.

Return to agenda

Return to agenda

Identification of viral factors regulating host translation during SARS-CoV-2 infection

Basmah Zahid and Eric J. Yager, PhD

SARS-CoV-2 is an emerging coronavirus responsible for COVID-19 that continues to cause public health concerns around the world. As a novel virus, the biology and pathogenesis of SARS-CoV-2 is an active area of research. Coronavirus belongs to the Coronaviridiae family and consists of crown-like spikes on the outer surface of the virus. Previous studies on related coronaviruses have revealed that interactions between viral proteins and cellular factors can impact the host cell function and help the virus evade immune defenses. These glycoprotein spike proteins, including the coronavirus, causes acute respiratory syndrome (SARS) by attaching and entering into the host cells. Another factor of the coronavirus that has shown to affect the host cells is the nucleocapsid protein. The nucleocapsid protein (N) has a multifunctional role in enhancing the efficiency of viral transcription and assembly. The N protein of SARS-CoV has been shown to deregulate the cell cycle, shutdown host translation, and induce apoptosis. Based on our preliminary data, our central hypothesis is that the interactions between SARSCoV-2 N proteins and the initiation factor eIF4H favors viral protein impact host cell translation and favor viral protein production.

Return to agenda

Return to agenda

Investigating the mechanisms involved in HIV associated neuroinflammation

Arthur H. Worrad, Irfan Khan PhD, Vir Singh, PhD

Human Immunodeficiency Virus-1 (HIV-1) has remained the cause a worldwide epidemic since its emergence in early 1980s and actively affects more than 37.9 [32.7−44.0] million people globally. However, with the advent of Combine Anti-Retroviral Therapy (cART), the number of deaths due to HIV has decreased dramatically. There are 23.3 million people on antiretroviral therapy as per data collected in 2018. People undergoing cART are experiencing very low or undetectable viral load and significantly improved life expectancy with a very low risk of developing AIDS. With the extended lifespan of HIV infected individuals, we can observe other comorbidities with the disease. One common comorbidity of HIV infection is the early onset of neurological impairment which exhibit Alzheimer's like symptoms in severe cases. This pathology is referred to as HIV Associated Neurological Disorder (HAND). HAND is attributed to the persistent Central Nervous System (CNS) inflammation that occurs regardless of cART. A known contributor to HAND is HIV-Tat protein that induces neuroinflammation and increase permeability by downregulating the Blood Brain Barrier (BBB). We have previously shown that HIV-1 can cause BBB damage by downregulating Sonic hedgehog (Shh) signaling, thereby altering the expression of tight junction proteins. In this study, we investigated if pharmacological induction of the Sonic Hedgehog (Shh) pathway in astrocytes and Human Brain Microvascular Endothelial Cells (HBMECs) can rescue the adverse effects caused by HIV-Tat. The Neuroprotective effects of Shh induction were measured using RT-qPCR to analyze the expression of inflammatory markers IL-6, IL-1B, and CCL2. Additionally, western blots were performed to measure expression of tight junction proteins Claudin and Occludin. Overall, our results suggest that pharmacological induction of Shh can alleviate neuroinflammation and rescue BBB integrity by recovering tight junction proteins.

Return to agenda

Return to agenda

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.

Return to agenda

Return to agenda

Sulforaphane promotes activation of the antiviral protein SAMHD1 to protect macrophages from HIV infection

Dakota Paine, Vincent Fazzari, and H. John Sharifi, PhD

We have previously reported that Sulforaphane (SFN), a natural compound found in cruciferous vegetables, protects macrophages from HIV infection. The exact mechanism of this protection is unclear. Here we show that pre-treatment of macrophages with SFN activates the restriction factor SAMHD1 in a dose-dependent manner. SAMHD1 is a cellular protein with potent anti-HIV activity. The ability of SAMHD1 to restrict HIV infection is regulated by phosphorylation of the protein. Here we show that SFN triggers removal of an inhibitory phosphate from SAMHD1 in a dose-dependent manner. Further, we identify several cellular proteins that potentially contribute to the dephosphorylation (activation) of SAMHD1. Some of these candidate proteins have been previously implicated in influencing SAMHD1 phosphorylation while others have not and may therefore represent novel contributors to SAMHD1 activation. Ultimately the goal of this work is to characterize previously unrecognized cellular pathways that can be manipulated to protect against HIV infection.

Return to agenda

Return to agenda

The antiviral protein SAMHD1 is a major driver of sulforaphane-mediated protection of macrophages from HIV

Vincent Fazzari, Dakota Paine, and H. John Sharifi, PhD

We have previously reported that Sulforaphane (SFN), a natural compound found in cruciferous vegetables, protects macrophages from HIV infection. In unpublished follow up work, we show that pre-treatment of macrophages with SFN activates the restriction factor SAMHD1 in a dose-dependent manner through removal of an inhibitory phosphate. SAMHD1 is a cellular protein with potent anti-HIV activity. The ability of SAMHD1 to restrict HIV infection is regulated by phosphorylation of the protein. SFN mediated removal of this inhibitory phosphate from SAMHD1 is therefore likely a major contributor to the protective effect of SFN against HIV. To test this, we employed macrophage cell lines devoid of SAMHD1 and found that SFN failed to protect these SAMHD1-deficent cells from HIV infection. These data, in combination with data showing the ability of SFN to promote SAMHD1 dephosphorylation, strongly implicate SAMHD1 activation as the major mechanism by which SFN protects macrophages from HIV infection. Ultimately the goal of this work is to characterize previously unrecognized cellular pathways that can be manipulated to protect against HIV infection.

Return to agenda

Return to agenda

Scanning Subclones to Identify Monocytic Cells that Maximal Produce Wild Type and Mutant SAMHD1

Alexandria Tipple, Robert Bautz, Dakota Paine, Vincent Fazzari, and H. John Sharifi, PhD

SAMHD1 impairs the infection of macrophages with HIV. SAMHD1 can exist in two different forms: the active dephosphorylated form or the inactive phosphorylated form. SAMHD1 can also be present in a tetramer form, which prevents reverse transcription by reducing the nucleotides that are required for the process. The genetic sequence that encodes for SAMHD1 was modified in ways that limit its ability to form certain disulfide bonds, or make it unable to be phosphorylated, or mimic permanent phosphorylation. The SAMHD1 gene was deleted from the THP1 monocytic cell line using CRISPR-Cas9 targeting. These SAMHD1 KO THP1 cells were then infected with viral vectors carrying either the wild type or modified SAMHD1 genetic sequences. The cells were then grown under selection, subcloned, and individual clones were scanned via western blot to identify those that maximally produce SAMHD1. Cells from wells identified as being able to produce the desired SAMHD1 were then further cultured and expanded. The ultimate goal of this work is to test the effect these alterations to SAMHD1 on HIV infection.

Return to agenda

Return to agenda

Review: The Biosynthesis of Recombinant Human Insulin by Escherchia coli

Alexis M. Parry, Alexa R. Boni, and Ehsan Mahdinia, PhD

Insulin is a hormone that is secreted by the pancreas which allows us to convert carbohydrates and fats into glucose so that our bodies can use it as energy. However, when the pancreas does not secrete enough insulin or when there is a problem with insulin production, the individual may suffer from a disease called diabetes. Type 1 Diabetes is a hereditary autoimmune disease which attacks the insulin-producing islet cells of the pancreas, therefore halting insulin production and resulting in the individual's need for exogenous insulin. Type 2 Diabetes is a disease of the metabolic processes and occurs when the glucose levels in the blood are too high, due to unhealthy eating habits and a sedentary lifestyle. In the early to mid 1900s, diabetes was treated by either a very restrictive diet, like starvation, or by giving the individual insulin derived from a pig or cow pancreas. Clearly each of these methods were not efficient and most people living with the disease did not survive very long. What started as giving patients pig insulin to treat their diabetes, has now evolved to treating diabetic patients with human insulin that is produced by recombinant strains of the bacteria, Escherichia coli. The main method for producing recombinant insulin from E. coli is by genetically engineering the human insulin gene and inserting it into a plasmid vector. Then the bacteria is transformed with the vector and grown under optimized conditions for both rapid growth and insulin production. The objective of this paper is to discuss the development of various production methods for insulin that have been made over the past few decades, with a focus on the optimized fermentation conditions which are used specifically in the bioprocessing industry for the production of human insulin. A brief analysis of the Human Insulin Drug Market will also be discussed.

Return to agenda

Return to agenda

Nuclear trafficking of cell death molecules during the hyperglycemic shift from apoptosis to necroptosis

Nafisa Arfan, William D. McCaig, Phillip Truong, and Timothy J. LaRocca, PhD

Necroptosis plays a significant role in the pathogenesis of several neurological conditions such as ischemia-reperfusion injury, including neonatal hypoxia-ischemia. Unlike apoptosis, necroptosis is a caspase-independent pathway of programmed cell death (PCD). Necroptotic molecules like MLKL (Mixed Lineage kinase domain-like) and RIP1 (Receptor-Interacting Protein 1) usually drive the process of necroptosis. Many necroptotic molecules accumulate in the nucleus could explain why necroptosis is coincident with inflammation. Reactive oxygen species (ROS) are one of the cell-damaging agents which are released from the mitochondria due to such accumulations, which further elevate the cell-destruction process of necroptosis. Our previous work has demonstrated that the assembly of necroptotic machinery occurs more readily during hyperglycemic conditions, thereby promoting necroptosis over apoptosis. Further investigation is required to understand whether the accumulation of specific death proteins (RIP1, RIP3 and MLKL), especially in the nucleus, manipulates the necroptotic pathway. Additionally, the mechanism by which the necroptotic molecules, especially RIP1, prepare the cells for necroptosis preferentially remains unclear. Evidences suggest that RIP1 may a play an essential role in gene regulation during hyperglycemia, while RIP3 and MLKL start to form signaling platforms that are later exported from the nucleus and into the cytosol. Therefore, we believe that the nuclear trafficking of specific death proteins is vital for necroptosis to occur. We will investigate the role of RIP1 and its kinetics using antioxidants as well as inducers of RIP1 in nuclear trafficking during the necroptotic shift. We will examine this using qPCR in RIP1 mutant U937 cells as well as subcellular fractionation.

Return to agenda

Return to agenda

Role of RIP1 in cellular trafficking during the hyperglycemic shift to necroptosis

Kevin R. Metz, Melanie Vugelman, Phillip V. Truong, Nafisa V. Arfan, Will D. McCaig, Payal S. Patel, Timothy J. LaRocca, PhD

Hyperglycemia has been shown to potentiate a shift to necroptosis in TNF-induced cell death. Increased levels of reactive oxygen species (ROS) interact with receptor-interacting protein 1 (RIP1) to facilitate downstream effects of necroptosis, including RIP3 phosphorylation and MLKL oligomerization. These downstream targets of RIP1 perform various actions during necroptosis, including membrane pore formation, ROS production, and mitochondrial disruption. In our present research, we determine the role RIP1 holds in the cellular trafficking of these necroptosis proteins.

Determining these relationships involved producing RIP1 knockout (KO) cells and non-targeting controls (NTC) via CRISPR-Cas9 genome editing. This was done in U937 monocytes as these cells are primed for necroptosis. After treating the cells in 10 mM and 50 mM concentrations of glucose enriched media, these cells were treated with TNF-α to induce programmed cell death. Fractionations were then performed to isolate the cytoplasm and mitochondrial samples. Protein levels were then assessed via western blotting; the cytoplasmic samples were normalized to housekeeping protein GAPDH, and the mitochondrial samples were normalized to gatekeeping mitochondrial protein VDAC.

The results begin by showing the successful KO of RIP1 and an increased presence of RIP1 in the cytoplasm and the mitochondria in the NTC. Firstly, the KO of RIP1 ended the movement of MLKL and p-MLKL to the mitochondria in the high sugar condition. These data also supported that the oligomerized pore-forming form of MLKL was trafficked to the membrane during necroptosis, as shown in a non-reducing gel. Next, due to the absence of RIP1, there was a reduction of Bak and Bax in the 50 mM in the mitochondria, there was a decrease in the activation of Drp1 and no movement to the mitochondria, and less cytochrome c was released. Similar to MLKL, there was a reduction in the oligomerization of Bak and Bax in the mitochondria, as shown on the non-reducing gel. Lastly, RIP1 did not affect caspase movement to the mitochondria, but its removal in the KO caused an increase in caspase activation in the cytoplasm.

Necroptosis is heavily dependent on the autophosphorylation of RIP1. The knocking out of RIP1 stops necroptosis shifting the mechanism of cell death back to apoptosis. Thus, we can see the effects RIP1 has on the trafficking of components during necroptosis. In our data, we demonstrated that RIP1 is associated with the movement of MLKL, p-MLKL, and o-MLKL to the mitochondria, suggesting a novel function for the protein in necroptosis. Additionally, the transfer of Drp1 to the mitochondria also seemed to function in a RIP1-dependent manner.

Return to agenda

Return to agenda

The role of VDUP1 in neural stem cells: analyses of the relationships between Brat, VDUP1, and Miranda

Andrew Fiorica and Richard Dearborn, PhD

Vitamin D3 Upregulated Protein 1 (VDUP1) also known as thioredoxin interacting protein TXNIP is a tumor suppressor protein that's expression in downregulated in certain types of cancer. VDUP1 actively binds to thioredoxin which reduces its ability to inhibit reactive oxygen species. It is also a redox protein that has numerous in the regulation of cellular homeostasis. Drosophila melanogaster is used for its fast reproductive systems along with its VDUP1 homolog that is similar to humans so the functions in the neuroblasts can be studied. VDUP1 is important because its expression and the role it plays in the central nervous system development. The relationship between VDUP1, Brat and Miranda within the NB genetic circuitry was looked at since both Brat and Mira have specific functions in these cells where VDUP1 does not. This was done through the immunostaining of Brat null mutants and through the clonal analysis of the VDUP1 loss of function in the developing CNS. When looking at Brat LOF resulted in the INP proliferation of the NB but with no differentiation. The phenotype of this mimic's tumorigenesis. This INP proliferation doesn't allow for differentiation of the cell so all the cells are the same cell. The VDUP1 LOF on Mira expression are seen to be colocalized in NB which raises a question of what their interaction together is. The next steps with this study is to find out whether Brat LOF disrupts the VDUP1 expression or that Brats expression is localized upstream of VDUP1. Also the identification of if VDUP1 expression is related to Mira expression or if it is upstream. If neither of these come to be true then the results would show that VDUP1 is independent of both Brat and Mira. The final thing that has to be done is for the images to be analyzed waiting on the confocal microscope.

Return to agenda

Return to agenda

Analysis of VDUP1 expression Patterns in Asence mutants and using hexameric GFP reporter constructs

Gianna Flint and Richard Dearborn, PhD

The tumor suppressor protein VDUP1 (Vitamin D3 Up-regulated Protein 1), also known as thioredoxin (TRX) interacting protein (TXNIP), has important clinical implications in tumorigenesis and various other pathological conditions. While VDUP1's role in modulating reactive oxygen species (ROS) via TRX, mediating apoptosis, and controlling cell-cycle progression are all well-studied, its function in stem cell biology has not been characterized. Using Drosophila melanogaster, a model in which VDUP1 has been shown to be critical to central nervous system development, we have explored the role of VDUP1 in neural stem cell proliferation and differentiation through two separate studies. In the first study, VDUP1-GAL4 lines, which should mediate gene expression in patterns mimicking VDUP1 expression were tested for their ability to drive expression of a GFP reporter gene. In parallel, the VDUP1-GAL4 lines were also used to drive VDUP1 gain-of-function and loss-of-function expression in normal VDUP1 developmental expression patterns. In the second study, we examined how VDUP1 expression changes in neural stem cells deficient for Asense, a pro-neural transcription factor whose expression shifts cells from a stem cell state to a differentiated state. Results from both studies, which rely on confocal analysis of immunocytochemical stained tissue, will provide insights into the functionality of VDUP1 in neural stem cells and validate tools for developmental studies in the future.

Return to agenda

Return to agenda

Adipsin and its downstream components as ER target genes

Daniel Galke and Kideok Jin PhD

In ER+ breast cancer, the expression of the estrogen receptor allows for the use of targeted endocrine therapies, however, some populations that undergo this treatment become endocrine resistant and can become metastatic as a result. Of this population, the ERα 1 (ESR1) gene was more frequently mutated than in non-resistant population. The most prevalent mutations, Y537S (YS) and D538G (DG), cause constitutive ER activity that is ligand independent. Adipsin is a complement system component and has been linked to increased proliferation and cancer stem cell-like properties in ER+ breast cancer when secreted by adipocytes. In our previous studies, the cytokine adipsin was found to be upregulated in the secretome of YS and DG mutant MCF7 and T47D cells by human cytokine array analysis. Adipsin mRNA and protein levels were also found to be elevated in these cells by RT-qPCR and ELISA. The complement components C3 and C3aR had increased mRNA levels and C3a had increased protein levels in all ESR1 mutants. Treatment of ESR1 mutants with the C3aR inhibitor SB290157 on cellular viability and cytotoxicity were assessed using a CyQUANT assay and flow cytometry with annexin-V staining. Cellular viability and apoptosis were significantly decreased in all ESR1 mutants compared to WT when treated with SB290157. ESR1 mutant cells were treated with SB290157 and tamoxifen (4-OHT) in combination which revealed an increased sensitivity of these cells to tamoxifen.

Adipsin and other alternative pathway complement components being ER target genes would provide a direct explanation for the upregulation of mRNA overexpression. In this study, we examined that the levels of adipsin, C3, and C3aR mRNA were assessed using RT-qPCR for MCF7-ESR1 mutant cells cultured in estrogen supplement and estrogen deprived media for 4 days. The results showed that the expression of adipsin, C3, and C3aR was significantly upregulated in YS and DG ESR1 mutant cells compared to the wild type of ESR1-MCF-7 cells in the estrogen supplement conditions. Furthermore, we found that the level of adipsin, C3, and C3aR mRNAs were significantly increased in YS and DG mutant cells in estrogen deprived media for 4 days while they were decreased in the wild type of ESR1 cells.

Return to agenda

Return to agenda

Identification and validation of secreted factors in the cross talk 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.

Return to agenda

Return to agenda

Crosstalk between stromal components and endocrine resistant breast cancer via secreted factors enhances tumor growth and metastasis

Sneha Pandithar and Kideok Jin

Breast cancer is currently targeted using various endocrine therapies that include use of Selective Estrogen Receptor modulators (SERM), Selective Estrogen Receptor Downregulator (SERD) and Aromatase inhibitors (AI). Tamoxifen (TAM) is a primary choice in treating early-stage estrogen receptor positive breast cancer in post-menopausal women. Despite the proven therapeutic efficacy and safety profile of TAM as a SERM, resistance to the drug and reoccurrence of tumor appears to be a complication that many patients deal with. Molecular pathways underlying the development of resistance is being widely studied. Tumor microenvironment is being perceived to play vital role in in multiple stages of disease progression, development of resistance, immune-escaping ability. Our previous work shows that the critical molecular and cellular components secreted from a crosstalk between breast cancer cells and stromal cells can regulate the tumor growth and process of metastasis in breast cancer.

We have established four different tamoxifen resistant breast cancer (TAMR) cells to simulate pre- and post-menopausal conditions. We screened the secreted factors from normal fibroblast co-cultured with TAMR cells using cytokine antibody arrays targeting 105 cytokines simultaneously and ranked the expression of each of the cytokines using real time qPCR. CXCL1 and IL-6 were our top candidates, which we further confirmed using U-Plex (MSD) assay. Our data showed increased proliferation of TAMR cells co-cultured with fibroblasts when compared to monoculture. To study the role of the cytokines in metastasis we studied cell migration of TAMR cells. We observed an increased migration of TAMR cells when cultured with tumor conditioned media-induced fibroblasts. Furthermore, TAMR cell migration, a key step in tumor metastasis, was promoted by conditioned medium (CM) from TCM-induced fibroblasts. Further, 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 also yielded in diminished growth and migration of the TAMR cells.

Return to agenda