Graduate Research Forum 2022

Abstract Booklet

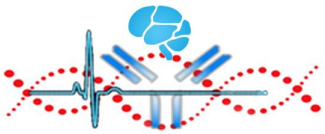
Council of Biomedical Graduate Students

March 16th and March 17th 2022

Presented by
UToledo College of Medicine and Life Sciences
and
UToledo College of Pharmacy and Pharmaceutical Sciences

COLLEGE OF MEDICINE AND LIFE SCIENCES
THE UNIVERSITY OF TOLEDO
Biomedical Science Program

BIOMEDICAL SCIENCE GRADUATE PROGRAM



THE UNIVERSITY OF
TOLEDO

COLLEGE OF PHARMACY AND PHARMACEUTICAL SCIENCES
THE UNIVERSITY OF TOLEDO

Contents

I Forum Program Information 1

Dean's Message 2

Welcome 3

About CBGS 5

Keynote Speaker 6

Keynote Speech 8

Program 9

Virtual Conference Links 10

Group Assignments 11

Guidelines 13

II Oral Presentations 15

The FDA-approved drug auranofin degrades IRF3 to prevent fatty liver diseases (*Shumin Fan*) 17

Targeting survivin to overcome gemcitabine resistance in pancreatic cancers (*Caoqinglong Huang*) 18

Characterizing Behaviors Induced by Bremelanotide in Female Mice (*Mitchell Harberson*) 19

Perturbation of Cell-subtype specific Active Kinome Networks in Schizophrenia (*Ali Imami*) 20

Platelet Responses Against Hypermucoviscous Klebsiella pneumoniae (*Iluja Gautam*) 22

Sepiapterin mediates immunogenic shift in breast cancer via reprogramming of Tumor–Associated Macrophages (*Veani Fernando*) 23

Beyond the Gastrointestinal Tract: Sex-Specific Skin Microbiota Are Associated With Hypertension in Rats With Genetic Disparities (*Xue Mei*) 24

RIKA, a new mechanism to prevent viral inflammation (*Sukanya Chakravarty*) 25

An examination of the Antiviral Properties of Retinoic Acid (RA) Treatment during Coronavirus infection (*Justin Franco*) 27

Targeting MCTs to overcome Enzalutamide resistance in prostate cancer (*Sayani Bhattacharjee*) 28

The Dark Kinome: Identification of Novel Substrates of Dark Kinases and Their Associations with Schizophrenia (*Khaled Alganem*) 29

Mechanisms by which Factor H protects Trypanosoma cruzi from the Alternative Pathway of Complement. (*Smrithi Menon*) 30

III Poster Presentations 31

Social Transmission of Negative Valence in Prairie Voles (*Daniella Pabon*) 33

Machine Learning as an Aid for Allergy Diagnosis using the Gut Microbiome (*Leah Stevenson*) 34

Investigate the role of RKIP in mammary gland development and neoplastic transformation (*Christopher Figy*) 35

Osteocytic PPARG supports prostate cancer growth in bone (*Emily Crowe*) 36

Assessing Factor H-Fc Fusion Proteins for Controlling Burkholderia pseudomallei Infection (*Kelly Morgan*) 37

Meta-Analysis of Human Renal-Transplant RNAseq Data (*Elizabeth Shedroff*) 38

Prosocial Consoling Behavior in Mice (*Farzaneh Naghavi*) 40

Harmful Algal Bloom Impacts on Human Health: An Analysis of National Emergency Department Data in the U.S. from 2016 to 2018 (*Kathryn Helminiak*) **41**

Developmental pesticide exposure in rodents as a model of Autism (*Nilanjana Saferin*) **42**

Differential Cytokine Expression Responses to Coronavirus Infection in Asthmatic Cells (*Benjamin French*) **43**

Vibrio cholerae Phage Shock Protein Response (*Kelli Devanna*) **44**

Identifying Transcriptional Gene Signatures of Suicide Across Psychiatric Disorders (*Evelyn Bates*) **45**

Inhibitory Neurons Within the Infralimbic-Medial Prefrontal Cortex (IL-mPFC) (*Nicole Bell*) **47**

Analysis of protein kinase activity though Kinopedia, an interactive application for kinome array data (*Alex Joyce*) **48**

Targeting the thioesterase of FASN using lansoprazole derivatives in triple negative breast cancer cells (*Shreyasi Ganguly*) **49**

Novel ligand of the Na/K-ATPase revealed by computational and experimental analysis (*Fathima Dhilhani Mohammed Faleel*) **50**

AMPK in Alzheimer's Disease: A Biochemical and Bioinformatic Intersection (*Nicholas Henkel*) **51**

Quinoline derivative, IND-2, potently inhibits prostate cancer cell proliferation by causing oxidative stress, inducing apoptosis, and inhibiting topoisomerase II (*Swapnaa Balaji*) **52**

Examination of Differential Cancer Rates in Schizophrenia: Identification of Novel Off Target Effects of Typical Antipsychotics (*Abdul-Rizaq Hamoud*) **54**

Incorporating Single Cell Transcriptomic Data into a Hippocampal Computer Model to Understand Epilepsy Pathophysiology (*Vineet Reddy*) **55**

Targeting Dynamics of 14-3-3-σ for the Treatment of Pancreatic Cancer (*Deepti Gurung*) **56**

Ketone body β-hydroxybutyrate is a novel epigenetic antihypertensive metabolite (*Juthika Mandal*) 57

High antioxidant environment in Peromyscus leads to tick-borne flavivirus attenuation. (*Ryan Harris*) 58

Development of LC-MS method to study whole body uptake and elimination of amphetamines and synthetic cathinones (*Kabita Gwachha*) 59

Dysregulated Kinase Networks in Major depressive Disorder (*Rawan Alnafisah*) 61

Identifying Candidate Loci Towards Correlates of Predictive Coding in C. elegans (*William Ryan*) 62

Novel effect of Selumetinib-mediated autophagy via HSF1 in K-Ras mutant pancreatic cancer (*Shruti Ghai*) 63

Dynamics of antimicrobial immune protein quantities in milk (*Ahmed Abokor*) 64

Aerosolized Harmful Algal Bloom Derived Microcystin-LR Exacerbates Inflammatory Mediators of Asthma in Asthmatic Human Airway Epithelium (*Joshua Breidenbach*) 65

Author Index 66

Part I

Forum Program Information

Dean's Message

Dr. Christopher J. Cooper, M.D.

The Health Science Campus Graduate Research Forum is a student-led forum organized by the Council of Biomedical Graduate Students since 1979. This forum has been instrumental in creating a collaborative atmosphere that allows students to share their research with their peers, while improving their own presentation skills.

Beginning in their second year, all students in the Biomedical Science Program are expected to participate either with oral talks or poster presentations of their research projects. The oral and poster presentations are judged by faculty and postdoctoral fellows, with 1st, 2nd, and 3rd place cash awards subsequently presented in each of the two presentation categories.

In addition, every year, the Council of Biomedical Graduate Students collaboratively chooses and invites distinguished scientific keynote speakers to share their stories and inspire our students. This year, the students admirably adapted to the current situation and turned the forum completely virtual. These student-led decisions are also wonderful examples of how the Council of Biomedical Graduate Students reinforces leadership qualities to our trainees.

Dr. Christopher J. Cooper, M.D.
Dean
College of Medicine and Life Sciences
University of Toledo

Welcome

Rachel Golonka

The Council of Biomedical Graduate Students (CBGS) has been organizing the Graduate Research Forum (GRF) in collaboration with the College of Pharmacy over the last four decades. GRF is a great platform for students and faculty to learn about the research being done in other tracks. This event provides important practice for students to explain their research to an audience outside of their field. Not only does this help the student develop better communication skills, but the awareness of ongoing projects at the University of Toledo can help foster internal collaborations.

As with everyone from 2020, CBGS has had to adapt in continuing this student-run forum in a virtual format. We are happy to see such remarkable participation for GRF 2022, including 58 students registered, 41 of those students giving either an oral or poster presentation, and 26 volunteer judges from faculty and postdoctoral fellows. We are also thankful for the 8 moderators that will be leading each poster and oral session. Based on my own personal experience and watching others live in the presentation moment, I have strong faith that everyone who participates as either a presenter, judge, or audience member will learn something new.

We are excited to have a keynote speech by Dr. Douglas A. Lauffenburger, who is a Ford Professor of Engineering at the Massachusetts Institute of Technology (MIT). Speaking about learning something new, Dr. Lauffenburger is going to provide some novel insight to the engineering and computational approaches in molecular biology that are advancing medicine. His speech will be a great eye opener and bring awareness about other techniques available that students can learn and apply to their research. I am thankful to Dr. Lauffenburger for graciously accepting our invitation and patiently supporting us while we figured out the several intricacies of this virtual format.

I am thankful for having such a brilliant team of graduate students without whom organizing this forum would have been impossible. Finally, I would like to thank Drs. Kandace Williams and David Giovannucci for guiding the council and providing us with their invaluable advice. I hope all attendees enjoy the forum and that our second virtual Graduate Research forum is a big success with hope that next year we will have this forum back to being live again.

Rachel Golonka
President
Council of Biomedical Graduate Students
University of Toledo

About CBGS

The University of Toledo Council of Biomedical Graduate Students consists of officers and representatives from the College of Medicine and Life Sciences and the College of Pharmacy and Pharmaceutical Sciences at the University of Toledo. This includes the Biomedical Science Graduate Program and related graduate programs in Pharmacy, and from the Center of Excellence in Biomarker Research & Individualized Medicine (BRIM) at the Health Science Campus.

The overarching goals of the council include:

- Facilitate discussion and collaboration among the graduate student body
- Represent the interests of BMSP and other graduate programs before UT Faculty, the Graduate Students Association and University Administration
- Organize social and professional events to enrich the graduate student life experience

We meet regularly, at least once per month, to discuss any current issues that need to be addressed and to plan and organize upcoming events. The meetings are open to all graduate students to encourage discussion of ideas and concerns pertaining to graduate student life. However, only elected members of the Council may vote during the meetings.

Annual events organized by the CBGS include

- Graduate Student Picnic A summer social event for new and current students
- Career Forum Held in autumn to help guide students for career decisions
- Graduate Research Forum Held during the spring semester to allow students to showcase their research and get helpful advice from faculty and fellow students

Visit us at http://www.utoledo.edu/med/grad/biomedical/cbgs/

Keynote Speaker

Dr. Douglas A. Lauffenburger, PhD

Dr. Douglas A. Lauffenburger is a Molecular Cell Bioengineer who is currently a Ford Professor at the Massachusetts Institute of Technology (MIT). He has dedicated 41 years to supervising research at the interface of engineering, quantitative cell biology, and systems biology with a focus toward integrating experimental/computational applications in various types of cancers (including breast, colon, lung, and pancreatic), inflammatory pathologies (such as Crohn's disease, rheumatoid arthritis, and Alzheimer's disease), and the immune system (mainly for vaccines against AIDS, malaria, tuberculosis, and Covid). A major focus of his research has been understanding the fundamental aspects of cell dysregulation, complemented by translational efforts in identifying and testing new therapeutic ideas.

Dr. Lauffenburger received his B.S. in Chemical Engineering from the University of Illinois and his Ph.D. in Chemical Engineering from the University of Minnesota. Shortly after arriving at MIT, Dr. Lauffenburger became the founding Head of the Department of Biochemical Engineering and served in that capacity from 1998 until 2019. His dedication to creating an innovative education program that prepares students to become future engineering leaders earned him the prestigious Bernard M. Gordon Prize for Innovation in Engineering & Technology Education from the National Academy of Engineering. Accordingly, more than 130 doctoral students and postdoctoral associates have undertaken research education under his supervision.

Over the past decade, Dr. Lauffenburger has increasingly focused on translating systems biology approaches to in vivo studies, including mouse models and human patients, as well as to more complex in vitro bioengineered systems. A major emphasis is in developing and applying a principled framework for translation between species, using *omics-*

based machine learning modeling frameworks to enhance the likelihood of success in moving potential treatments from pre-clinical through clinical stages. His research program is focused on receptor-mediated cell communication and intracellular signal transduction with application to drug discovery and development. The dedication Dr. Lauffenburger has is motivated by the goals to advance pharmaceutical and biotechnological companies and promote multi-disciplinary collaborative interactions between academia and industry.

Keynote Speech

Dr. Douglas A. Lauffenburger, PhD
Ford Professor of Engineering
Massachusetts Institute of Technology

1 PM – 2 PM

Click Here to Join

Program

Date	Session	Time	Notes
March 16th, 2021	Poster Session 1 Poster Session 2 Poster Session 3 Poster Session 4 Poster Session 5	10 AM – 12 PM	All Poster Sessions will happen simultaneously
	Oral Session 1	1 PM - 2 PM	
	Oral Session 2	2 PM - 3 PM	
	Oral Session 3	3 PM - 4 PM	
March 17th, 2021	Poster Session	10 AM – 11 AM	
	Oral Session	11 AM – 12 PM	
	Lunch	12:00 PM – 1:00 PM	Only for registered Students
	Keynote Speech	1 PM to 2 PM	
	Speaker Meet and Greet	2 PM to 3 PM	

Virtual Conference Links

Oral Presentations

- Oral Session 1: click here to join
- Oral Session 2: click here to join
- Oral Session 3: click here to join

Poster Presentations

- Poster Session 1 click here to join
- Poster Session 2 click here to join
- Poster Session 3 click here to join
- Poster Session 4 click here to join
- Poster Session 5 click here to join

Keynote Speech

- Keynote Speech click here to join
- Speaker Meet and Greet click here to join

Group Assignments

Oral Presentations

Group 1	Shumin Fan Caoqinglong (Jackson) Huang Mitchell Harberson Ali Imami
Group 2	Iluja Gautam Veani Fernando Xue Mei Sukanya Chakravarty
Group 3	Justin Franco Sayani Bhattacharjee Khaled Alganem Smrithi Menon

Poster Presentations

Group 1	Daniella Pabon
	Leah Stevenson
	Christopher Figy
	Emily Crowe
	Kelly Morgan
	Elizabeth Shedroff
Group 2	Farzaneh Naghavi
	Kathryn Helminiak
	Nilanjana Saferin
	Benjamin French
	Kelli Devanna
	Evelyn Bates
Group 3	Nicole Bell
	Alex Joyce
	Shreyasi Ganguly
	Fathima Dhilhani Mohammed Faleel
	Swapnaa Balaji
	Nicholas Henkel
Group 4	Abdul-Rizaq Hamoud
	Vineet Reddy
	Deepti Gurung
	Juthika Mandal
	Ryan Harris
	Kabita Gwachha
Group 5	Rawan Alnafisah
	William Ryan
	Shruti Ghai
	Ahmed Abokor
	Joshua Breidenbach

Guidelines

Virtual Oral Presentation Guidelines

- PowerPoint will be used for the oral presentations. There is no restriction for the number of slides per presentation and no restriction on the slide design. However, please make sure all text, figures and diagrams on the slides are clear and legible.
- Each oral talk will be given a maximum of 10 minutes for their presentation followed by a maximum of 5 minutes for questions.
- Each presenter will be given an assigned *Group* for which each group will be given a specific WebEx link for their presentation. When it is your turn for presenting, the moderator will allow you access to *Share Screen*.
- Practice the *Share Screen* function on the computer you plan to present the talk BEFORE the event day. It is advised to use a school computer because the University has good internet (home internet can sometimes unexpectedly fail).
- Judging will be based on knowledge of the subject, organization and clarity of the talk, conclusions supported by data, ability to finish the talk in allotted time, ability to answer questions, and overall presentation.

Virtual Poster Presentation Guidelines

- This year, the format of the virtual poster will be a *flash talk*. The presentation will comprise of no more than five slides of research information in a PowerPoint file (a Title Slide and an Acknowledgement Slide does NOT count toward the five slide limit). The flash talk is a condensed shortened version of an oral presentation and should contain the following information: background, methods/results, and conclusion. Please make sure all text, figures and diagrams are clear and legible.
- Each poster presenter will be given a maximum of 5 minutes for their presentation (1 minute per slide) followed by a maximum of 7 minutes for questions.

- Each presenter will be given an assigned "Group" for which each group will be given a specific WebEx link for their presentation. When it is your turn for presenting, the moderator will allow you access to *Share Screen*.

- Practice the *Share Screen* function on the computer you plan to present the talk BEFORE the event day. It is advised to use a school computer because the University has good internet (home internet can sometimes unexpectedly fail).

- The judging will be based on knowledge of the subject, explanation of background and significance, organization of poster, clarity of presentation, conclusions supported by data, ability to finish presentation in allotted time, ability to answer questions, and overall presentation.

Part II

Oral Presentations

Group 1

The FDA-approved drug auranofin degrades IRF3 to prevent fatty liver diseases

Oral-1

Shumin Fan

Shumin Fan, Sukanya Chakravarty, Anna Glanz, Ritu Chakravarti, Saurabh Chattopadhyay

Inflammation-induced cell death is an important pathologic hallmark in liver injury, involved in many liver diseases such as steatosis and hepatitis. Therefore, keeping inflammation under control and preventing excessive cell death is critical in slowing down the progression of liver pathogenesis such as fibrosis, etc. Interferon (IFN) regulatory factor 3 (IRF3) is well recognized as an antiviral factor and could induce the expression of a variety of antiviral genes such as Type I IFN, etc., it also fights against virus infection by activating RIG-I induced pathway of apoptosis (RIPA). However, dysregulated IRF3 is involved in excessive inflammation and pathogenic cell death. Evidence has shown that IRF3-induced inflammation and apoptosis are associated with exacerbated fatty-acid induced liver pathogenesis. We have identified an FDA-approved anti-rheumatic drug, auranofin, as an inhibitor of RIPA through a high-throughput screen. Our study indicated that auranofin also inhibits the transcriptional activity of IRF3. We further studied the mechanism by which auranofin inhibited the transcriptional and proapoptotic activities of IRF3 and revealed that auranofin activates the cellular autophagy pathway to degrade the IRF3 protein. Our study suggested that auranofin could reduce fatty-acid-induced hepatocyte cell death by using an in vitro model with palmitic acid (PA) treated human liver cells and uncovered the potential therapeutic use of auranofin against fatty liver disease. Moreover, given the involvement of IRF3 in the immune response against virus infections, the auranofin-activated autophagic degradation of IRF3 protein could confer protection against liver injury caused by hepatic viruses such as hepatitis A virus (HAV), hepatitis C virus (HCV), etc.

Targeting survivin to overcome gemcitabine resistance in pancreatic cancers

Oral-1

Caoqinglong Huang

Caoqinglong (Jackson) Huang, Jian-Ting Zhang

Pancreatic cancer is one of the deadliest diseases worldwide, and its 5-year overall survival rate is only 10%. Despite the advancement in immuno- and targeted therapy, traditional radiation and chemotherapy are still the major treatments for pancreatic cancers with gemcitabine as a first line chemotherapeutic drug for localized pancreatic cancer. However, gemcitabine resistance often develops, resulting in disease relapse. Thus, understanding the mechanism of this resistance and the development of novel approaches to overcome this resistance are clearly in dire need. Survivin, a member of the inhibitor of apoptosis family, has been shown to contribute to drug resistance by enhancing survival pathways, and inhibiting survivin has been thought to have a great attribute to enhance chemotherapeutics by inducing apoptosis of cancer cells. To meet the demand, we are investigating if survivin possibly contributes to gemcitabine resistance in pancreatic cancer cells. However, we found that survivin knockdown increased the sensitivity to gemcitabine in both MIA PaCa-2 and BxPC-3 pancreatic cancer cells. In those survivin-knockdown cells, we also observed down-regulation of Rad51, a DNA damage repair protein. Our goal is to determine whether this sensitization due to survivin knockdown can partly be explained by survivin regulating Rad51.

Characterizing Behaviors Induced by Bremelanotide in Female Mice

Oral-1

Mitchell Harberson

Mitchell T. Harberson, Jennifer W. Hill

Bremelanotide is a cyclic heptapeptide drug that was approved by the FDA in 2019 for the treatment of hypoactive sexual desire disorder (HSDD) in women. This exogenous peptide belongs to a larger drug class called melanocortins that activate melanocortin 4 receptors in the brain. One of the best studied melanocortins, Melanotan-II, induces desirable effects like increasing sexual desire for the treatment of HSDD and reducing food intake for the treatment of obesity; however, it has many side effects in rodents and humans including yawning, stretching, and grooming. Not only have these behaviors never been studied using bremelanotide, but they have never been studied together to compare the doses at which these behaviors occur. This study aims to establish a dose-response relationship between bremelanotide and each of its behaviors in female mice. Wild-type, female mice that have been ovariectomized will be estradiol- and progesterone-primed prior to all matings. After four practice pairings to gain sexual experience, females will receive a drug treatment (Saline, 3 mg/kg melanotan-II, or bremelanotide (0.05, 0.3, 2, or 10 mg/kg)) moments before each of their six sexual studies. These studies are "paced," meaning that females will be allowed to choose how much time they spend with the male. Paced experiments gauge sexual receptivity by analyzing the frequency that females leave the male chamber after mounts, intromissions (penile insertion), and ejaculation. Lordosis will also be studied. Additional filmed studies will analyze the frequency of yawning, stretching, grooming, and feeding after drug treatment. We expect that bremelanotide will improve sexual receptivity at lower concentrations (0.05-0.3 mg/kg) but will decrease receptivity at higher concentrations (2-10 mg/kg) due to the onset of excessive grooming, scratching, and yawning. We also expect feeding will gradually decrease with increasing bremelanotide concentrations.

Perturbation of Cell-subtype specific Active Kinome Networks in Schizophrenia

Oral-1

Ali Imami

Ali S. Imami, Elizabeth Shedroff, Alex W. Joyce, Smita Sahay, Khaled Alganem, William G. Ryan, Nicholas D. Henkel, Robert E. McCullumsmith

Schizophrenia is an illness with significant consequences for patients and their families. Since the disease affects executive function, the Dorsolateral Pre-frontal Cortex (DLPFC) has been an area of interest. We previously reported perturbed protein kinase activity of kinases, including AKT, in postmortem brain samples of patients with schizophrenia. We are now extending this hypothesis to the cell level, with a focus on frontal cortical pyramidal neurons. We used laser capture micro dissection to isolate DLPFC pyramidal neurons from matched pairs of schizophrenia and control PM brain samples (n = 20). We then utilized the PamChip STK kinome array assay for high throughput characterization of kinase activity in the samples. We then deployed well-characterized bioinformatics approaches to identify upstream kinases implicated in schizophrenia. We identified several kinases of interest, including c-Jun N-terminal kinases (JNK), extracellular signal-regulated kinases (ERK) and p38 mitogen-activated protein kinases (P38). These kinases belong to the mitogen-activated protein (MAP) kinase family and are associated with inflammatory responses. We then applied a novel technique of identifying the kinase interaction networks in high throughput kinase activity data. This modelling allowed us to identify the network-level changes in pyramidal neurons in schizophrenia. This is the first study to examine the subkinome at the cellular level in schizophrenia and to identify active kinome network changes in this often-devastating illness. Our data provides an important new starting point for understanding how signaling networks are perturbed in severe mental illness.

Group 2

Platelet Responses Against Hypermucoviscous Klebsiella pneumoniae

Oral-2

Iluja Gautam

Iluja Gautam, Chadwick Huss, Zachary Storad, Michelle Krebs, Leah M. Wuescher, Randall G. Worth

Klebsiella pneumoniae is an opportunistic pathogen widely known for its ability to cause infections in hospital acquired settings. With bacterial evolution to evade host immune system, hypervirulent strains of K. pneumoniae are emerging which can cause severe infections in immunocompetent individuals. Combined with the increase in antibiotic resistance, it is important to understand how K. pneumoniae affects components of the immune system. We studied the interactions of human platelets with hypervirulent, encapsulated strain and non-hypervirulent, non-capsulated strain of K. pneumoniae. A significant thrombin-dependent bacterial growth inhibition was observed for both strains in human blood. Similar experiments showed the thrombin-dependent killing was due to the presence of platelets. K. pneumoniae killing assays were then repeated with thrombin-stimulated or unstimulated washed platelets to show that platelets cannot directly kill K. pneumoniae. Furthermore, we investigated the actions of K. pneumoniae on platelet activation. Non-capsulated strain was shown to exert a significant increase in activation of both unstimulated and thrombin stimulated human platelets. However, the encapsulated strain could only enhance the response of thrombin stimulated human platelets. Additionally, only the non-capsulated strain increased the rate of platelet aggregation in response to ADP. Overall, this study highlights the role of platelets in mediating a protective response against K. pneumoniae. It also reinforces the importance of capsule in bacterial pathogenicity.

Sepiapterin mediates immunogenic shift in breast cancer via reprogramming of Tumor–Associated Macrophages

Oral-2

Veani Fernando

Veani R. Fernando, Xunzhen Zheng, Vandana Sharma, Saori Furuta

Immunotherapy is an emerging therapeutic strategy for many types of cancer. Yet, it yields only moderate responses in breast cancer owing to its immunosuppressive tumor microenvironment (TME). Tumor–Associated Macrophages (TAMs), major resident cells in the TME, contribute to such immunosuppressive milieu, in particular by acquiring the immunosuppressive M2 type rather than the immunogenic M1 type. Thus, reprogramming M2 towards M1 TAMs would induce an overall immunogenic shift in TME and could serve as an immunotherapeutic strategy. To this end, we sought to alter arginine metabolism since differential usage of arginine greatly contributes to the phenotypic difference between M1 and M2 TAMs. M1 TAMs utilize arginine to produce nitric oxide (NO) that boosts anti–tumor immunity, while M2 TAMs produce polyamines that induce pro–tumorigenic responses. Such difference is due to the reduced synthesis of tetrahydrobiopterin (BH4), the essential cofactor of NO synthase (NOS), in M2 TAMs. In this study we supplement M2 TAMs with Sepiapterin (SEP), the endogenous precursor of BH4, to redirect their arginine metabolism towards NO production and to reprogram them to M1 TAMs. Our in vitro (cell line and primary cell derived) results showed that supplementing SEP restored NO production in M2 TAMs and led to a significant decrease in M2 specific markers while increasing M1 specific markers. SEP–treated M2 TAMs produced higher level of IL12 (M1–TAM specific) and lower level of IL10 (M2–TAM specific). Furthermore SEP-treated M2 TAMs demonstrated the increased capabilities of antigen presentation and cytotoxic T cell proliferation. The results corroborate that SEP treatment induces an immunogenic shift in the breast TME through metabolic reprogramming of M2 TAMs to M1. These findings strongly suggest that SEP treatment could be utilized as a safe and efficient immunotherapeutic strategy for breast cancer.

Beyond the Gastrointestinal Tract: Sex-Specific Skin Microbiota Are Associated With Hypertension in Rats With Genetic Disparities

Oral-2

Xue Mei

Xue Mei, Blair Mell, Xi Cheng, Ji-Youn Yeo, Tao Yang, Nathaline Chiu, Bina Joe

Current knowledge of microbiota in hypertension is restricted to the gut and mostly to males. Other than the kidney, the skin is the largest organ playing a role in sodium homeostasis and the mouth is the first site exposed to dietary salt, whereby, we hypothesized that microbiota in these sites associate with salt-sensitive blood pressure (BP). To test this hypothesis, we compared microbiota from the skin, mouth and feces of both male and female young Dahl salt-sensitive (S) rats and 4 genetically disparate congenic strains (RNO1, RNO5, RNO9 and RNO10). Skin microbiota was from 4-6 days old rats before they developed their coats. Oral and fecal samples were collected at 4 weeks old. Microbiota were profiled by 16S rRNA sequencing. Compared to the gut, which is an anerobic site, microbiota from the aerobic skin and oral sites were consistently different in composition across all the strains. Unlike in the fecal samples where Firmicutes and Bacteroidetes were enriched, Cyanobacteria and Actinobacteria were enriched in the skin and oral samples, respectively. Female S rats had greater abundance of skin Cyanobacteria than male S rats. Strain effects were not observed in any of the strains or sites except for RNO5, where a strain $\times$ sex $\times$ site interactive effect was specifically noted for skin Cyanobacteria. Cyanobacteria contain sodium transporters and are salt-tolerant microbiota, whereby they could potentially regulate sodium excretion and contribute to BP regulation. In summary, our study is the first to discover microbiota beyond the gut as being associated with BP.

RIKA, a new mechanism to prevent viral inflammation

Oral-2

Sukanya Chakravarty

Sukanya Chakravarty, Sonam Popli, Ritu Chakravarti, Saurabh Chattopadhyay

The COVID-19 pandemic has shown us that in addition to viral load, our body's fight for survival can be fatal for us. Virus infection activates our immune system resulting in the recruitment of various immune cells that secrete proteins, called cytokines, that cause inflammation to fight off the infection. However, a delicate balance in the secretion of cytokines is critically important to avoid an overreaction of the host inflammatory response. An unwanted surge in the body's inflammatory response to virus infections can result in fatal cytokine storms, a manifestation of viral pathogenesis and independent of the viral load. This has been a major cause of mortality during the recent COVID-19 pandemic. We study respiratory virus infections and have uncovered a novel cellular mechanism to regulate viral inflammation, RIKA, the Repression of IRF3-mediated NF-κB Activity. Using the knockout cells and mice, we demonstrate that RIKA inhibits the inflammatory signaling pathways and prevents the production of the pro-inflammatory cytokines in response to respiratory virus infections. The presentation will highlight the molecular mechanisms of RIKA and its role in preventing viral disease.

Group 3

An examination of the Antiviral Properties of Retinoic Acid (RA) Treatment during Coronavirus infection

Oral-3

Justin Franco

Justin H. Franco, Zhixing K. Pan

The ongoing Coronavirus Disease 2019 (COVID-19) pandemic has revealed the limit of currently available antiviral therapeutics. With over 5 million case fatalities globally, viral sepsis represents a significant complication of Severe COVID-19 and a major cause of patient morality. Previously examined in the context of bacterial sepsis, all trans-retinoic acid (RA) exhibits both antiviral and immunomodulatory effects. When applied to L929 mouse fibroblast cells infected with a related coronavirus, mouse hepatitis virus strain A59 (MHV-A59), RA treatment significantly attenuated viral replication. The upregulation of various IFIT proteins was also seen during RA treatment, highlighting a novel signaling pathway whereby RA directly promotes the activity of the Type I interferon (IFN) response. Initiation of the Type I IFN response is necessary for clearance of viral pathogens especially in a coronavirus infection where the antiviral response is inhibited by viral proteins. Further study of RA treatment in the context of viral sepsis will elucidate whether it may be efficacious as a novel antiviral against coronavirus disease.

Targeting MCTs to overcome Enzalutamide resistance in prostate cancer

Oral-3

Sayani Bhattacharjee

Sayani Bhattacharjee, Jonathan Doan, Rebecca Wynn, Nagalakshmi Nadiminty

Prostate cancers (PCa) exhibit a unique metabolic profile with reliance on different forms of glucose metabolism at different stages of disease progression. Early stage PCa cells use the more efficient TCA cycle, while metastatic PCa cells switch to glycolysis (Warburg effect), leading to the accumulation of lactate. Lactate is exported out of the cells by monocarboxylate transporters (MCTs) to maintain redox balance. Such metabolic reprogramming can lead to gain-of-function mutations and affect drug sensitivity. Enzalutamide is a second-generation antiandrogen used for the treatment of metastatic castration-resistant PCa. However, resistance to Enzalutamide develops in most patients within 9-15 months. Since Warburg effect is a hallmark of metastatic PCa, we sought to understand whether lactate transport has any effect on Enzalutamide-resistance in prostate cancer. We measured the expression of MCTs in parental and Enzalutamide-resistant C4-2B and 22RV1 cells and found that they are overexpressed in the Enzalutamide resistant subtypes. Based on this observation, we hypothesized that targeting lactate transport might be a potential strategy to overcome Enzalutamide-resistance in prostate cancer cells. Using cell survival and cell proliferation assays, we found that MCT antagonists resensitized Enzalutamide resistant C4-2B and 22Rv1 cells to treatment with Enzalutamide. Using the Seahorse based glycolytic rate assay we found that these combination treatments significantly reduced the extracellular acidification rate by reducing the level of glycolysis in the Enzalutamide-resistant prostate cancer cells. We also found that treatment with these antagonists either singly, or in combination with Enzalutamide suppressed xenograft growth in SCID mice bearing subcutaneously injected parental or Enzalutamide-resistant C4-2B cells. These findings led to the conclusion that targeting MCTs could be an attractive strategy to overcome Enzalutamide resistance in prostate cancer.

The Dark Kinome: Identification of Novel Substrates of Dark Kinases and Their Associations with Schizophrenia

Oral-3

Khaled Alganem

Khaled Alganem, Abdul-Rizaq Hamoud, Nicholas D. Henkel, William G. Ryan, Justin F. Creeden, Michael Morran, Robert E. McCullumsmith

There are over 500 protein kinases in the human genome. Protein kinases have been the center of research efforts that span several decades to investigate their structure and function. As a result, protein kinases are well known for their involvement in many critical biological processes and have strong associations with several diseases. However, research shows that there are some kinases that are studied more than others. Thus, there is a subset of kinases that are regarded to be understudied, and these are titled to be "dark" kinases. Recent research efforts, from different consortiums and institutions, have been aiming to "illuminate" these understudied kinases and explore their roles in various diseases and develop drugs to target these kinases. Here we use a "functional" approach to extend the current knowledge of five "dark" tyrosine kinases utilizing a multiplex functional kinase activity profiling platform (PamStation12). We use purified recombinant kinases, with different protein concentrations, to identify novel kinase-substrate interactions. The scale of protein concentrations allows us to map different degrees of affinity between kinases and reporter substrates printed on the PTK PamChip4. Using these newly identified substrates, we perform a pathway analysis followed by amino acid sequence motif analysis. To explore the associations of these "dark" kinases with schizophrenia, we query several publicly available databases to examine the expression level and regulation of these kinases across brain-focused and schizophrenia-related signatures. These databases include GTEx, Brain Atlas, Kaleidoscope, and iLINCS. We also examine the enrichment scores of these newly identified substrates compared to schizophrenia kinome signature.

Mechanisms by which Factor H protects Trypanosoma cruzi from the Alternative Pathway of Complement.

Oral-3

Smrithi Menon

Smrithi S. Menon, Galia Ramirez-Toloza, Keith L. Wycoff, Jutamas Shaughnessy, Sanjay Ram, Viviana P. Ferreira

Chagas disease is a neglected tropical disease caused by the protozoan Trypanosoma cruzi (T. cruzi). Complement is a critical arm of the immune system. Various pathogens, including T. cruzi, hijack host regulators such as Factor H (FH) to inactivate complement. FH regulates the alternative pathway (AP) of complement in blood and on cell surfaces. FH contains 20 short consensus repeat (SCR) domains and binds to complement activation products (i.e., C3b/C3d) or polyanionic host cell markers (e.g., sialic acid, glycosaminoglycans) on cell surfaces, and uses its 4 N-terminal SCRs to inactivate the AP. T. cruzi potentially binds FH by transferring host sialic acids onto its surface using its trans-sialidase enzyme. This study asked whether FH binds directly to T. cruzi in the absence of C3b and defines the FH domains involved. Only trypomastigotes (infective forms, highly resistant to complement), but not epimastigotes (non-infective forms, sensitive to complement), bound FH directly in a dose-dependent manner. Although SCR 19-20 was hypothesized as important for this interaction given it is the only known sialic acid-binding region, domain mapping indicated SCR 5-8 competitively inhibited FH binding by >35% with lower contributions of other regions. FH related protein-5 (FHR-5), whose SCRs bear sequence homology to all known polyanion-binding regions (6-7, 10-14, 19-20), fully competitively inhibited FH binding to trypomastigotes. In addition, competitive survival experiments with serum and FHR-5 led to >80% killing when the AP or all complement pathways were active. This is the first study to show consequences of completely inhibiting FH protection on T. cruzi and to suggest multiple FH domains are involved in the interaction. These data may be harnessed for developing therapeutics and vaccine candidates against T. cruzi.

Part III

Poster Presentations

Group 1

Social Transmission of Negative Valence in Prairie Voles

Poster-1

Daniella Pabon

Daniella G. Pabon, Shivangi Patel, James Burkett, Elissar Andari

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by significant deficits in social interaction, social communication, and repetitive behaviors. Social deficits encompass dysfunctions in social recognition, empathy, and social learning. In this study, we used prairie voles which are highly social species characterized by long-term affiliations and monogamous bond formation with partners. Here, we are developing a social learning behavioral assay inspired by the social transmission of fear paradigms used in rats. Social transmission of negative valence is a two-day paradigm that assesses social learning in the prairie voles while watching their partner being stressed because of fear conditioning. We assess social learning by freezing behavior during social conditioning. We also measure partner-directed grooming as a proxy of consoling behavior in voles. Our results indicate that observers show increased freezing to the tones after being exposed to the stressed partner compared to control observers who were exposed to the non-stressed partner. Also, prairie voles show increased partner-directed grooming towards the stressed partner following fear conditioning. Lastly, we tested the observer's anxiety levels in the open field test, a highly recognized behavioral paradigm following social learning. We were able to find a significant decrease in levels of zone entries, movement, and exploratory behavior in socially stressed observers compared to the control. These preliminary findings suggest that prairie voles can be socially conditioned via the stress of the partner and that fear and stress of the conditioned partner can be transferred to observers in different facets, such as fear and anxiety. In future experiments, we foresee increased c-fos activity in social learning areas such as the amygdala, anterior cingulate cortex, and anterior insula during the social learning event in prairie voles. Our work provides new outcome measures to leverage the study of social cognition in rodents and pharmaceutical therapies.

Machine Learning as an Aid for Allergy Diagnosis using the Gut Microbiome

Poster-1

Leah Stevenson

Leah Stevenson, Sachin Aryal, Ishan Manhandar, Sanjay Koka, Xi Cheng, Bina Joe.

The current most effective of diagnosing a food allergy is a food challenge test. A food challenge test is when a patient is given small amounts of the suspected allergen then monitored for a reaction. This can cause both physical and emotional discomfort to the patients as an allergic reaction is purposely being induced. The key to improved food allergen diagnostics may lie in the gut as a means to decrease the discomfort that a patient experiences during testing. It has been observed that there is a decreased richness in the gut microbiota of individuals with specific allergies, and this study moves to expand upon these findings. This study aims to utilize Machine Learning algorithms and Artificial Intelligence (A.I.) to fabricate a diagnostic aid to analyze the gut microbiome and allergy association. The data analyzed was obtained from the American Gut Project (AGP) collection of participants' gut microbiomes. The data used from the AGP was filtered to only include data from the allergies being studied. We included control data for the different allergies along with the data that included allergies. This data was run in a Machine Learning program that separated the data into 70

Investigate the role of RKIP in mammary gland development and neoplastic transformation

Poster-1

Christopher Figy

Christopher Figy, Paige Cubberly, Taylor Conrad, Claire Tipton, Miranda Yeung, Kam Yeung

Background: Raf Kinase Inhibitor Protein (RKIP) has been identified as a metastatic suppressor of multiple cancers in cancer cell mouse transplantation models. However, the use of transformed cancer cell lines does not empower these models to assess the impact on cancer initiation and progression upon the loss of RKIP. Methods: To investigate the role of RKIP in early steps of cancer transformation and progression, we generated RKIP germ line conditional knockout mice (RKIPf/f) and studied the effect of loss of RKIP on neoplastic transformation. Because of our past experience, we first focused on breast cancer. Results: Animals with RKIP deficiency in mammary epithelial cells (RKIPf/f;MMTV-cre) were viable and appeared normal up to 18 months of age. Conclusions: Since the RKIPf/f;MMTV-cre mice were viable until the age of 18 months, we concluded that in the absence of other tumor-promoting mutations RKIP deficiency alone is not sufficient to induce breast cancer within the lifetime of mice. We therefore hypothesized that RKIP may play a regulatory role inhibiting breast cancer initiation and progression. To test this hypothesis we examine the effect of loss of RKIP expression in mammary glands on tumorigenesis in genetically engineered mouse breast cancer models. Several mouse models of breast tumorigenesis based on gain-of-function oncogenes have been described. We begin to study the effect of mammary gland specific knockout of RKIP on the progression of breast cancer initiated and driven by polyoma middle T antigen (PyMT). PyMT breast cancer model (also known as MMTV-PyMT model) is robust, with a high degree of tumor penetrance. Our study allows us to investigate if loss of RKIP in mammary epithelial cells impacts primary tumor development in a transgene mouse model of breast cancer. In addition, the specific deletion of RKIP alleles from mouse mammary glands also provide us with a handle to investigate whether RKIP loss influences mammary epithelial duct development.

Osteocytic PPARG supports prostate cancer growth in bone

Poster-1

Emily Crowe

Emily Crowe, Piotr Czernik, Sudipta Baroi, Izabela Podgorski, Beata Lecka-Czernik

Bone, the most common site of prostate cancer (PCa) metastasis, provides a favorable microenvironment for malignant cancers to grow. Previous studies have focused on two bone remodeling cells, osteoclasts and osteoblasts, in regard to PCa colonization and proliferation at skeletal sites. However, little is known about the most abundant cells in this process, osteocytes (OTs). Here we observed that osteocytic peroxisome proliferator activated receptor gamma (PPARG) protein plays an important role in the tumor growth and bone destructive phenotype of PCa bone metastasis. Tibiae of WT mice injected with PCa cells (RM-1) displayed tumor burdens, bone destruction, and loss of marrow adiposity. In contrast, mice with osteocyte-specific deletion of PPARG (Dmp1CrePpar γflfl or γOTKO) were protected from these effects. Transwell-coculture (CC) experiments of CRISPR/Cas9 PPARG-edited osteocytic MLO-Y4 (Y4-γKO) cells with RM-1 cells revealed that expression of pro-inflammatory and pro-osteoclastic factors, PGES and COX2, decreased in OTs deficient in PPARG. Further, cytokine array analyses of conditioned media collected from Y4-γKO cells grown in CC conditions with RM-1 cells (CM-CC), showed a reduction in levels of IL11 and LIF cytokines, previously recognized for their activities supporting bone resorption. Moreover, proliferation assays using CM-CC collected from Y4-γKO cells indicated a decreased RM-1 cell growth rate as compared to CM-CC collected from unedited MLO-Y4 cells. Indeed, the cytokine array analyses of CM-CC from Y4-γKO cells revealed a reduction in cytokines CXCL5 and GDF-15 which have been found to support PCa cell proliferation. These findings suggest that osteocytic PPARG controls tumor growth and bone destruction in PCa bone metastasis. Therefore, osteocytic PPARG, as a pertinent pharmacological target, should be considered for treatment of PCa in bone.

Assessing Factor H-Fc Fusion Proteins for Controlling Burkholderia pseudomallei Infection

Poster-1

Kelly Morgan

Kelly Morgan, Mark Wooten

Burkholderia pseudomallei is the causative agent of the disease melioidosis, a major cause of septic death. As a Tier 1 select agent, B. pseudomallei has potential as a bioweapon. Melioidosis cases are rising globally, but no vaccines or antibiotic treatments exist for this disease due to the bacteria's robust immune evasion strategies. Previous work in our lab has shown B. pseudomallei to survive in normal human serum, indicating a resistance to complement, an innate immune response of cascading proteins activated by antibodies and sugars. Complement is tightly regulated in the host by Factor H (FH), a chain-like protein that many bacteria recruit to evade opsonization. Opsonization tags the pathogen for destruction by phagocytes and complement-mediated direct killing by formation of membrane pores on the bacteria surface. We have identified Factor H (FH)-binding proteins in B. pseudomallei's genome. FH-Fc fusion proteins, which are constructs engineered to conjugate particular domains of FH to the Fc region of IgG, have been shown to control infections of bacteria expressing FH-binding proteins by outcompeting native FH and promoting complement activation with the Fc portion. This in vitro therapeutic study will assess FH-Fc binding by B. pseudomallei after incubating cultured bacteria with various FH-Fc constructs. Binding assays are performed via flow cytometry and analyzed by mean fluorescence intensity after detecting FH-Fc-bound bacteria by rat-anti-human IgG Fc-FITC. Indirect ELISAs are performed in tandem within a 96-well plate and analyzed at an absorbance at 490 nm. We hypothesize that B. pseudomallei will bind to various FH-Fc constructs, and our follow-up studies will show Fc-mediated activation of classical complement, opsonization, direct killing via formation of pores on B. pseudomallei's surface, and opsonophagocytosis by human neutrophils. This therapeutic study would expand the repertoire of pathogens FH-Fc constructs are effective against and facilitate development of therapeutics to control B. pseudomallei infection.

Meta-Analysis of Human Renal-Transplant RNAseq Data

Poster-1

Elizabeth Shedroff

Elizabeth Shedroff, William G. Ryan, Ali S. Imami, Jarek Meller, Lance Dworkin, Puneet Sindhwani, Nagalakshmi Nadiminty, Kunal Yadav, Stanislaw Stepkowski, Robert E. McCullumsmith

Patients with End-Stage Renal Disease (ESRD) and/or Chronic Renal Failure (CRF) are often dependent on lifelong dialysis or renal transplantation for survival. Although transplantation improves quality of life, it comes with a risk of rejection or chronic renal dysfunction. In order to better understand this risk, we performed a meta-analysis to examine the differential gene expression (DEG) signatures of renal transplant transcriptomic datasets to understand the variability in rejection rates and the effects of immunosuppression on these signatures. Standard pathway analyses typically do not integrate multiple tools, and only consider the most dramatic changes in gene expression. This transcriptomic study allows for a more elaborate pathway analysis that can account for subtle changes in gene expression, granting a deeper level of understanding into the mechanics of organ rejection. We used a '3-Pod' approach to analyze data obtained from the NCBI Gene Expression Omnibus (GEO) database as well as supplementary datasets from literature. This approach involves a combination of GSEA, enrichR, and iLINCS for both transcriptomic and targeted pathway analysis as well as perturbagen analysis. By utilizing this 3-Pod transcriptome pathway analysis approach, we hope to observe expression level and activity differences in renal transplant blood and tissue samples as they relate to functional proteomics, particularly kinomics. This study may suggest that an active kinome profile will reveal kinomic biomarkers for rejection/acceptance/drug response in PBMCs. Our transcriptomic analysis allows for a more elaborate pathway analysis that can account for subtle changes in gene expression, granting a deeper level of understanding into the mechanics of organ rejection.

Group 2

Prosocial Consoling Behavior in Mice

Poster-2

Farzaneh Naghavi

Farzaneh Naghavi, James Burkett

The term empathy is used for any type of process in which one is affected by the emotional state of others and for more than a century it was applied only to describe high-level, cognitive phenomenon in humans. However, recently, numerous studies on non-human animals suggested that empathy might be a phylogenetically continuous phenomenon which has varied presentations across a wide range of species. Burkett et al. (2016) demonstrated that prairie voles (Microtus ochrogaster) have capacity for empathetic behavior. Allogrooming of a stressed vole by an unstressed observer was characterized as a consolation behavior since this behavior shared similar behavioral characteristics and conserved biological mechanisms with human empathy. So far, this phenomenon has been studied in voles but has not as widely been researched in other rodents. Here, in this study we hypothesized mice also exhibit allogrooming as a comforting behavior toward stressed partner. Mice were habituated to human handling and to the behavioral procedures for three consecutive days. On the test day, the partner was removed from the home cage and transferred to a foot-shock chamber and received 20 foot shocks (0.7 mA, 1s) with a random interval between 20 and 40 s, before being returned to the home cage. Baseline was recorded for 30 minutes prior to removal and reunion was recorded for 10 minutes after return. Although in normal situation mice exhibit only occasional allogrooming towards unstressed partners, our results showed that this behavior was substantially increased towards distressed partners. This increase in allogrooming was observed in both male and female subjects.

Harmful Algal Bloom Impacts on Human Health: An Analysis of National Emergency Department Data in the U.S. from 2016 to 2018

Poster-2

Kathryn Helminiak

Kathryn Helminiak, Joshua D. Breidenbach, Sadik Khuder, Steven T. Haller, David J. Kennedy

Numerous harmful algal bloom (HAB) cyanobacterial species produce toxins that disrupt ecosystems and are harmful to both human and animal health. We sought to determine trends and patterns in diagnostic codes relating to HAB exposures from the Healthcare Cost and Utilization Project's (HCUP) Nationwide Emergency Department Sample (NEDS). We analyzed years 2016 to 2018 as these represented the years in which complete data was available using the World Health Organization (WHO) International Classification of Diseases-10 diagnosis codes for HAB exposure. For each year's grouping, statistical analysis was performed to uncover patterns and trends. Each patient occurrence was screened for the most prevalent comorbidities associated with HAB exposures. Over the 3-year period studied, there were 118 reported patient admissions to the Emergency Department. Respiratory related illness accounted for the majority of comorbidities and were present in 53% of patients, including 30% as the primary diagnostic code. These data represent one of the first attempts to analyze HAB exposure related illness presenting to Emergency Departments in the United States. The predominance of respiratory related diagnostic codes in these patients suggests greater attention to these conditions in the risk characterization of HAB exposure in the development of evidence-based prevention and treatment strategies.

Developmental pesticide exposure in rodents as a model of Autism

Poster-2

Nilanjana Saferin

Nilanjana Saferin, Melissa A. Curtis, Khaled Alganem, Justin F. Creeden, Rammohan Shukla, James Burkett

Deltamethrin is a synthetic type II pyrethroid widely used worldwide in agriculture, aquatic systems, household pest control etc. It is a potent neurotoxin for insects, however the effect of chronic exposure in humans are poorly understood. Recent studies demonstrated potential adverse effects of deltamethrin in mammalian brain and harmful bioaccumulation in other organs including the liver, kidneys, and reproductive organs. Epidemiological studies have shown that pregnant women exposed to pyrethroid pesticides are at a greater risk for their unborn child to be later diagnosed with autism. Pyrethroid exposure studies in mice have demonstrated deficits in communication and learning, hyperactivity, and repetitive behavior which are representative of neurodevelopmental disorders such as Autism, ADHD, and Developmental Delay (DD). These behaviors in mice were causally related to changes in dopamine transporter (DAT) expression in the striatum, accompanied by increases in dopamine uptake. Previously our lab has studied the effect of developmental exposure to low dose deltamethrin in mice to explore behavioral changes, followed by a transcriptomics and kinomics approach to explore its effects at a gene transcription and proteomic level. The study showed differential expression of kinases and small molecule metabolites that are important in metabolic pathways such as biosynthesis of Folic acid, 5-hydroxytyptamine, CoA, and DAT. In this study we propose to expose female prairie voles to a low dose of deltamethrin throughout pregnancy and lactation along with another group of exposed animals supplemented with Folic acid. We expect to delineate the effect of developmental deltamethrin exposure on autism-relevant behavioral changes and molecular changes in the brain, as well as possible treatment with folic acid supplementation. Results from this experiment will help us understand whether the use of supplements during pregnancy can help in xenobiotic detoxification.

Differential Cytokine Expression Responses to Coronavirus Infection in Asthmatic Cells

Poster-2

Benjamin French

Benjamin W. French, Sara Kazmi, Joshua D. Breidenbach, Andrew L. Kleinhenz, James C. Willey, Jeffrey R. Hammersley, Mark Wooten, Erin Crawford, Nikolai Modyanov, Deepak Malhotra, Steven T. Haller, David J. Kennedy

The coronavirus disease 2019 (COVID-19) has resulted in substantial morbidity and mortality worldwide, and is caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Human Corona Virus OC43 (OC43) is also known to cause respiratory infections such as the common cold. Both viruses are members of the Coronaviridae family Betacoronavirus genus and are enveloped, positive-sense, single-stranded RNA viruses. While SARS-CoV-2 and OC43 share some regions of high homology, including the S2 subunit of the spike protein and its S2' cleavage site, OC43 is a biosafety level 2 (BSL-2) pathogen making it a more readily available model than SARS-CoV-2, which has been designated as a BSL-3 pathogen. Asthma is a very common chronic inflammatory airway condition, with an estimated 8% of Americans being afflicted. Asthma is one of many pre-existing conditions that has been identified by the CDC as a risk factor for worsened coronavirus infection outcomes. However, not all the data from COVID-19 hospitalizations supports this. Further research is needed to understand if/how coronaviruses disproportionately impact asthmatic patients. By exposing a 3D airway epithelium model pooled from healthy patients or asthmatic patients to vehicle or OC43 for 24 hours, we were able to utilize RNA-sequencing and RT-PCR to develop gene expression profiles for each cell type post infection. These profiles showed that the healthy and asthmatic cells only share 14% (roughly 400) genes that were either upregulated or downregulated in both cell types, differing in the remaining 86% of genes that were differentially expressed due to OC43 infection. Additionally, there were significant differences in cytokine, lung fibrosis, and RNA polymerase II related gene expression. The results of this study suggest that key differences in response to beta coronavirus OC43 infection exist in asthmatic vs healthy airway epithelium.

Vibrio cholerae Phage Shock Protein Response

Poster-2

Kelli Devanna

Kelli DeVanna, Jyl Matson

Vibrio cholerae is the Gram-negative bacterium that causes epidemic cholera. The phage shock protein (Psp) response is an extra-cytoplasmic stress response. There has been very limited study on the Psp response in V. cholerae. The V. cholerae system includes the pspFABCD operon, and unlinked pspG and pspE genes, and the latter resides on a separate chromosome. PspF is localized to the cytoplasm, and acts as a transcriptional activator. Without an inducing stress PspF is bound to PspA, which acts as a negative regulator. Previous work found that the GspD secretin from the type II secretion system induces the V. cholerae Psp response. Due to the limited previous studies on this system in V. cholerae, we hypothesize that the V. cholerae phage shock protein response can be induced by other proteins and external factors that have yet to be identified. To identifying additional protein inducers of the phage shock protein response in V. cholerae, we will perform transposon mutagenesis in V. cholerae using the TnAraOut mariner-based transposon system. This transposon contains an arabinose-inducible promoter that can be used to identify genes that, when overexpressed, induced the Psp response. A similar strategy was used to identify several unexpected protein inducers in Yersinia enterocolitica. We will mutagenize the strain of V. cholerae containing a pspA-lacZ fusion and identify mutants with increased pspA promoter activity on MacConkey lactose agar containing arabinose. Colonies that indicate promoter activity will be verified using a β-galactosidase assay. Identified genes will be cloned and overexpressed from a plasmid to verify they are responsible for inducing the response. Improved understanding of the V. cholerae Psp response can provide better insight into the pathogen's virulence and response to external factors.

Identifying Transcriptional Gene Signatures of Suicide Across Psychiatric Disorders

Poster-2

Evelyn Bates

Evelyn A. Bates, William G. Ryan, Sinead M. O'Donovan, Robert E. McCullumsmith

Suicide is defined as the act of self-injurious behavior that results in an outcome of death. Nearly 90% of suicide victims are reported to have suffered from a neuropsychiatric condition, such as major depressive disorder (MDD), schizophrenia (SCZ), and bipolar disorder (BP). Despite the amount of public data associated with suicide, large-scale analyses to identify a genetic signature for suicide has not been attempted. Here, we carry out a meta-analysis of postmortem brain transcriptomic datasets from patients diagnosed with a neuropsychiatric disorder who died by suicide and those who died of natural causes. Publicly available RNAseq (n=3, GSE102556, GSE78939, GSE101521) and microarray (n=4, GSE92538, GSE5389, GSE5388, GSE53897) datasets, generated in frontal cortex brain tissue, were identified from NCBI. Subjects are grouped based on cause of death (suicide v natural) with a secondary group based on presence of a comorbid neuropsychiatric disorder (MDD, SCZ, BP). We employ parametric and nonparametric statistical analyses to select features (i.e. genes) that contribute significantly to clustering of subjects based on death by suicide versus natural death. Using generalized mixed linear models on selected features, we assess the effects of cause of death on gene expression. The resulting differentially expressed genes (DEGs) are used as input for gene expression exploration tools, including Weighted Gene Correlation Network Analysis (WGCNA) and Gene Set Enrichment Analysis (GSEA), to identify hub genes and significantly perturbed biological, cellular, and molecular pathways. We will compare gene signatures generated from mouse models of aggression and impulsivity, behaviors associated with suicide, to our suicide gene signature. This study addresses a pressing need in suicide research and provides a biocomputational approach to assess gene expression associated with a suicidal death.

Group 3

Inhibitory Neurons Within the Infralimbic-Medial Prefrontal Cortex (IL-mPFC)

Poster-3

Nicole Bell

Nicole A. Bell, Ipe Ninan

Anxiety disorders are the most prevalent of the adolescent onset mental disorders and seen to effect females at higher rates than males. This statistic, alongside recent findings of development-dependent neurobiological changes from preadolescence to adulthood, has emboldened attention towards the possible development-dependent mechanisms in the increased incidence of anxiety disorders in adolescents. Anxiety disorder prevalence during adolescence coincides with a diminished threat extinction, an inhibitory learning necessary to regulate anxiety behaviors. Synaptic inhibition is mediated by local GABAergic neurons, which play a role in sculpting cortical circuitry, synaptic plasticity, and the generation of cortical rhythms. Therefore, it is important to understand how GABAergic neurons in the infralimbic medial prefrontal cortex (IL-mPFC), a brain region analogous to the human ventromedial prefrontal cortex, develops from pre-adolescence to adulthood and whether it is possible to identify adolescence-specific events in the IL-mPFC pertinent to anxiety-related behaviors. The most abundant GABAergic neurons are the parvalbumin interneurons (PVINs) and somatostatin interneurons (SSTINs), which inhibit local excitatory pyramidal neurons (PyNs). These PyNs project from the IL-mPFC to the basolateral amygdala and are critical for fear memory suppression. One of the main inhibition mechanisms on PVINs and SSTINs relies on the vasoactive intestinal peptide interneurons (VIPINs) via direct GABAergic synaptic transmission. This study focuses on the development of VIPIN-mediated GABAergic synaptic transmission to PVINs and SSTINs in preadolescent, adolescent, and adult mice. We utilize optogenetic techniques to stimulate VIPINs while using whole cell patch clamping to measure the GABAergic currents in PVINs and SSTINs to observe age-dependent differences in both the inhibitory post synaptic current (IPSC) amplitudes and diminishment of excitability. These studies aim to shed light on how VIPINs contribute to the prolonged development in the mPFC throughout adolescence and their potential effect on the regulation of threat memory and anxiety-related behaviors.

Analysis of protein kinase activity though Kinopedia, an interactive application for kinome array data

Poster-3

Alex Joyce

Alex W. Joyce, Khaled Alganem, Ali S. Imami , Abdul-Rizaq Hamoud, Justin F. Creeden, Elizabeth Shedroff, Evelyn A. Bates, Rawan Alnafisah, Gordon Meares, Robert E. McCullumsmith

Despite the prevalence of protein kinases in cellular functions and their role in complex issues such as cancer, diabetes, and Alzheimer's disease, treatments involving kinases can be hard to develop due to their highly integrated network of interactions and the dynamic cellular ecosystem. As such, it is important to gain a better sense of protein kinase activity when designing treatments. Here we present a unique way of looking at protein kinase activity by combining the PamStation12 kinome assay with Kinopedia, a web-based application developed in R Shiny. This study will analyze the similarities and differences between two types of kinase data and perform pathway analysis with this data using enrichR. The first data type consists of a particular recombinant kinase assayed in isolation which produces an empirical activity profile that shows that kinase's expected peptide targets. The second data type is a cellular 'fingerprint' obtained by assaying a cell culture treated with the activator or inhibitor for a specific kinase. Comparisons between these datasets can generate inferences regarding the nature of that kinase's peptide targets. Similarities between datasets can be used to reinforce existing kinase-peptide interactions while notable differences can indicate that intercellular effects such as other kinases, molecules, or the treatment method are altering the behavior of the target kinase. These findings will prove useful in revealing existing, novel, and altered kinase-peptide interactions as well as showing how these interactions impact the landscape of complex diseases and how we treat them. Acknowledgments: I would like to thank PamGene for providing recombinant kinase data for this project and feedback on the methodology of this project.

Targeting the thioesterase of FASN using lansoprazole derivatives in triple negative breast cancer cells

Poster-3

Shreyasi Ganguly

Shreyasi Ganguly, Qingbin Cui, Jacob Danielson, JingYuan Liu, Jian-Ting Zhang

Cancer cells show the ability to modify cellular metabolism to promote neoplastic proliferation. Increased de novo fatty acid synthesis, which is observed in many cancers, is catalyzed by Fatty Acid Synthase (FASN), a cytosolic dimeric enzyme containing seven domains, which condenses Acetyl CoA and Malonyl CoA to form palmitate using ATP and NADPH. However, non-lipogenic normal cells depend on dietary lipids for their lipid demand and, thus, do not express FASN. This makes FASN an ideal anti-cancer drug target. FASN has also been reported to regulate DNA repair activity via the NHEJ pathway by increasing PARP1 expression. Recently, Proton Pump Inhibitors (PPIs) have been shown to also inhibit FASN by binding to the thioesterase (TE) domain of FASN. FASN is shown to be overexpressed in Triple Negative Breast Cancer and here we hypothesize that chemical modification of the PPIs may make them better FASN inhibitors. To test this hypothesis, lansoprazole as the parent PPI was modified in collaboration with a medicinal chemist, resulting in nine new analogues (Lan D-1 to Lan D-9). Of these analogues, Lan D-8 and Lan D-9 inhibit TE activity of FASN with Ki at 1.35 and 1.41 µM and TNBC cell survival IC50 at 6.72±0.97 and 9.53±4.35 µM, respectively, compared with the Ki at 12.197 µM and IC50 of 14.106±2.65 µM for lansoprazole. These compounds will be tested for their activities in inhibiting FASN-mediated DNA repair activities. Interestingly, Lan D-1 and Lan D-5 inhibited TNBC cell survival with an IC50 of 2.85±0.68 and 2.17±0.40 µM, respectively but did not inhibit the TE activity. These compounds will be tested if they inhibit any other domains of FASN. Finally, Lan-D2, Lan-D3, Lan-D4, Lan-D6 and Lan D-7 do not effectively inhibit cell survival and do not inhibit TE activity. Based on these findings, we conclude that modification of the benzene ring with strong halogen groups joined by ether links improves the activity of lansoprazole.

Novel ligand of the Na/K-ATPase revealed by computational and experimental analysis

Poster-3

Fathima Dhilhani Mohammed Faleel

Dhilhani Faleel, Shungang Zhang, Abayomi E. Adegboyega, Jacob A. Connolly, Travis W. Stevens, Deepak Malhotra, Steven T. Haller, John R. Falck, David J. Kennedy

Arachidonic acid metabolites such as 20-Hydroxyeicosatetraenoic acid (20-HETE) promote chronic inflammation in the kidney, which leads to chronic kidney disease (CKD). We and others have demonstrated both clinical and experimental evidence that stimulation of NKA-Src signaling complex by endogenous NKA ligands, such as cardiotonic steroids, leads to persistent renal inflammation and fibrosis in settings such as CKD. However, whether 20-HETE directly interacts with the NKA and promotes pro-inflammatory NKA signaling is unknown. We sought to determine the ability of 20-HETE to bind with the NKA relative to other known NKA ligands and stimulate NKA mediated signaling using molecular modeling and experimental approach. First, we performed induced fit docking using glide and prime tools (Schrodinger 2021-2) to predict the binding free energy of both 20-HETE and its stable analog, 5,14-20-HEDE, in comparison with the well-established cardiotonic steroid NKA ligand Telocinobufagin. This docking analysis predicted that 20-HETE and 5,14-20-HEDE interact with the NKA with similar binding free energy as cardiotonic steroids (Predicted binding free energies: Telocinobufagin =-9.2; 20-HETE= -8.5 and 5,14-20-HEDE = -8.18). Further this computational modeling demonstrated that all of these molecules interact in the same binding pockets of the NKA. Next, we performed in-vitro experiments on NKA signaling were performed with both 20-HETE and its stable analog, 5,14-20-HEDE, in renal LLC-PK1 proximal tubule cells. The result showed that 20-HETE and its analog 5,14-20-HEDE increased MAPK activation in a dose dependent manner from 10 nM to 10 uM in LLC-PK1 cell lines. This MAPK activation was significantly reduced after pretreatment with pNaKtide, a specific inhibitor of the NKA-Src signaling complex (1uM pNaKtide, 30 minutes). The result of these study suggests that 20-HETE interacts with NKA in similar manner as cardiotonic steroids and is capable of inducing NKA signaling in renal proximal tubules

AMPK in Alzheimer's Disease: A Biochemical and Bioinformatic Intersection

Poster-3

Nicholas Henkel

Nicholas D. Henkel, Elizabeth Shedroff, Alex W. Joyce, Xiaojun Wu, Khaled Alganem, Justin F. Creeden, Gordon Meares, Zhexing Wen, Robert E. McCullumsmith

Background

Adenosine-monophosphate activated kinase (AMPK) is a master regulator of energy and nutrient sensing in cells. AMPK is implicated in the pathophysiology of neurodegenerative disorders, like Alzheimer's dementia (AD). However, there have been no comprehensive studies to characterize AMPK in human substrates of the disorder.

Methods

Using postmortem dorsolateral prefrontal cortex (DLPFC), we performed PamGene12 Kinome Array experiments, RT-qPCR, enzyme activity assays (Cyclex AMPK Kinase Activity Assay), capillary electrophoresis, and subcellular fractionation to define changes in AMPK in AD. We used control (n = 20 (11M/9F)) and Alzheimer's subjects (n = 20 (8M/12F)) from the NIH NeuroBioBank.

Results

Using the PamGene12 Kinome Array platform, AMPK was identified as a "hit" kinase. Confirming our bioinformatic findings, we found decreased AMPK activity in male and female AD DLPFC. Moreover, we found lower AMPK activity in control v. MCI, and even lower AMPK activity in MCI vs. AD. Inspired by our changes in enzyme activity, we measured changes in transcript and protein expression. Both transcript and protein expression of the regulatory subunits of AMPK were changed. With the PamGene12 Kinome Array, we identified 15 peptides that differentially reported AMPK's activity when either activated or knocked down. Interestingly, increased phosphorylation was observed at Lamin-B1 (LMNB1) in both male and female subjects. As LMNB1 is a nuclear protein, is performed subcellular fractionation on postmortem DLPFC. In a preliminary study (CTL = 2, AD = 2), AMPK 1 was decreased in a chromatin-bound nuclear fraction, compared to controls.

Conclusions

The regulatory subunits of AMPK may play a key role in the pathophysiology of AD. In the AD brain, AMPK may be mislocalized with loss of nuclear localization and activity. Future work will define these changes in the DLPFC.

Quinoline derivative, IND-2, potently inhibits prostate cancer cell proliferation by causing oxidative stress, inducing apoptosis, and inhibiting topoisomerase II

Poster-3

Swapnaa Balaji

Swapnaa Balaji, Rabin Neupane, Saloni Malla, Rahul Khupse, Haneen Amawi, Shikha Kumari, Diwakar B. Tukaramrao, Charles R. Ashby Jr, Sai H.S. Boddu, Karthikeyan Chandrabose, Piyush Trivedi, Dayanidhi Raman, Amit K. Tiwari

Prostate cancer (PC) is the most commonly diagnosed cancer among men, causing an estimated 375,000 deaths worldwide. Due to drug resistance and adverse effects, existing treatments for prostate cancer, particularly metastatic cancer, have limited efficacy. Consequently, it is imperative to discover and develop prostate cancer treatments that are effective and do not produce intolerable side effects. Quinolines are naturally occurring anticancer compounds. The purpose of this study was to examine the in vitro efficacy of IND-2 (4-chloro-2-methylpyrimido[1,2":1,5]pyrazolo[3,4-b]quinolone) in prostate cancer lines PC-3 and DU-145. IND-2 significantly inhibited the proliferation of PC-3 and DU-145 cells, and reduced the mitochondrial membrane potential in PC-3 cells. Furthermore, IND-2 increased the expression of cleaved caspase-3, caspase-7, and poly (ADP-ribose) polymerase (PARP). Incubating PC-3 cells with IND-2 significantly reduced the expression of apoptotic proteins, Bcl-2-associated X protein (BAX) and B-cell lymphoma 2 (BCL-2). Additionally, IND-2 caused morphological changes characteristic of apoptosis in PC-3 cells. The mitotic catastrophe in PC-3 cells caused by IND-2 was also characterized by accumulation of multinuclei. The incubation of DU-145 cells with IND-2 significantly increased reactive oxygen species levels and inhibited the catalytic activity of topoisomerase IIα. Overall, we found that IND-2 may be useful in the development of more effective prostate cancer drugs.

Group 4

Examination of Differential Cancer Rates in Schizophrenia: Identification of Novel Off Target Effects of Typical Antipsychotics

Poster-4

Abdul-Rizaq Hamoud

Abdul-Rizaq Hamoud, Jacob Rethman, Sadik Khuder, Jarek Meller, James Reigle, Robert E. McCullumsmith

Interestingly, overall cancer rates in schizophrenia are lower than the general population. We sought to confirm lower cancer rates in schizophrenia and explore mechanisms responsible for this unexpected observation. We hypothesize that typical antipsychotics are competitive inhibitors of PIM kinases. We postulate that inhibition of these kinases by antipsychotics accounts for reduced cancer incidence in patients with schizophrenia. We applied a three-pronged approach to answer this question: 1) epidemiological confirmation of decreased cancer incidence rates in schizophrenia, 2) bioinformatics analyses of perturbagen structural moieties and gene expression data, and 3) in vitro kinase activity drug screening. The Healthcare Cost and Utilization Project (HCUP, n=140476/group) and the TriNetX (N=36,695) datasets were used to identify health outcomes for patients with schizophrenia. Schizophrenia patients were matched to controls by age and sex in HCUP and stratified in TriNetX. Structure-Activity relationship analyses were conducted to assess antipsychotics antineoplastic properties. 53 antipsychotics were virtually screened for similarity in gene expression and structural similarity across a library of 41,000 LINCS small molecules. Protein kinase activity arrays and assays were used to assess the effects of study drugs on recombinant PIM1 activity. Cancer incidence risk for patients with schizophrenia were decreased across all cancer subtypes in the HCUP dataset (Imputed lung cancer RR = 0.15) and confirmed in the TriNetX datasets (bladder and lung cancer when accounting for smoking, RR=0.24, 0.22). In our bioinformatics analyses, a known PIM1 inhibitor, 10-DEBC, was identified as structurally and transcriptionally similar to several phenothiazine antipsychotics. Thioridazine tanimoto structural similarity coefficient as compared to 10-DEBC was 0.87 and the transcriptional similarity in A549 NSCLC cells was 0.475. PIM1 activity was inhibited on the protein kinase activity array in a concentration dependent manner by typical antipsychotics. Our data suggest that phenothiazine antipsychotics act directly on protein kinases to lower lifetime cancer risk

Incorporating Single Cell Transcriptomic Data into a Hippocampal Computer Model to Understand Epilepsy Pathophysiology

Poster-4

Vineet Reddy

Vineet Reddy, Mohamed Sherif, Craig Kelley, Robert E. McCullumsmith, Rammohan Shukla

Single-cell transcriptomics provides cellular and molecular characteristics of brain changes in great detail, but this information is merely a snapshot of cell states. To accurately estimate the non-linear contribution of these transcriptomically detailed neurons in disease phenotypes, we propose the integration of single-cell transcriptomic data with biophysically realistic microcircuit models. To demonstrate the feasibility of such integration, we used single cell RNAseq (scRNAseq) level data collected from Temporal Lobe Epilepsy (TLE), a penetrant phenotype characterized by recurrent seizures. After incorporating the magnitude (in fold changes) and direction (up or down regulation) of cell specific expression of Alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazole Propionic Acid (AMPA), N-methyl-D-aspartate (NMDA), and Gamma-aminobutyric acid type A (GABAA) receptor subunits into a microcircuit computer model of the hippocampus composed of 800 PYR, 200 PV, and 200 OLM neurons, we measured changes in the gamma oscillation range, a known marker for seizure initiation. Increased pyramidal AMPAR density increased the gamma power generated by the model, suggesting increased seizure susceptibility. However, the magnitude of this increase was modulated by PV GABAAR density scaling. Additionally, augmenting PV GABAAR and reducing pyramidal GABAAR scaling reduced gamma power, suggesting a possible homeostatic effect. Several optogenetics-based prior studies validate our findings. Our work, for the first time, illustrates how microcircuit computer modeling can maximize information gained from single-cell RNAseq experiments.

Targeting Dynamics of 14-3-3-σ for the Treatment of Pancreatic Cancer

Poster-4

Deepti Gurung

Deepti Gurung, Catherine Rajendran, Jacob Danielson, JingYuan Liu

Background

The overexpression of 14-3-3-σ mRNA in pancreatic cancer patients is associated with the worse prognosis and treatment failure. Therefore, it is recognized as a potential therapeutic target for pancreatic cancer treatment. There are seven isoforms of 14-3-3 protein that are highly conserved and share similarities in their primary sequences, secondary structures as well as ligand binding sites. Consequently, the currently available 14-3-3-protein inhibitors cannot target 14-3-3-σ selectively and have been shown to perform poorly in clinical trials because of toxicity. Proteins including 14-3-3 are dynamic entities and can undergo structural transitions to reach different conformational states. We have shown that the sigma isoform exhibits different unique dynamic properties compared with other isoforms. Therefore, we aim to understand the mechanism of the unique dynamic properties of 14-3-3-σ and utilize this knowledge to selectively inhibit 14-3-3-σ in pancreatic cancer.

Methods

Molecular dynamics simulation were performed using Amber force field for all seven 14-3-3 isoforms. The simulation data was further analyzed using computational tools developed by our lab. In vitro BioSAXS experiment was performed to validate our findings from in silico analysis.

Results

All unbound 14-3-3 isoforms displayed more open conformational states compared with their peptide-bound complexes. Unbound sigma isoform, particularly, showed unique, wide - open conformation in comparison to other isoforms. We were able to identify residues and the mechanism that are potentially responsible for the open-close conformation transition of 14-3-3-σ . These residues will be served as targeting site for in-silico drug screening to identify sigma isoform specific inhibitors.

Conclusions

Understanding the mechanism of dynamic properties of protein may open a new window in identifying inhibitors with high specificity and potency in the targeted therapy for pancreatic cancer.

Ketone body β-hydroxybutyrate is a novel epigenetic antihypertensive metabolite

Poster-4

Juthika Mandal

Juthika Mandal, Saroj Chakraborty, Blair Mell, Bina Joe

Hypertension or elevated blood pressure (BP) is the number one risk factor for significant illness and death due to stroke, kidney failure, and ischemic heart failure. Recent reports increasingly demonstrate that aberrant energy metabolism is a significant feature of hypertension. In support, we previously demonstrated that the ketone body β-hydroxybutyrate (BHB) is depleted during hypertension. Further, nutritional repletion of circulating BHB ameliorates HTN. Two mechanisms for the observed anti-hypertensive effect of this hepatic metabolite were discovered to be due to (1) inhibition of the renal NLRP3-inflammasome and (2) potent autophagy dependent vasodilatory activity. BHB has been previously described as an epigenetic inhibitor of histone deacetylation. A more recent study identified BHB as a new epigenetic modifier of histones wherein histones are chemically modified at lysine residues by covalent bonding of β-hydroxybutyrate. The resultant H3K9- β-hydroxybutyrylation of histone H3 is upregulated during fasting. Because intermittent fasting lowers hypertension, we hypothesized that H3K9- β-hydroxybutyrylation of histones is an epigenetic mechanism contributing to the antihypertensive property of BHB. Methods: Renal and hepatic tissues from a group of hypertensive Dahl Salt-Sensitive (S) rats and S rats protected from hypertension with nutritional correction of hypertension by raising circulating levels of BHB were compared for epigenetic modifications of histone 3 by 1) H3K9-β-hydroxybutyrylation, and 2) acetylation. Results: (1) H3K9- β-hydroxybutyrylation was significantly elevated in the group of S rats with lower BP compared to control S rats (kidney: p=0.0095, liver: p=0.0455). (2) Histone deacetylase (HDAC) activity was lower and accompanied by increased acetylation of Histone 3. The locations of these enhanced acetylation were mapped to lysine residues at H3K9 (kidney: p=0.0095, liver: ns), as well as at H3K14 (kidney: p=0.0303, liver: p=0.0095,), H3K18 (kidney: p=0.0498, liver: p=0.0190) and H3K23 (kidney: p=0.0173, liver: p=0.0095). Conclusion: These results are the first to reveal that BP, a cardiovascular risk factor, is epigenetically regulated by a hepatic metabolite. This epigenetic effect imparted by BHB was mechanistically traced to be due to a combination of histone β-hydroxybutyrylation and inhibition of HDAC activity.

High antioxidant environment in Peromyscus leads to tick-borne flavivirus attenuation.

Poster-4

Ryan Harris

Ryan A. Harris, Ada Izuogu, Michael Haddad, Travis R. Taylor

Tick-borne flavivirus (TBFV) are emerging viruses that cause hemorrhagic fever and encephalitis if the virus crosses the blood brain barrier. These complications can lead to prolonged morbidity and death with a case fatality rate of 1-30%. There are currently no treatments to protect against these complications. Therefore, there's an urgent need to uncover new therapeutic targets to combat infection. In the natural life cycle of TBFV, reservoir hosts enable maintenance of the virus allowing low level infection without debilitating symptoms. Peromyscus leucopus (white-footed mouse) is an example of such reservoir host. Uncovering the molecular mechanism by which these mice restrict viral infection may lead to development of drugs that can translate this restriction to humans. Therefore, we hypothesize that P.leucopus coevolution with flaviviruses has led to the development of a potent antiviral pathway that restricts viral replication preventing viral-induced pathogenesis. Previous work showed that P.leucopus fibroblasts display a 10,000-fold decrease in TBFV virion production when compared with susceptible mice, M.musculus. Restriction is also flavivirus-specific as a non-flavivirus, vesicular stomatitis virus (VSV) replicated to comparable levels between the two rodent species. Consistent with reduced virus replication, P.leucopus also showed a significant reduction in viral-induced oxidative stress, a known contributor to viral disease. Reactive oxygen species (ROS) are reduced through the action of the transcription factor Nrf2 and subsequently induced antioxidant proteins. Consistently, Nrf2 and select antioxidants are abundantly expressed in P.leucopus compared to M.musculus. Susceptible human and mouse cells treated with drugs that activate the Nrf2 pathway show a significant reduction in viral replication, similar to virus replication in resistant P.leucopus cells. Therefore, restriction found in Peromyscus is shown to be linked to its resistance to oxidative stress and this can be replicated in susceptible cells. Using KO cell lines, we will confirm the importance of antioxidant production for viral restriction.

Development of LC-MS method to study whole body uptake and elimination of amphetamines and synthetic cathinones

Poster-4

Kabita Gwachha

Kabita Gwachha, Alexander Wisner, Tue Q. T. Chau, Frederick E. Williams, Scott Hall, Isaac T. Schiefer

Synthetic cathinones (a.k.a. "bath salts) are popular drugs of abuse that are being used in place of amphetamine-like stimulant drugs such as methamphetamine. There are limited studies on pharmacokinetics of cathinones in mammals, and no studies in zebrafish, which are now being used to evaluate the pharmacological and toxicological effects of these drugs. To aide this research, pharmacokinetic profiles are needed for these drugs based on the emersion route in zebrafish. To develop these methods, methamphetamine was used as a prototypical drug to examine its absorption, distribution, and metabolic profiles. A matrix-matched (zebra fish homogenate) LC-MS method for quantification of methamphetamine was developed and validated. The uptake and elimination profiles of methamphetamine in 5dpf zebrafish larvae was determined. An analytical method was developed and validated, and samples were analyzed in triple quadrupole LC-MS/MS on a X-terra C18 (150 *2.5*5). QueChers technique was used for the sample preparation and extraction. Matrix-matched calibration was determined by spiking blank 5 dpf zebrafish larvae with analyte and internal standard (D5 methamphetamine). For uptake study, 5dpf larva were exposed to 250µM and euthanized at different time points (0.25, 0.5, 1, 2, 3, 6, 12, 24 hours). In every time points, 10 larvae were pooled, and euthanized by snap freezing using dry ice and stored at -20°C. To study elimination from the zebrafish larvae, they were transferred into drug free medium after 24 hours of exposure and collected at 1, 2, 3, 6, 8, 12, 24, 30 and 48 hours of depuration following the same procedure as uptake. Samples for determination of uptake and elimination parameters were processed using QueChers technique. 10, 5 dpf zebrafish were pooled and analyzed using validated method. Samples were analysed in triplicates for each time points. Calibration curves showed good linearity (R2>0.99, 0.060µg/ml- 3.9 µg/ml). For repeatability, the %RSD of three replicates at three different concentration were found to be less than 10%. The concentration continued to increase up to 12 hours and remain at a steady state. Upon transfer to drug free medium, drug was eliminated completely within 24 hours.

Group 5

Dysregulated Kinase Networks in Major depressive Disorder

Poster-5

Rawan Alnafisah

Rawan Alnafisah, Khaled Alganem, Abdul-Rizaq Hamoud, Ali S. Imami, Justin F. Creeden, William G. Ryan, Alex W. Joyce, Robert E. McCullumsmith, Sinead M. O'Donovan

Major depressive disorder (MDD) is a chronic debilitating disorder with an unknown etiology. MDD is associated with the altered expression and activity of protein kinases, which regulate signaling in complex biological networks. Abnormalities in kinase signaling are involved in the pathophysiology of MDD. However, of the hundreds of kinases expressed in human brain, only a small number have been studied in MDD. To address this gap in our knowledge, we assayed the activity of the serine/threonine subfamily of protein kinases in postmortem tissue from the dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC) using an array-based platform (Pamgene12). Relative changes in kinase activity were assessed in MDD subjects, MDD subjects who died by suicide and non-psychiatrically ill controls (pooled samples, male n= 2-9/grp; female n= 3-9/grp). Kinome data was analyzed using multiple bioinformatic pipelines (UKA, KRSA) to identify kinases with significantly altered activity (peptide signal intensity threshold: log 2-fold change ± 0.2). Significant kinases like PKA (Z-score=2.42) and AKT (Z-score=2.31), which were previously implicated in MDD, were also identified in our study. We also identified novel kinases including FRAY (Z-score=2.14), whose role in MDD has yet to be elucidated. In the DLPFC, global kinase activity was downregulated in MDD compared to controls. Conversely, in the ACC global kinase activity was increased in MDD, suggesting that region-specific differences in kinase activity in MDD. Studying the activity of protein kinases, which are essential for cellular signal transduction, provides valuable insight dysregulated biological processes that are implicated in the neurobiology of MDD disorder.

Identifying Candidate Loci Towards Correlates of Predictive Coding in C. elegans

Poster-5

William Ryan

William G. Ryan, Ali S. Imami, Sinead M. O'Donovan, Rammohan Shukla, Robert E. McCullumsmith, Bruce Bamber

Cortical function comprises the gestalt of information processing mechanisms inthe brain giving rise to higher-order sensory perception, cognition, and behavior thatunderlie consciousness. However, traditional feed-forward theories of neurosciencefail to capture the complexity of interactions within the neural connectome leadingto these phenomena. Prevailing theory now posits a generative, predictive computa-tional framework to better describe the architecture of the cortex.C. eleganspermits high-content behavioral phenomics and assessment of activa-tion kinetics in response to external stimuli across all 302 neurons simultaneously.As such,C. eleganspresents a tractable model to facilitate learning about neuralnetworks and to test the assumptions of cortical theory. To that end, we performedwhole-brain recording in tandem with behavioral phenotyping of behavingC. elegansin response to both aversive stimuli eliciting backwards locomotion and food-likestimuli eliciting attraction.Calcium imaging profiles revealed highly dynamic neurons with activity patternswherein a feed-forward model does not fully explain neuronal nor behavioral responseto stimuli. Suggestive of a complex sensory integration, we sought to apply a data-driven approach to characterize these nonlinear interactions and assess the complexityof neural dynamics and sensory plasticity withinC. elegans.We demonstrated an oscillating global brain state dependent on stimulus. Fur-iii thermore, functional connectivity analysis showed disparate network organization inunstimulated and stimulated systems. These functional network differences drive var-ied responses to stimulus and represent candidate loci towards correlates of predictiveprocessing mechanisms inC. elegansposing avenues for further study.As schizophrenia, autism, major depression, Alzheimer's disease, and age-relatedcognitive decline are linked to impairments in sensorimotor processing, this workrepresents an important step forward in advancing our understanding of the cortex.

Novel effect of Selumetinib-mediated autophagy via HSF1 in K-Ras mutant pancreatic cancer

Poster-5

Shruti Ghai

Shruti Ghai, Alex Young, Kuo-Hui Su

K-Ras mutant pancreatic cancer are a third cause of cancer-related death and are difficult to treat, with a five-year survival rate of less than 6%. It is reported that oncogenic K-Ras signaling passes through RAF/MEK/ERK pathways. Selumetinib (AZD6244) is a selective MEK inhibitor for K-Ras mutant cancers and is currently in phase II trials. However, the underlying mechanisms of action are not well known. Autophagy, a self-digest pathway to degrade cellular organelles and macromolecules in maintaining proteome homeostasis, is suppressed by the mammalian target of rapamycin complex 1 (mTORC1). mTOR activity is known to be inhibited by c-Jun N-terminal kinase 1/2 (JNK1/2) signaling. Autophagy has protective and suppressive effect in cancer; however, the molecular basis for the relationship between the induction of autophagy and the initiation of pancreatic malignancy is currently unknown. Heat shock factor 1 (HSF1), a key transcription factor involved in proteotoxic stress response via protein-folding, plays a pro-oncogenic role in tumorigenesis. HSF1 is reportedly hyperactive in pancreatic cancers. AZD6244 is known to decreases HSF1 phosphorylation at Ser326 and HSF1 expression in melanomas and therefore abates cancer development. However, the detailed mechanism by which HSF1 engages in AZD6244-mediated autophagy in K-Ras mutant human pancreatic cancer is not fully understood. This study aims to investigate the mechanism of HSF1 in AZD6244-mediated autophagy in K-Ras mutant human pancreatic cancer. We found that, in human pancreatic cancer cells, AZD6244 induces robust autophagy response. Simultaneously, it reduces HSF1 phosphorylation at Ser326 and HSF1 expression. In addition, AZD6244 induces phosphorylation of JNK1/2 T183/Y185 and decreases mTORC1 activity in human pancreatic cancer cells. To summarize, inhibition of HSF1 by AZD6244 induces autophagy and JNK1/2 phosphorylation in human pancreatic cancer cells.

Dynamics of antimicrobial immune protein quantities in milk

Poster-5

Ahmed Abokor

Ahmed A. Abokor, Piu Saha, Beng San Yeoh, Rachel M. Golonka, Matam Vijay-Kumar

Breast milk is the preferred food for the newborns and considered an 'edible immune system'. Breast milk contains macro and micronutrients required to maintain infant growth that are absent in commercial infant formulas. In addition, breast milk is also an excellent source for antimicrobial innate and adaptive proteins which not only protects the naïve infant gastrointestinal tract from enteropathogens but also aids in the initial colonization of gut microbiota. In this study, we analyzed several antimicrobial immune proteins in the milk of C57BL/6 dams in our colony at several time points, and observed significant changes in the immune protein composition of milk throughout the lactation period. The presence of innate immune proteins serum amyloid A (SAA), CD14, and notably lipocalin-2 (Lcn2) were observed in high quantities with Lcn2 and SAA milk quantities present at microgram levels suggesting a potential niche for these proteins during neonatal immune development. Indeed we observed the presence of adaptive immune proteins immunoglobulins (Ig) A and G with IgG present in milk at higher quantities compared to IgA at day 5 post-delivery but interestingly reversing by day 15 indicating a selective adaptive immune preference toward IgA, which could be due to gut colonization for which secretory IgA is the predominant Ig in the gut. Additionally, milk from IgA-deficient dams allowed for robust proliferation of E. coli compared to wild-type milk further signifying the involvement of IgA during the early colonization of microbes in the neonatal gut. Collectively, our findings provide insight to the various immune proteins present in milk for future studies investigating the role of milk proteins in the immune development of neonates.

Aerosolized Harmful Algal Bloom Derived Microcystin-LR Exacerbates Inflammatory Mediators of Asthma in Asthmatic Human Airway Epithelium

Poster-5

Joshua Breidenbach

Joshua D. Breidenbach, Tamiya T. Gordon, Shivani C. Patel, Steuart A. Besly, Benjamin W. French, Andrew L. Kleinhenz, James C. Willey, Jeffrey R. Hammersley, Mark Wooten, Erin Crawford, Nikolai Modyanov, Deepak Malhotra, David J. Kennedy, Steven T. Haller

Harmful algal blooms are on the rise globally with the potential to pose serious adverse health effects in humans. Microcystin-LR, one of the most abundant and toxic HAB-derived cyanotoxins, has recently been detected in aerosols from HAB water. We previously reported that aerosol MC-LR exposure has a pro-inflammatory influence on the airways. Asthma, which is an extremely prevalent airway disease afflicting approximately 8% of the U.S. population, is largely driven by inflammation. However, the impact of MC-LR aerosol exposure on this at-risk population is unknown. In this study, a 3D primary human airway epithelium model was utilized, in which cells were isolated from healthy and asthmatic donors. An environmentally relevant concentration of MC-LR (1 μ M) was aerosolized and delivered to the cell surface, before the cells were harvested for transcriptome analysis. Strikingly, 1% of the genes upregulated (log2FC $>$ 0.25) by asthma alone, were further upregulated by MC-LR exposure including inflammation mediators, such as CXCL11 (log2FC = 0.63); and TLR4 (log2FC = 0.31). These genes had significant associations with pathways, such as "immune cytokine binding" (FDR = 0.015).Again, when comparing MC-LR exposed vs unexposed asthma derived epithelium, MC-LR exposure further downregulated a subset (9%) of genes that were downregulated (log2FC $<$ -0.25) by asthma alone including extracellular matrix (ECM)-related and cadherin signaling-related mediators, such as PCDHGA7 (log2FC = -1.46); MMP3 (log2FC = -0.37); and COL6A1 (log2FC = -0.44). These genes had significant associations with localization to "ECM" (FDR = 0.004). Airway epithelium of asthmatic patients demonstrates a gene transcriptional profile that is distinguishable from its healthy counterpart. Importantly, the results of this study show that aerosolized MC-LR further amplifies these differences leading to the exacerbation of inflammatory mediators of asthma such as toll-like receptors and decreases in ECM proteins, suggesting a potential for MC-LR exposure to worsen asthma severity.

Author Index

Abokor
Ahmed A., 64
Adegboyega
Abayomi E., 50
Alganem
Khaled, 20, 29, 42, 48, 51, 61
Alnafisah
Rawan, 48, 61
Amawi
Haneen, 52
Andari
Elissar, 33
Aryal
Sachin, 34

Balaji
Swapnaa, 52
Bamber
Bruce, 62
Baroi
Sudipta, 36
Bates
Evelyn A., 45, 48
Bell
Nicole A., 47
Besly
Steuart A., 65
Bhattacharjee
Sayani, 28
Boddu
Sai H.S., 52
Breidenbach
Joshua D., 41, 43, 65
Burkett
James, 33, 40, 42

Chakraborty
Saroj, 57
Chakravarti
Ritu, 17, 25
Chakravarty
Sukanya, 17, 25
Chandrabose
Karthikeyan, 52
Chattopadhyay
Saurabh, 17, 25
Chau
Tue Q. T., 59
Cheng
Xi, 24, 34
Chiu
Nathaline, 24
Connolly
Jacob A., 50
Conrad
Taylor, 35
Crawford
Erin, 43, 65
Creeden
Justin F., 29, 42, 48, 51, 61
Crowe
Emily, 36
Cubberly
Paige, 35
Cui
Qingbin, 49
Curtis
Melissa A., 42

Czernik
Piotr, 36

Danielson
Jacob, 49, 56
DeVanna
Kelli, 44
Doan
Jonathan, 28
Dworkin
Lance, 38

Falck
John R., 50
Faleel
Dhilhani, 50
Fan
Shumin, 17
Fernando
Veani R., 23
Ferreira
Viviana P., 30
Figy
Christopher, 35
Franco
Justin H., 27
French
Benjamin W., 43, 65
Furuta
Saori, 23

Ganguly
Shreyasi, 49
Gautam
Iluja, 22
Ghai
Shruti, 63
Glanz
Anna, 17
Golonka
Rachel M., 64
Gordon
Tamiya T., 65
Gurung
Deepti, 56
Gwachha
Kabita, 59

Haddad
Michael, 58
Hall
Scott, 59
Haller
Steven T., 41, 43, 50, 65
Hammersley
Jeffrey R., 43, 65
Hamoud
Abdul-Rizaq, 29, 48, 54, 61
Harberson
Mitchell T., 19
Harris
Ryan A., 58
Helminiak
Kathryn, 41
Henkel
Nicholas D., 20, 29, 51
Hill
Jennifer W., 19
Huang
Caoqinglong (Jackson), 18
Huss
Chadwick, 22

Imami
Ali S., 20, 38, 61, 62
Izuogu
Ada, 58

Joe
Bina, 24, 57
Joe.
Bina, 34
Joyce
Alex W., 20, 48, 51, 61
Jr
Charles R. Ashby, 52

Kazmi

Sara, 43
Kelley
Craig, 55
Kennedy
David J., 41, 43, 50, 65
Khuder
Sadik, 41, 54
Khupse
Rahul, 52
Kleinhenz
Andrew L., 43, 65
Koka
Sanjay, 34
Krebs
Michelle, 22
Kumari
Shikha, 52

Lecka-Czernik
Beata, 36
Liu
JingYuan, 49, 56

Malhotra
Deepak, 43, 50, 65
Malla
Saloni, 52
Mandal
Juthika, 57
Manhandar
Ishan, 34
Matson
Jyl, 44
McCullumsmith
Robert E., 20, 29, 38, 45, 48, 51, 54, 55, 61, 62
Meares
Gordon, 48, 51
Mei
Xue, 24
Mell
Blair, 24, 57
Meller
Jarek, 38, 54
Menon
Smrithi S., 30
Modyanov
Nikolai, 43, 65
Morgan
Kelly, 37
Morran
Michael, 29

Nadiminty
Nagalakshmi, 28, 38
Naghavi
Farzaneh, 40
Neupane
Rabin, 52
Ninan
Ipe, 47

O'Donovan
Sinead M., 45, 61
O'Donovan
Sinead M., 62

Pabon
Daniella G., 33
Pan
Zhixing K., 27
Patel
Shivangi, 33
Shivani C., 65
Podgorski
Izabela, 36
Popli
Sonam, 25

Rajendran
Catherine, 56
Ram
Sanjay, 30
Raman
Dayanidhi, 52
Ramirez-Toloza
Galia, 30
Reddy

Vineet, 55
Reigle
James, 54
Rethman
Jacob, 54
Ryan
William G., 38
William G., 20, 29, 45, 61, 62

Saferin
Nilanjana, 42
Saha
Piu, 64
Sahay
Smita, 20
Schiefer
Isaac T., 59
Sharma
Vandana, 23
Shaughnessy
Jutamas, 30
Shedroff
Elizabeth, 20, 38, 48, 51
Sherif
Mohamed, 55
Shukla
Rammohan, 42, 55, 62
Sindhwani
Puneet, 38
Stepkowski
Stanislaw, 38
Stevens
Travis W., 50
Stevenson
Leah, 34
Storad
Zachary, 22
Su
Kuo-Hui, 63

Taylor
Travis R., 58
Tipton
Claire, 35
Tiwari
Amit K., 52
Trivedi
Piyush, 52
Tukaramrao
Diwakar B., 52

Vijay-Kumar
Matam, 64

Wen
Zhexing, 51
Willey
James C., 43, 65
Williams
Frederick E., 59
Wisner
Alexander, 59
Wooten
Mark, 37, 43, 65
Worth
Randall G., 22
Wu
Xiaojun, 51
Wuescher
Leah M., 22
Wycoff
Keith L., 30
Wynn
Rebecca, 28

Yadav
Kunal, 38
Yang
Tao, 24
Yeo
Ji-Youn, 24
Yeoh
Beng San, 64
Yeung
Kam, 35
Miranda, 35
Young
Alex, 63

Zhang
Jian-Ting, 18, 49
Shungang, 50
Zheng
Xunzhen, 23