Graduate Research Annual Forum 2024

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Council of Biomedical Graduate Students

March 21st and 22nd, 2024

COLLEGE OF MEDICINE AND LIFE SCIENCES
THE UNIVERSITY OF TOLEDO

BIOMEDICAL SCIENCE GRADUATE PROGRAM
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About the Council of Biomedical Graduate Students

The University of Toledo Council of Biomedical Graduate Students (CBGS) 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 (BMSP) 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 to the Graduate Students Association and University Administration

• Organize social and professional events to enrich the graduate student life experience

We meet 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 the autumn to help guide students' career decisions

• Graduate Research Annual 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/

Dean’s Message

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. These student-led decisions are also wonderful examples of how the Council of Biomedical Graduate Students reinforces leadership qualities to our trainees.

Christopher Cooper, MD
Dean
College of Medicine & Life Sciences
University of Toledo

Welcome to 2024 Graduate Research Annual Forum

I would like to personally welcome you to the 2024 Graduate Research Annual Forum (GRAF 2024)! The Council of Biomedical Graduate Students (CBGS) has been organizing GRAF in collaboration with the College of Pharmacy and Pharmaceutical Sciences over the last four decades. This event is a great time for students to present their work and get invaluable feedback from expert post-docs and faculty. GRAF also gives all our research tracks a chance to learn about the other research projects taking place at the University of Toledo, helping foster interdisciplinary collaborations.

Our students are very excited for this year's forum, including 42 poster and oral presentations and 15 volunteer judges from faculty and postdoctoral fellows. We are also thankful for the moderators who will be leading each poster and oral session. We want GRAF to benefit scientists and trainees at all levels, whether that is in the form of presenting to a new audience, organizing the event, or evaluating the presentations.

We are excited to have this year's keynote speech delivered by Dr. Joshua Starmer. Dr. Starmer was an Assistant Professor at the University of North Carolina Chapel Hill when he founded the StatQuest YouTube channel. The channel's original purpose was to help educate his colleagues on statistics, but has since evolved to be a massive, freely available educational resource on math and statistics that has garnered over 1 million subscribers. Beyond being the Founder and CEO of StatQuest, Dr. Starmer also holds the title of Lead AI Educator at Lightning AI.

I would like to thank all the Council members who helped plan this event, with special thanks to those on the executive committee for their diligent planning and effort. I would also like to thank Drs. Kandace Williams and David Giovannucci for guiding the Council and providing us with their advice. This event would not have been possible without the support of the UT COMLS Foundation, the COMLS Alumni Affiliation, and ThermoFisher, and we are very grateful for their contributions. Further, we cannot thank the departments in our program enough for their support, with their donations to GRAF and all the resources they provide that allow us to host this event year after year. Lastly, I would like to express our gratitude for Margaret Hoogland and the journal *Translation* for working with us to get the abstracts published in a special GRAF issue. I hope all attendees enjoy the forum and walk away richer for the experience!

Benjamin W. French
President
Council of Biomedical Graduate Students
University of Toledo

Schedule

Thursday, March 21, 2024

Poster Presentations: 9:00 am – 12:00 pm

Location: Collier Building Room 2409

Lunch: 12:00 pm – 1:00 pm

Location: Collier Building Room 2409

Oral Presentations Group 1: 1:00 pm – 2:30 pm

Oral Presentations Group 2: 2:30 pm – 4:00 pm

Oral Presentations Group 3: 4:00 pm – 5:30 pm

Location: Collier Building Room 1200

Friday, March 22, 2024

Final Poster and Oral Presentations: 9:00 am – 11:30 pm

Location: Collier Building Room 1200

Lunch with Keynote Speaker: 12:00 pm – 1:00 pm

Location: Collier Building Room 2409

Keynote Reception: 3:00 pm – 3:30 pm

Location: Health Education Building 105

Keynote Lecture & GRAF Winner Announcements: 3:30 pm – 5:00 pm

Joshua Starmer, PhD: *"Neural Networks: Where they are now, where they came from, and where they might go"*

Location: Health Education Building Room 105

Keynote Speaker: Joshua Starmer, PhD

Dr. Joshua Starmer is the Lead AI Educator at Lightning AI and the CEO and Founder of StatQuest, an educational resource on YouTube for statistics, math, and machine learning. Dr. Starmer has sat on the Board of Directors for the Society for Scientific Advancement, was the co-founder and head of software development at SeqQuest.

Dr. Starmer received his PhD from The University of North Carolina at Chapel Hill, where he proceeded to also complete a Postdoctoral Fellowship working on X chromosome inactivation and nuclear organization. He then went on to become an Assistant Professor studying novel statistics and visualization methods for high-throughput sequencing technologies. During his time as an Assistant Professor at UNC Chapel Hill, Dr. Starmer started his StatQuest channel with the original intention of helping his colleagues understand the math behind their experiments, giving them more powerful ways to conduct studies and draw results. Since then, he has continuously built up the resources available on his channel, which now boasts more than 250 videos covering a wide variety of statistics-based concepts and has gained over 1.1 million subscribers.

Dr. Starmer has published papers covering a wide variety of topics, including immunology and autoimmunity, protein synthesis, genetics and DNA methylation, stem cells, and cellular differentiation. One of his most prominent publications, in *Genetic Epidemiology*, illustrated a multiple testing correction method for single nucleotide polymorphisms in genetic association studies and has been cited over 700 times.

Beyond being an excellent scientist, Dr. Starmer is an excellent educator to both students and the public. He has appeared on podcasts like “The Engineered Mind Podcast” (Ep. 52) “Decisions Now” (Ep. 11) and “Super Data Science” (Ep. 553) to help discuss and pick apart complicated topics and exciting developments in statistics and machine learning. Dr. Starmer has also published a book, entitled “The StatQuest Illustrated Guide to Machine Learning,” which aims to help audiences of all types to understand the complicated concepts built into machine learning. Outside the world of science, Dr. Starmer is a very talented musician; since January of 2013, he has recorded 8 albums, and has been the composer and performer of electro-acoustic cello music for the modern dance company Gaspard and Dancers.

The Council of Biomedical Graduate Students is thrilled to have such an enthusiastic Keynote speaker who has such a wide-ranging career for our 42nd Graduate Research Annual Forum.

Poster and Oral Presentation Procedure

Preliminary Session:

Each group will be judged by three faculty members/post-doctoral fellows and one finalist will be selected from each group.

Final Session:

Finalists will be presented to a special panel of judges, consisting of:
Dr. Joshua Starmer, 2024 Keynote Speaker
Dr. Kandace Williams, Senior Associate Dean, College of Medicine & Life Sciences Graduate Programs
Dr. Stanislaw Stepkowski, Professor of Medical Microbiology and Immunology

Prizes:

The top three participants (from each oral and poster sessions) will be awarded:
$300 for first place
$200 for second place
$100 for third place

Poster Presentations: March 21st, 2024

Note: Both Group 2 and Group 3 will fill the same time slot but are judged separately.

Group 1 COB 2409, 9:00 – 10:30	Christopher Figy Nicole Bell Matthew Hathaway Ryan Harris Iluja Gautam Shruti Ghai Sanjana Kumariya Jaya Bhandari Brooke Ring Subhra Kanti Dey
Group 2 COB 2409, 10:30 – 12:00	Adriana Alviter Plata Hugo Sigona Gonzalez Somayeh Darzi Emily Crossley Saroj Khadka Dipesh Pokharel Sydnee P Dressel Bivek Timalsina Azeezat Osikoya Marziyeh Salehi Jahromi
Group 3 COB 2409, 10:30 – 12:00	Jennifer Nguyen Daniella Gamboa Ali Imami Nilanjana Chakrabarti Saferin Rejina Shrestha Smita Sahay Emily Kinney Dhilhani Faleel Augustine Kwabil William George Ryan

Oral Presentations: March 21st, 2024

Group 1 COB 1200, 1:00 – 2:30	Hemaa Sree Kumar Gabby Vento Ishan Manandhar John B. Presloid
Group 2 COB 1200, 2:30 – 4:00	Mir Himayet Kabir Taylen O. Arvay Jessica M. Jiron Mrunmayee R. Kandalgaonkar
Group 3 COB 1200, 4:00 – 5:30	Emma Elizabeth Sabu Kattuman Kesha Dalal Deepti Gurung Sachin Aryal

Streamlining High Throughput Kinome Analysis: Introducing KADL, a Comprehensive Kinome Analysis Description Language

Imami Ali Sajid[1], Robert McCullumsmith, MD, PhD[2,3], William George Ryan V[1], Hunter Eby[1], Jennifer Nguyen[1]

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Introduction: We deployed the PamGene kinome array platform for use with postmortem brain samples and iPSCs from schizophrenia (SZ) and Alzheimer's dementia (AD). The kinome array platform provides a read-out of protein kinase activity across hundreds of peptide substrates, measuring global protein kinase activity across serine/threonine and tyrosine subkinomes. We used this omics-based platform to generate novel hypotheses for the pathophysiology of severe neuropsychiatric disorders with cognitive dysfunction.

Methods: We used the PamGene kinome array platform to assess protein kinase activity in disease (AD, MDD, and SZ) and control (SZ) samples. We also evaluated protein kinase activity in stem cell cultures for these disorders. We used R programs (KRSA and UKA) to deconvolve the generated kinome array datasets to identify specific protein kinases altered across these disorders. Information for deconvolution of datasets was supplemented with recombinant kinase and kinase perturbation studies.

Results: A joint hit for AD and SZ was adenosine monophosphate kinase (AMPK), a master regulator of insulin signaling pathways. Subsequent studies of AMPK in AD and SZ reveal subunit-specific deficits in the frontal cortex in AD, with changes in the regulatory subunits for AMPK. Bioinformatics analyses revealed several novel pathways and several candidate drugs that might be repurposed for treating cognitive deficits in these disorders.

Conclusions: We used a hypothesis-free, kinome-based approach to extend our understanding of the pathophysiology of SZ and AD and provide novel leads to advance the diagnosis and treatment of these often-devastating illnesses.

Reduced Colonic Expression of Fat/CD36 is Associated with Elevated Fecal Calorific Values and Enrichment in Bacterial Fatty Acid Metabolism in a Mouse Model Characterized by Reduced Diet-Induced Adiposity and Weight Gain

Adriana Alviter-Plata[1], Christopher Martyniuk[2], Jennifer Gadient[3], Jasenka Zubcevic[4]

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Introduction: Dysfunctional neuro-immune axis is a hallmark of cardiometabolic disease. Our previous research showed that genetic ablation of beta-adrenergic receptors 1 and 2 (ADRB1/2) selectively on bone marrow (BM) hematopoietic cells reduced immune responses and altered gut microbiota in a novel BM chimera mouse model. This was correlated with lower expression of colonic fatty acid (FA) transporter Fat/CD36, suggesting decreased dietary fat absorption.

Methods: To measure this, male C5BL/6 wild type (WT) mice were irradiated and reconstituted with whole bone marrow cells from C57BL/6 WT controls or ADRB1/2 knock-out mice (KD) to generate the BM chimera. Post-recovery mice were administered either a control (CNT) or high-fat diet (HFD) for two weeks ad libitum. Weekly food intake and body weight, and endpoint retroperitoneal fat weight were measured in all mice. Metagenomic/16S sequencing analyses, and measurements of fecal FAs and residual calorific values were performed at endpoint.

Results: Despite similar food intake, WT on HFD presented with elevated body weight and retroperitoneal fat compared to the KD chimera. In addition, the WT presented with elevated gut bacterial α-diversity compared to the KD on HFD, while an enrichment in p_Bacteroidetes and bacterial FA metabolism was observed in the KD mice, suggesting elevated utilization of dietary FAs by the gut bacteria. Moreover, increased fecal calorific values were observed in the KD compared to WT on HFD, with no difference in bacterial short chain FAs, suggesting reduced dietary fat absorption in the KD mice.

Conclusions: Reduced colonic Fat/CD36 expression correlated with reduced diet-induced weight gain and body fat. This may be due to reduced dietary fat absorption, as suggested by higher residual fecal calorific values. An enrichment in Bacteroidetes, reportedly negatively correlated with obesogenic diets, and enhanced bacterial FA metabolism in the KD on HFD suggest a potential for a probiotic anti-obesity therapy.

PACAP-mediated Regulation of Chromaffin Cell Secretion

Nicole Bell[1], Arun Anantharam[2], David Giovannucci[2]

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Introduction: The adrenal medulla is an important effector of the sympathetic nervous system in the periphery. An increase in sympathetic tone causes the medulla to release epinephrine, norepinephrine, and other hormones in the bloodstream for circulation throughout the body. The secretory units of the adrenal medulla are chromaffin cells. Chromaffin cells synthesize and store catecholamines and peptide hormones in dense core granules and release these agents as a result of exocytosis. In situ, chromaffin cell exocytosis is triggered by the neurotransmitters, acetylcholine and pituitary adenylate cyclase activating polypeptide (PACAP). However, the mechanisms by which PACAP causes exocytosis in chromaffin cells is poorly understood.

Objectives: The goal of this study is to further our understanding of how PACAP activation of chromaffin cells causes exocytosis.

Methods: We used time-resolved membrane capacitance measurements in combination with perforated patch clamp methods in primary cultured adrenomedullary chromaffin cells from adult mice to measure and determine the relationship between calcium entry and secretory activity. In addition, we used pharmacological and genetic approaches to elucidate how PACAP affects secretory output.

Results: We showed that PACAP treatment increased the calcium sensitivity of exocytotic activity and increased the readily releasable pool size. This PACAP-mediated enhancement of depolarization-evoked secretion is mitigated when the cell is pretreated with PKC antagonist NPC-15437, indicating that PACAP activation of PKC is critical for the enhancement of chromaffin cell secretion.

Conclusions: PACAP application during depolarization-induced exocytosis appears to enhance both the calcium sensitivity and the number of vesicles available for exocytotic release. This PACAP-mediated enhancement appears to rely on the activation of PKC. Understanding how PACAP affects chromaffin cell secretory activity provides new insights into how splanchnic signaling tunes the neuroendocrine stress response.

Role of 14-3-3ζ in the Activation-Induced Cell Death

Jaya Bhandari[1] and Ritu Chakravarti, PhD[2]

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[2]Department of Physiology and Pharmacology, The University of Toledo, Toledo, Ohio 43614

Introduction: Immune cell dysfunction is a critical step in the pathogenesis of autoimmune diseases. Activation-induced cell death (AICD) occurs in various immune cells, especially T cells, following antigen receptor ligation. AICD plays a significant role in maintaining peripheral immune tolerance. We showed that 14-3-3ζ is an autoantigen in human aortitis.

Methods: To investigate the immunological functions and role in autoimmune conditions, we generated 14-3-3ζ knockout Lewis rats. Under two distinct experimental models, 14-3-3ζ knockout rats showed their crucial role in alleviating inflammatory arthritis (IA). To elucidate the mechanisms underlying 14-3-3ζ anti-inflammatory action, we studied its role in the AICD of immune cells. We investigated the CD3/CD28 activation of primary splenocytes isolated from wild-type and 14-3-3ζ knockout rats.

Results: Our data showed that the viability of primary splenocytes upon T cell receptor activation is reduced in the presence of 14-3-3ζ. We extended these results to explore whether 14-3-3ζ modulates AICD in macrophages, employing various inducers such as TNF-a, LPS, and IFN-g. Preliminary results suggest that the AICD in macrophages operates independently of 14-3-3ζ.

Conclusions: This study is innovative in demonstrating that 14-3-3ζ is implicated in the AICD of T cells but not in macrophages, signifying cell-type-specific effects. Ongoing research is directed at understanding how AICD influences the pathogenesis of inflammatory arthritis and the potential implications of 14-3-3ζ -regulated cell death in its anti-inflammatory role.

Profound Non-Randomness in Dinucleotide Arrangements within Ultra-Conserved Non-Coding Elements and the Human Genome.

Emily R. Crossley[1], Larisa Fedorova[2], Oleh A Mulyar[2], Shuhao Qiu (Faculty)[3], Ryan Freeman[1], Alexei Fedorov (PI)[1,2,3]

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Introduction: Ultra-conserved non-coding elements (UCNEs) remain fascinating genomic fragments that have maintained almost perfect sequence identity for millions of years. UCNEs are defined as regions of DNA that are longer than two hundred nucleotides in length and at least 95% conserved between humans and chickens. Previous research found that UCNEs have an excess of GpC dinucleotides but a decrease in GpG/CpC dinucleotides compared to the human genome. However, similarities between UCNES or other characteristics that make them unalterable have yet to be unveiled.

Objectives: Based on these findings, we hypothesize that UCNEs have a distinct dinucleotide composition that may contribute to a unique DNA structure. We calculate the distance between all dinucleotide pairs within UCNEs and the human genome to identify patterns in dinucleotide arrangements.

Methods: We purified a publicly available UCNE database, which includes a total of 4,272 sequences, and human genome sequences with masked repetitive elements. Randomly generated UCNE and human genome sequences were created using the dinucleotide frequencies from the real perspective sequences. Statistical analysis was performed to assess the non-randomness in dinucleotide spacing arrangements using relative percentage difference (RPD).

Results: Remarkable non-randomness in dinucleotide spacing arrangements was observed within the entire human genome and UCNEs. Approximately 83% of all dinucleotide pairs within UCNEs showed significant (>10% RPD) non-random genomic arrangements when compared to the rest of the human genome. Most non-random arrangement of dinucleotide pairs occurred at short distances, 2-6 nucleotides. Non-randomness in dinucleotide spacing distances deviated up to 40% from the expected values and were frequently associated with GpC, CpG, ApT, GpG, and CpC dinucleotides.

Conclusion: The described peculiarities in dinucleotide arrangements have persisted for hundreds of millions of years within vertebrates. These distinctive patterns may suggest that UCNEs form a unique DNA structure with distinct properties that contribute to their extraordinary conservation.

The Role of Cysteinyl Leukotrienes and Their Receptors in EC-Macrophage Interaction and Therapeutic Implications for Atherosclerosis

Somayeh Darzi[1], Lakshminarayan Reddy Teegala[2], Emma Elizabeth Sabu Kattuman[1], Sailaja Paruchuri[3]

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Introduction: Cardiovascular disease (CVD) remains a global health threat with atherosclerosis at the forefront. Atherosclerosis involves low density lipoprotein oxidation, monocyte infiltration through endothelial cells (EC), smooth muscle cell (SMC) proliferation leading to plaque formation and chronic inflammation. During inflammation, cysteinyl leukotrienes (cys-LTs; LTC4, LTD4, LTE4) are released from the membrane via the 5-lipoxygenase pathway and exert their effects via cysteinyl leukotriene receptors (CysLTR) 1 and 2. Since these receptors transduce inflammatory signals and regulate EC and macrophage dysfunction, we hypothesized that they play vital role in macrophage-EC interactions in a co-culture and contribute to atherosclerosis.

Methods: Mouse dermal ECs were co-cultured with bone marrow-derived macrophages (BMDM) in the presence or absence of CysLT1R and CysLT2R antagonists MK571 and BayCysLT2 respectively for 6-hours. Culture supernatant and cells were collected for ELISA and qPCR analysis, respectively. EC contraction was determined by F-actin staining.

Results: We observed a significant increase in pro-inflammatory cytokine Interleukin (IL)-6 in the EC-BMDM co-culture. Further, BMDM in co-culture upregulated IL-6, IL-1β, Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) and Oxidized Low-Density Lipoprotein Receptor-1 (OLR-1) transcripts with a simultaneous reduction in the Vascular Endothelial Growth Factor (VEGF) transcript. ECs in co-culture exhibited an increase in IL-6, and upregulation of adhesion molecules like Intercellular Adhesion Molecule-1 (ICAM-1) and Vascular Cell Adhesion Molecule-1 (VCAM-1) transcripts and a contractile response revealed by gap formation. Importantly, IL-6 was significantly inhibited by both MK571 and BayCysLT2 suggesting that CysLTR signaling mediate EC-macrophage interactions.

Conclusion: Our results suggest that blocking CysLTR may offer a promising therapy to prevent plaque initiation during atherosclerosis.

Blocking Parathyroid Hormone 1 Receptor Inhibits Prostate Cancer Metastases

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Introduction: Patients with metastatic castration-resistant prostate cancer (mCRPC) have 30% of the 5-year survival rate and contribute significantly to prostate cancer-related death. Parathyroid hormone-related protein (PTHrP), secreted by cancer cells, is shown to be a driver for cancer-induced bone metastasis, including mCRPC. However, clinical trials using PTHrP monoclonal antibodies demonstrated only palliative effects. On the other hand, PTHrP affects through the only known receptor, parathyroid hormone 1 receptor (PTH1R), which is a member of the G protein-coupled receptor that consists of up to 35% of all clinical drug targets. Therefore, we will investigate the role of PTH1R in prostate cancer metastasis using genetic and pharmacological approaches.

Methods: To investigate the paracrine effect of PTH1R on advanced prostate cancer metastases, we crossed the floxed PTH1R ($PTH1R^{FloxE2}$) mouse with the Col1α2 CreERT mouse. Following tamoxifen administration, the Cre-positive mouse's mesenchymal cell-specific PTH1R gene is deleted, producing a $PTH1R^{ColCreERT}$ KO mouse. The Cre-negative littermates with the same tamoxifen injections were used as the control $PTH1R^{FloxE2}$ mouse. Pharmacologically, we tested the effect of blocking PTH1R on prostate cancer cell growth and viability.

Results: Human prostate cancer cells, PC3 cells (luciferase labeled), were intracardially injected into both $PTH1R^{FloxE2}$ and $PTH1R^{ColCreERT}$ KO littermates. We found PC3 metastases in various organs, including the liver, kidney, and bones. The overall and organ-specific metastases, such as bone metastases, were significantly inhibited in the $PTH1R^{ColCreERT}$ KO, compared to the $PTH1R^{FloxE2}$ mice, suggesting that blocking the paracrine effects of PTH1R effectively inhibits prostate cancer metastases. In vitro, we used a small molecule inhibitor called XC039 and a commercially available PTH1R peptide antagonist (Asn10, Leu11, D-Trp12)-PTHrP(7-34) amide to block PTH1R. Both antagonist and XC039 can suppress ligand-dependent cAMP production and XC039, but not the peptide antagonist, significantly inhibited prostate cancer cell growth.

Conclusions: These data suggest that inhibiting PTH1R could effectively inhibit prostate cancer metastases.

Synthetic Psychoactive Cathinones (SPCs): Predicting Toxicity using *In Vitro* and *In Vivo* Models

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Introduction: In 2021 a staggering 33,000 American lives were lost to psychostimulant overdoses, accounting for over 30% of all drug overdoses that year. Synthetic psychoactive cathinones (SPCs) are novel psychoactive substances with effects like cocaine, methamphetamine, and methylenedioxymethamphetamine (MDMA). SPCs are of great concern because their abuse liability and potential for adverse effects, including lethal overdose, are largely unknown. Cell culture can help streamline toxicity assessment of new drugs of abuse.

Methods: For zebrafish studies, 5-day post fertilization (dpf) wildtype larval fish were exposed to various concentrations of SPCs to determine lethal dose 50 (LD50). For In vitro studies cell viability was assessed using 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT). HepG2 (hepatic), AC-16 (cardiac), and SH-SY5Y (neural) cells were exposed to each SPC to determine the half-maximal inhibitory concentration (IC50).

Results: The LC50 values in zebrafish exhibited a correlation with the IC50 values of both the HepG2 (R^2= 0.8653, F(1,6)= 38.53, p= 0.008) and SH-SY5Y (R^2= 0.5762, F(1,6)= 8.158, p= 0.0289) cells. However, there was no significant correlation between zebrafish lethality and AC-16 toxicity (R^2= 0.3182, F(1,6)= 2.801, p= 0.1452).

Conclusion: The toxicity evaluation of SPCs in HepG2 and SH-SH5Y cell lines predict lethal toxicity in zebrafish. The similarity in toxicity patterns between these cell lines and zebrafish strengthens the potential utility of predictive *in vitro* models of SPC-induced lethal toxicity. The correlation between the toxicity in both cell lines and zebrafish indicates that the adverse and lethal effects of SPCs involves cellular mechanisms rather than physiological factors. Therefore, cell culture can provide insights into the cause of *in vivo* lethality and reduce the number of vertebrate subjects necessary for the study of the toxicity of these drugs, in line with the 3R principle of animal research.

Interplay Between Cardiotonic Steroids, Paraoxonases, and 20-Hydroxyeicosatetraenoic Acid in Chronic Kidney Disease: Insights from Molecular Simulation and In Vivo Studies.

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Introduction: Chronic kidney disease (CKD) progression is strongly linked to chronic inflammation. Cardiotonic steroids (CTS), elevated in volume-expanded conditions like CKD, induce renal inflammation by binding to Na+/K+-ATPase. Concurrently, Paraoxonases (PON-1, PON-2, and PON-3), endogenous enzymes hydrolyzing α-pyrone structures like CTS, play a crucial role. Arachidonic acid metabolites, particularly 20-Hydroxyeicosatetraenoic acid (20-HETE), are known pro-inflammatory mediators elevated in CKD.

Objective: This study explores the molecular interactions between PON-1-CTS, 20-HETE, and Na+/K+-ATPase (NKA), aiming to elucidate the connection between these proinflammatory molecules.

Methods: Molecular simulations analyzed the NKA-CTS interaction, revealing consistent binding with NKA, especially with ASN 168 via hydrogen bonds for 75% of the simulation. Other residues, including ASN 224, ASP 69, and ASN 270, exhibited variable interactions at different time points, while His 115 interacted briefly. Subsequently, the study investigated whether CTS activates 20-HETE production in vivo. PON-1 knockout (KO) and wild-type (WT) rats were subcutaneously injected with TCB for one week. LC/Ms/Ms analysis of TCB-infused rats assessed 20-HETE production in the kidneys.

Results: The molecular simulations demonstrated sustained interactions between CTS and NKA, suggesting the potential involvement of His 115 in CTS hydrolysis. In vivo analysis revealed a significant increase in 20-HETE production in TCB-infused rats, establishing a plausible link between CTS, NKA, and 20-HETE in CKD progression.

Conclusion: This study provides novel insights into the molecular interactions between CTS, PONs, and 20-HETE, emphasizing their roles in CKD. The combination of molecular simulation and in vivo experimentation enhances our understanding of the intricate pathways involved in renal inflammation. These findings open avenues for targeted therapeutic interventions in CKD, addressing the complex interplay of these molecules in disease progression

Cdk1-mediated phosphorylation of RKIP regulates spindle checkpoint in breast cancer

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Introduction: RKIP was identified initially as a physiologically relevant inhibitor of Raf-MEK-Erk signaling cascade. It has since been found to modulate several additional signaling pathways including NF-□B, GPCR, GSK, and IFR signaling pathways.

Methods: To understand how the pleiotropic actions of RKIP are specified in cells, we set out to identify novel phosphorylation sites that may determine RKIP's effects on different pathways. We interrogated post-translational modification database PhosphoSite Plus® and identified Thr-42 as a residue in RKIP explicitly phosphorylated during M-phase of cell cycle. RKIP has been previously implicated in regulating spindle checkpoint during mitosis.

Results: However, the mechanism is not well defined. We now show that Thr-42 phosphorylation is required for RKIP to function as a mitotic spindle checkpoint regulator. With Thr-42 phosphorylation specific monoclonal Ab we show that RKIP is only phosphorylated during G2/M phase of cell cycle. We show that the expression of M-phase cdk1/cyclin B1 is important for Thr-42 phosphorylation. We show that RKIP depletion decreases transversal times from nuclear envelope breakdown to anaphase. The reverse is true when the expression of RKIP is augmented by ectopic expression. We also show that the shortened mitotic transversal times due to a knockdown of RKIP expression can be rescued with wild-type but not Thr-42 non phosphorylatable RKIP mutant.

Conclusions: These results indicate that Thr-42 phosphorylation is required for RKIP to function as a spindle checkpoint regulator. The molecular mechanism of how phosphor-RKIP regulates spindle checkpoint is currently under investigation.

Social transmission of negative valence in prairie voles using a new behavioral assay that assesses social learning.

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Introduction: Autism Spectrum Disorder (ASD) is a neurodevelopmental condition marked by notable deficiencies in social interaction, social communication, and repetitive behaviors. The intricate nature of these behaviors and the underlying molecular basis of ASD remain challenging to comprehend. A lack of behavioral assays that assess social learning deficits in animal models is one of the biggest challenges in the field.

Objectives: The study aims at developing a new behavioral assay in prairie voles, an animal model that exhibits prosocial behaviors, that aims to measure social learning aptitudes based on the observation of subtle social cues in their stressed partners following a fear conditioning paradigm.

Methods: Same-sex adult pairs of prairie voles (housed together since weaning, ntotal = 50, two groups) were tested on the newly developed paradigm called social transmission of negative valence in voles (STNV), which is an adaptation of a previous paradigm. STNV is a two-day behavioral paradigm that consists of fear conditioning demonstrators on day 1 to the tone, by associating 15 tones (30s, 6KHz) to 15-foot shocks (1s, 1mA) or no shocks (in the control group). Next, the partner (observer) is brought to the experimental cage to observe demonstrators (through a clear barrier) freeze to the tone during a fear memory recall task. On day 2, we measured freezing behavior in observers during the re-introduction to the experimental cage.
Social learning was assessed by the percentage of observers' freezing, rearing, self-grooming, and by ultrasonic vocalizations they exhibit in both groups (fear conditioned demonstrators and control demonstrators). Rodent ultrasonic vocalization serves as an indicator of social communication and conveys their emotional state.

Results: We found a significant increase in freezing behavior in observers in the experimental group as compared to the control group ($p<0.05$). Also, observers showed a significant increase in self-grooming, and rearing behaviors in the experimental group as compared to the control

group ($p<0.05$). Ultrasonic vocalizations also show a significant increase in frequency of calls in experimental observers (56-95 kHz) compared to controls (20-45 kHz, $p<0.05$).

Conclusion: STNV yields promising outcomes as a social learning paradigm, offering insights into how these rodent species learn through social transmission of subtle cues. Future directions consist of investigating the neural correlates of social learning and the effects of targeted drugs on social behavior.

Protective immune role of platelets during respiratory viral infection

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Introduction: Platelets are small, anucleate cells derived from megakaryocytes. Conventionally, they are known for an indispensable role in hemostasis. Additionally, research in the past decade has now established platelets as orchestrators of immune response. At a molecular level, platelets express receptors that allow them to interact with viruses leading to platelet activation. Activated platelets can directly affect viral replication and modulate leukocyte behavior. Interestingly, thrombocytopenia is commonly observed during viral infections and is associated with worse disease outcomes. Most studies on platelets and viral infections are focused on severe viremic infections. However, the role of platelets and their impact on pulmonary infections such as those caused by Respiratory Syncytial Virus (RSV) is not clear.

Methods: Sendai virus (SeV) was used as a model pathogen. Flow cytometry was used to show in vitro platelet activation (P-selectin expression – CD62P) and internalization of SeV. Survival experiments post intranasal SeV infections were conducted using a mouse model of platelet depletion developed in our laboratory. Tissue damage was assessed by histology and immunohistochemistry. Viral loads were measured using qRT-PCR.

Results: Upon intranasal challenge with virus, control mice with normal platelet counts exhibited mild symptoms with no mortality. However, platelet depleted mice were highly susceptible to infection, had severe weight loss and high mortality rates. Detailed analysis of infected lungs showed that platelets modulate neutrophil accumulation in the lungs without affecting viral loads significantly. Histological analysis also revealed high levels of myeloperoxidase positive cells and severe tissue damage post infection in absence of platelets.

Conclusion: This study identified a significant protective role of platelets in immune response against respiratory viruses. The outcome of this study reinforces platelets as a therapeutic target to combat severe pulmonary viral infection.

HSF1 inhibition induces pancreatic ductal adenocarcinoma autophagy through JNK1/2

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Introduction: Pancreatic ductal adenocarcinoma (PDAC) is currently the third leading cause of cancer-related death, with a 5-year survival rate of only 13%. The most common treatment challenge is chemoresistance due to autophagy and proteotoxic stress response (PSR). Autophagy regulated by mTORC1 contributes to cellular homeostasis. However, excessive autophagy leads to cell death. Heat shock factor 1 (HSF1), a crucial transcription factor in PSR, is a pro-oncogenic factor in tumorigenesis. HSF1 has been shown to regulate autophagy and contribute to the growth of PDAC, yet the connection between HSF1 and autophagy in PDAC is unknown. c-Jun N-terminal kinase1/2 (JNK1/2) signaling activation under chemotherapy is known to maintain the autophagy and apoptosis equilibrium. HSF1 suppresses JNK and maintains the mTOR activity to combat stress and promote cell proliferation. This study aims to explore the HSF1-JNK axis in regulating autophagy in PDAC.

Method: The autophagy markers, including lipidation of microtubule-associated proteins 1A/1B light chains (LC3) and autophagosome formation, were detected by immunoblotting and fluorescence microscopies in human PDAC cells. Autophagy flux was measured via RFP-GFP-LC3 reporter cells. Cell viability was detected by CellTiter-Blue® Cell Viability Assay, and apoptosis was detected by the Caspase-3 Activity Assay.

Results: In multiple PADC cell lines, HSF1 inhibitors (HSF1i) suppressed HSF1 phosphorylation at Ser326 and increased lipidation of LC3, while overexpression of HSF1 reversed this effect. Results from fluorescence imaging also revealed that HSF1 inhibition significantly increased autophagosome formation. HSF1i induced phosphorylation of JNK1/2 at Thr183/Tyr185 and decreased mTORC1 activity. Knockdown of JNK1/2 via JNK1/2 siRNA reversed the HSF1i-induced autophagy. Inhibition of autophagy via 3-methyladenine enhanced HSF1i-induced apoptosis in PDAC.

Conclusion: JNK1/2 is involved in HSF1 inhibition-induced PDAC autophagy. HSF1 inhibition-regulated autophagy could potentially be a novel target for treating pancreatic cancer.

Assessing the abilities of Factor H-Fc IgG fusion protein variants as a therapeutic against *Burkholderia pseudomallei*

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Introduction: *Burkholderia pseudomallei* (Bp) is a Gram-Negative bacterium and is emerging as a global health threat, including the America's, causing melioidosis and lethal sepsis. Because Bp has a LD_{50}≥organisms, it is designated as a Tier 1 select agent due to its bioweapon potential. Bp is naturally resistant to most antibiotics and there is no vaccine, thus there is a great need for therapeutics.

Methods: One of Bp's important virulence mechanisms is its ability to evade the host complement system. We have identified a surface protein expressed by Bp that can bind host Factor H, which is a negative regulator of the complement cascade and thus promotes immune evasion. Focusing on this mechanism, we are collaborating with Planet Biotech which has generated several chimeric molecules which contain the host binding site for Factor H and the other portion consists of the Fc region of human immunoglobulin G. Thus, this chimera should competitively bind to the bacterial surface, eliminating their ability to bind functional Factor H, and the IgG Fc region should activate the complement cascade to mediate direct and/or opsonophagocytic killing by immune cells.

Results: Our preliminary studies indicate that a subset of the initial constructs were able to bind to Bp, initiate C3 deposition, and generate membrane attack complexes (MAC) on their surface using ELISA. Based on these findings, we are now testing a second generation of constructs. Our current findings indicate that a subset of these new constructs are able to bind to, elicit C3 deposition, and generate MAC on Bp's surface better than the original construct. These chimera's are also able to promote direct killing of Bp strains.

Conclusion: Future studies will test their ability to promote opsonophagocytic killing by neutrophil/macrophages and protect mice from challenge with Bp.

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

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Introduction: Tick-borne flaviviruses (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 up to 20%. There are currently no treatments to protect against flaviviral disease complications. Therefore, there's an urgent need to uncover new therapeutic targets to combat infection. In the natural life cycle of TBFV, reservoir hosts, such as the white-footed mouse (*Peromyscus leucopus*), maintain virus without suffering debilitating symptoms. Uncovering the molecular mechanism by which these mice resist viral infection may lead to development of drugs that can translate this resistance to humans. Therefore, we hypothesize that *P.leucopus* coevolution with flaviviruses has led to the development of potent antiviral pathways that inhibit viral replication and pathogenesis.

Methods: Previous work from our lab has shown that *P.leucopus* fibroblasts display a 10,000-fold decrease in TBFV virion production when compared with susceptible mice, *M.musculus*. 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 neutralized through action of transcription factor Nrf2 and subsequently induced antioxidant proteins. Consistently, Nrf2 and antioxidants are abundantly expressed in *P.leucopus* compared to M.musculus during infection.

Results: Susceptible human and mouse cells treated with drugs that activate the Nrf2 pathway show a significant reduction in viral replication, similar to resistant *P.leucopus* cells. Therefore, restriction in *Peromyscus* is linked to resistance to oxidative stress and this can be replicated in susceptible cells. Comparative replication studies revealed that virus resistance to reservoir host restriction maps to mutations in the viral envelope protein, viral polymerase and protease enzymes.

Conclusion: Understanding how virus mutation overcomes Peromyscus-mediated restriction reveals insight into TBFV maintenance in nature and how best to leverage the host restriction for therapeutic design.

Investigation into TRAF6-NS3 functionality during tick-borne flavivirus infection

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Introduction: The increasing incidence of flavivirus infections have exacted an enormous burden on healthcare systems worldwide. This is exacerbated by the lack of therapeutics available and limited availability of vaccines. A recent publication by our lab explored the beneficial role of TRAF6 in tick-borne flavivirus (TBFV) infections through interaction with the viral protease NS3. TRAF6 plays a central role in Toll-like receptor (TLR) and RIG-I-Like receptor (RLR) signaling cascades. These antiviral pathways are critical for recognizing pathogens and activating the innate immune system; thus, it was surprising that the TRAF6-NS3 interaction evolved a proviral role for tick-borne flaviviruses and importantly identified a unique therapeutic target. The novelty of the TRAF6-NS3 complex was further highlighted by the fact that this interaction was strictly conserved only in TBFV and not mosquito-borne flaviviruses (MBFV). Previous research into TRAF6 interactions has demonstrated that many viruses attempt to prevent TRAF6 activation by degrading it. The proviral role of TRAF6 in TBFV also suggests that the canonical function is suppressed. Since TRAF6 is an E3 ubiquitin ligase we have explored how ubiquitination affects the NS3 protease and contributes to the suppression of canonical TRAF6 functionality.

Methods: To answer these questions, we identified lysine residues on the NS3 protease and performed site-directed mutagenesis on plasmid constructs. After confirming sequences, we performed ubiquitination assays in the presence of TRAF6 to determine phenotypic changes. We also performed luciferase using an NF-κB reporter to measure changes in TRAF6 activation in the presence of NS3.

Results: These results revealed that the NS3 protease had increased levels of total ubiquitination in the presence of TRAF6. We also found that NS3 protease suppressed TRAF6 activation and could be restored by attenuating the interaction with a mutation.

Conclusions: We have demonstrated that the TRAF6-NS3 interaction results in protein ubiquitination. Future studies will work to identify the specific residues involved and may identify potential therapeutic sites.

Role of Insulin Signaling in Prostaglandin Synthesis

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Introduction: This study explores the impact of the insulin/FOXO pathway on prostaglandin E2 (PGE2) synthesis in hypothalamic astrocytes. Previous research established insulin's role in activating FOXOs, leading to the expression of PTGS (COX-1, 2) and PTGES genes, ultimately driving PGE2 synthesis in astrocytes and influencing fertility. While earlier findings indicated insulin's ability to regulate PGE2 pathways in distinct astrocyte cell lines, questions remained about its specific effects on hypothalamic astrocytes.

Methods: To address this, astrocyte cell lines and primary astrocytes were isolated and treated with insulin or a control. Quantitative PCR and western blotting confirmed that 250nM of insulin induced Cox-2 expression within 30 minutes. Concerns about nonspecific signaling led to a decision to treat at 100nM for 6 hours. Transcriptomic analysis of RNAseq data revealed insulin's down-regulation of sterol and cholesterol biosynthesis pathway genes in male hypothalamic astrocytes. Kinome array analysis identified differentially phosphorylated kinases in the presence of insulin, with some sex-specific patterns.

Results: Notably, insulin phosphorylated AKT1, AR, P53, mTOR, RAF1, CDK1, GYS2, and MAPK10 in both sexes. MAPK1 showed male-specific phosphorylation, while MAPK3 and ISR2 displayed female-specific phosphorylation. In the presence of insulin, increased activity was observed in genes related to autophagy (more pronounced in males), various cancers, insulin resistance, type II diabetes, insulin signaling, FOXO signaling, and GnRH signaling and secretion (more prominent in females).

Conclusions: In conclusion, this study sheds light on how the insulin/FOXO pathway influences PGE2 synthesis in hypothalamic astrocytes. Insulin induces COX-2 expression and modulates pathways linked to sterol and cholesterol biosynthesis. Sex-specific phosphorylation patterns revealed by kinome analysis further contribute to our understanding of insulin and FOXO regulation in astrocytes, impacting PGE2 synthesis and associated pathways.

Klebsiella pneumoniae sugar import suppresses hypermucoviscosity

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Introduction: *Klebsiella pneumoniae* is significant cause of community- and hospital-acquired infections, impacting both immunocompromised and immunocompetent individuals. The co-emergence of drug-resistance and hypervirulence in *K. pneumoniae* has severely limited therapeutic options. Hypermucoviscosity (HMV) is an important *K. pneumoniae* virulence factor that manifests as a 'tacky' bacterial colony due to changes in capsule chain length. Two genetically encoded mechanisms regulating HMV are the regulator of mucoidy phenotype (*rmpD*) and *wzc* activity. We have previously shown that difference in growth medium also alters HMV, but specific nutrient signals and mechanisms involved are still unclear. To address this knowledge gap, we hypothesized that extracellular nutrients such as sugars distinctly induce changes in *K. pneumoniae* HMV without impacting capsule abundance.

Methods: To investigate, we cultured K. pneumoniae strain KPPR1 in M9 minimal medium supplemented with varying sugar concentrations, and measured HMV and capsule production using sedimentation resistance assay and uronic acid quantification, respectively.

Results: Our results demonstrated that all tested sugars, including metabolizable and non-metabolizable sugars, significantly suppressed the mucoidy, while capsule abundance was not impacted similarly. This finding indicates that sugar import in *K. pneumoniae* distinctly regulates HMV. Moreover, sugar supplementation led to significant downregulation of *rmpADC*. To further elucidate the mechanism tying sugar transport to *rmpD* transcription and mucoidy, we screened a transposon library covering ~70% of the KPPR1 genome to identify genes required for suppressing mucoidy in sugar-supplemented M9 medium. The transposon screen identified genes involved in carbohydrate and amino acid transport and metabolism, suggesting their role in sugar-mediated HMV suppression.

Conclusion: These findings collectively suggest that host-derived sugars could act as nutrient signals, selectively regulating *K. pneumoniae* hypermucoviscosity during infection. Further defining the mechanism by which sugars modulate hypermucoviscosity and how this observed phenotype manifests *in vivo* would contribute to better understanding of *K. pneumoniae* pathogenesis.

Effect of Capsule Properties on Infection for Classical and Hypervirulent *Klebsiella pneumoniae*

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Introduction: *Klebsiella pneumoniae* is a gram-negative pathogen that causes urinary tract infections, pneumonia, meningitis, and bloodstream infections. *K. pneumoniae* has two pathotypes – classical (cKp) and hypervirulent (hvKp). cKp cause nosocomial infections and have an increased frequency of antibiotic resistance. hvKp primarily cause invasive, community-acquired infections and have higher levels of capsule and mucoidy. We have recently found that hypermucoid hvKp strains have an increased average capsular polysaccharide chain length. Understanding how hypermucoid hvKp cause invasive infections could identify new therapeutic targets. Capsule has been shown to protect against complement and other host defenses, but there is a gap in knowledge regarding how the specific property of capsule chain length affects bacterial-host interactions.

Methods: The two aspects of capsule that we are studying are the capsular polysaccharide (CPS) chain length variations and released extracellular polysaccharides (EPS). The gene wzc is a regulator of capsule chain length and secretion. We have recently identified hvKp Wzc variants that increase the average CPS chain length and increase EPS release. In this study, we use these Wzc variants and cKp blood isolates as tools to study how specific CPS and EPS properties affect host interactions.

Results: The strains we used have the same K2 capsule composition, but the blood isolates have decreased capsule chain length, CPS abundance, and EPS abundance. We have observed that increased capsule chain length and decreased CPS attachment increase sensitivity to human serum for hvKp. No difference in EPS release was observed between the cKp isolates; however, increased capsule chain length decreases serum sensitivity for cKp. We are also investigating how changes in capsule chain length and CPS attachment in hvKp and cKp change interactions with host macrophages.

Conclusions: In future studies we will determine other roles capsule properties may play in promoting *K. pneumoniae* survival in the human host.

Characterization of the Role of Shikimic Acid in Vascular Smooth Muscle Cells

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Introduction: Vascular smooth muscle cell (VSMC) phenotypic modulation from a contractile to a synthetic state is central to the etiologies of multiple vascular wall diseases such as atherosclerosis, hypertension, and post-angioplasty restenosis. Shikimic acid (SA) is a chemical derived from a variety of plants and microorganisms and has been found to exhibit diverse pharmacological activities in multiple cell/tissue systems, such as antioxidant, anti-inflammatory, and pro-proliferative effects. However, the role of shikimic acid in the vascular system is unknown. The purpose of this study is to examine the effect of SA in regulating VSMC proliferation and migration.

Methods: Using human coronary artery smooth muscle cells (hCASMC), we investigated the effects of SA (10 mM) in vitro on (i) serum-induced VSMC phenotypic switching by immunoblotting or qRT-PCR analyses, (ii) platelet-derived growth factor-BB (PDGF-BB, 30 ng/ml) activation of proliferative signaling, (iii) serum- or PDGF-BB-induced VSMC proliferation by performing WST-1 and CyQUANT assays, and (iv) serum-induced VSMC migration by performing scratch wound assay

Results: SA enhanced the expression of CCND1, a proliferation marker, at both mRNA and protein levels. Consistently, SA enhanced the effects of PDGF-BB on proliferative signaling, including ERK1/2, Akt, and mTORC1 signaling. Conversely, SA activated antiproliferative signaling components including the AMPK/autophagy pathway. Proliferation and migration assays revealed no significant differences after SA treatment as compared to PDGF-BB or serum.

Conclusion: Our findings indicate that SA activates both pro- and anti-proliferative signaling components in VSMCs and suggest that the net outcome of activation of these diverse signaling pathways has little effect on VSMC proliferation or migration.

Osteoblasts-induce Prostate Cancer Cell Dormancy is Maintained by Tight Junction Proteins.

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Introduction: Bone metastasis remains the highest cause of prostate cancer (PCa)-related mortalities. Despite receiving curative treatments, a significant proportion of early-stage patients experience a relapse and progress to bone metastasis. This suggests that PCa cells might have disseminated early and remained dormant during cancer remission. Previous studies have demonstrated the dormancy-inducing ability of osteoblast in the bone microenvironment. The mechanism by which osteoblast induces prostate cancer dormancy is not well elucidated. Our lab has shown the increased expression of claudin-11(Cldn11) in dormant PCa cells.

Methods: Dormancy signatures were analyzed to identify enriched pathways. Public data sets were analyzed for the significance of dormancy signatures. We used a co-culture system to mimic osteoblast-induced- PCa dormancy in the bone. Dormancy phenotype characterization was carried out with qRT-PCR and immunoblotting. We employed shRNA knockdown and gene over-expression to validate the essence of tight junctional proteins in cell dormancy.

Results: Cell adhesion molecules, including Cldn11, were enriched in the analyzed dormancy signatures. Knockdown of Cldn11 rescued the proliferation of the dormant cells. We analyzed published patient datasets to reveal the potential role of Cldn11 in PCa patients. We found that Cldn11 mRNA level correlated positively with increased overall and disease-free survival and positively correlated with NR2F1, a dormancy marker. Cldn11 was high in primary tumors but low in bone metastatic tumors. Furthermore, in C4-2B PCa cells, NR2F1 knockdown reduced Cldn11 at both mRNA and protein levels. PF-271 is a focal adhesion kinase (FAK) inhibitor known to induce PCa dormancy, induced Cldn11 at the protein level.

Conclusion: Cldn11 mediates PCa dormancy, at least in part. Patient data support this. The dormancy marker NR2F1 positively regulates Cldn11 at the protein level, and kinase-active FAK correlates with decreased Cldn11 at the protein level.

Developmental pyrethroid exposure disrupts molecular pathways for circadian rhythms and MAP kinase in mouse brain.

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Introduction: Neurodevelopmental disorders (NDDs) are a category of pervasive disorders of the developing nervous system with few or no recognized biomarkers. A significant portion of the risk for NDDs, including attention deficit hyperactivity disorder (ADHD), is contributed by the environment, and exposure to pyrethroid pesticides during pregnancy has been identified as a potential risk factor for NDD in the unborn child. We recently showed that low-dose developmental exposure to the pyrethroid pesticide deltamethrin in mice causes male-biased changes to ADHD- and NDD-relevant behaviors as well as the striatal dopamine system.

Objective: Here, we used an integrated multiomics approach to determine the broadest possible set of biological changes in the mouse brain caused by developmental pyrethroid exposure (DPE).

Methods: Using a litter-based, split-sample design, we exposed mouse dams during pregnancy and lactation to deltamethrin (3 mg/kg or vehicle every 3 days) at a concentration well below the EPA-determined benchmark dose used for regulatory guidance. We raised male offspring to adulthood, euthanized them, and pulverized and divided whole brain samples for split-sample transcriptomics, kinomics and multiomics integration.

Results: Transcriptome analysis revealed alterations to multiple canonical clock genes, and kinome analysis revealed changes in the activity of multiple kinases involved in synaptic plasticity. Multiomics integration revealed a dysregulated protein-protein interaction network containing

primary clusters for mitogen-activated protein (MAP) kinase cascades, regulation of apoptosis, and synaptic function.

Conclusions: These results demonstrate that DPE causes a multi-modal biophenotype in the brain relevant to ADHD and identifies new potential mechanisms of action.

Role of Helicase Activity of Eif4a1 in Breast Cancer Stemness

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Introduction: Triple-negative breast cancer (TNBC) is the most aggressive subtype of metastatic breast cancer (BC) with poor prognosis. Neoadjuvant chemotherapy (NACT) is the first line of therapy involving taxanes, platinum compounds and/or anthracyclines. There is an initial treatment response but followed by drug resistance, tumor relapse and high proclivity to develop metastases. Recent evidence has shown that there is a small population of cells within the tumor known as breast cancer stem-like cells (BCSCs) that can indeed mediate chemoresistance. BCSCs are capable of self-renewal, tumor initiation and maintain pluripotency through the expression of transcription factors such as SOX2, OCT4 and NANOG. BCSCs play a pivotal role in primary tumor progression and metastases. BCSCs express elevated levels of cancer stemness markers such as aldehyde dehydrogenases (ALDH) and cluster of differentiation (CD44). TNBC patients often exhibit initial sensitivity to neoadjuvant chemotherapy, but eventually become refractory to such therapy (TNBC paradox). Innate or acquired drug resistance by BCSCs may be a crucial factor in therapy failure. Previously, we have demonstrated that the eukaryotic translation initiation factor, eIF4A1, can mediate resistance to paclitaxel in TNBC cell lines *in vitro*. Importantly, eIF4A1 supported the cancer stemness. In this study, we employ *in vitro* and *in vivo* models to definitively identify a causative role for eIF4A1 in cancer stemness.

Method: In order to identify a causal role for the helicase activity of eIF4A1 in contributing to cancer stemness, we will ectopically introduce wild type and mutant forms of eIF4A1 in the knockout background. Following this, bulk tumor cells and isolated BCSCs will be evaluated for the level of cancer stemness attributed by the helicase activity of eIF4A1 by a variety of approaches. Finally, tumor initiation, primary and metastatic tumor burden will be studied in a preclinical murine model when the helicase activity is compromised genetically.

Results: We have successfully generated the wildtype eIF4A1 clone in the lentiviral vector with different promoters. Upon test expression, mutations will be introduced that will abolish the helicase activity of eIF4A1 and evaluate the role of eIF4A1 in cancer stemness in TNBC.

Conclusion: Eliminating breast cancer stemness may pave way for combating clinically refractory tumors. This will eventually increase the quality of life and the longevity of mTNBC patients.

Exploring Hemispheric Dominance: Potential Implications for Success of Cell Transplantation in Parkinson's Disease

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Introduction: Hemispheric dominance refers to the distinct information-processing abilities exhibited by the cerebral hemispheres of the human brain. There is a well-established specialization of function in the two hemispheres, with the left hemisphere primarily specialized for language function in right-handed individuals. In contrast, the right hemisphere is mainly specialized for visuospatial function in right-handed individuals and ambidextrous individuals with no hand preference in humans. Evidence also suggests that paw preference in rats is similar to human handedness. Despite the evolutionary development of hemispheric dominance in humans, their role in cell transplantation for Parkinson's disease (PD) remains poorly studied. Previous studies have indicated that cell transplantation in the striatum of the dominant hemisphere, as opposed to the non-dominant hemisphere in 6-hydroxydopamine lesioned rats, resulted in improved motor behavior. However, the potential underlying factors for the improvement in motor behavior have not been explored. This experiment aims to investigate whether lateralization exists in the case of the substantia nigra pars compacta (SNpc) and striatum between the dominant and non-dominant hemisphere animal groups.

Methods: We hypothesize that animals with the dominant hemisphere will exhibit a higher population of dopaminergic neurons, as well as variation in volume in SNpc and striatum compared to the non-dominant hemisphere animal group. Sprague Dawley rats will be assessed for a paw preference test to determine the degree of handedness (right, left, or ambidextrous) as a measure of hemispheric dominance. Then euthanasia, followed by Cresyl violet (CV) staining and Tyrosine hydroxylase (TH)-immunohistochemistry. Stereological quantification of TH expression will be done on SNpc and striatum in each hemisphere using Stereo Investigator (MBF Bio.) software. Quantification of preliminary data (n= 2) on TH expression in SNpc and striatum showed higher population of dopaminergic neurons in the dominant hemisphere group compared to the non-dominant hemisphere group. Additionally, variation in volume was observed in both SNpc and striatum between the two groups. The potential finding of variation in intrinsic nigrostriatal dopaminergic factors between dominant hemisphere and non-dominant hemisphere animals might be crucial for understanding successful cell transplantation in PD patients.

Amino Acid-Mediated Control of Mucoid Phenotype in Klebsiella pneumoniae

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Introduction: Klebsiella pneumoniae is a Gram-negative bacterium known to cause a wide range of infections, including pneumonia, urinary tract infections (UTIs), and sepsis. Its ranking as the fourth leading cause of bacterial-associated human deaths is of significant concern, underscoring its substantial clinical impact and a profound burden on global public health. Two main pathotypes of K. pneumoniae exist: hypervirulent (hvKp) and classical (cKp). hvKp is associated with severe community-acquired infections and is characterized by a mucoid phenotype. Strains exhibiting mucoidy are associated with increased virulence. The current model is that increases in mucoidy are due to increased average capsular polysaccharide chain length. However, the specific exogenous signals that regulate mucoidy, and their contribution to invasive K. pneumoniae infections, remain unknown.

Methods: In our study, we aimed to explore the influence of amino acids on the regulation of mucoidy in K. pneumoniae. We conducted experiments by culturing the bacteria in low-iron M9 minimal media with varying amino acid compositions. To elucidate the amino acid-responsive pathways, we utilized a combination of techniques, including mucoidy quantification through low-speed centrifugation, capsule quantification based on uronic acid levels, and determination of average capsule chain length using SDS-PAGE visualization.

Results: We observed that the absence of a specific amino acid led to a decrease in mucoidy and changes in the average capsule chain length but does not change capsule abundance. We hypothesized that this amino acid is essential for mucoidy by regulating capsule chain length through genes involved in import and regulation of this amino acid.

Conclusion: Our findings provide insights into the mechanisms by which amino acids regulate mucoidy, contributing to a better understanding of how K. pneumoniae regulates virulence-associated behaviors in response to host cues.

MultiomicMenu: Streamlining Multiomics Data Interpretation for Insights into Neuronal Responses to Glutamate Treatment

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Introduction: The exponential growth of omics data poses a significant challenge to biomedical researchers. The development of high-throughput multiomics technologies has opened new avenues for understanding complex biological phenomena, yet the sheer volume of data often overwhelms human cognitive capacity. This "data deluge" has hindered the efficient interpretation of omics results, limiting their applicability in fields like precision medicine. To address this bottleneck, we present the "MultiomicMenu," an interactive web application designed for the interpretation of multiomics data. Our objective is to introduce the MultiomicMenu and demonstrate its utility in a practical use case. We applied this software package to analyze RNAseq and kinome array data obtained from rat neurons subjected to glutamate treatment. Utilizing the PCSF algorithm for multiomic data integration implemented by the Kinograte R package, we sought to confirm glutamate's known association with cytotoxicity, apoptosis, and stress pathways.

Methods: We employed the Kinograte R package to create an integrated protein-protein interaction (PPI) network from the transcriptomic and kinomic datasets. Node prizes and edge costs were assigned based on absolute log fold change or z score, respectively, and STRING-DB interaction confidence. Gene-set enrichment analysis was performed using the enrichR R package to identify dysregulated pathways. The MultiomicMenu facilitated functional interpretation through interactive network and pathway visualizations.

Results: The MultiomicMenu allowed us to identify and visualize significantly altered pathways, revealing clusters associated with cytotoxicity, apoptosis, and stress. The PPI network highlighted key "hub" genes involved in these pathways, providing valuable insights into the molecular mechanisms underlying glutamate-induced neuronal responses.

Conclusion: Our study demonstrates the effectiveness of the MultiomicMenu in streamlining the interpretation of complex multiomics data, enabling researchers to uncover biologically relevant insights with ease. This interactive web application holds promise for accelerating discoveries in the field of systems biology and advancing the application of omics data in precision medicine and beyond.

Effect of developmental pyrethroid exposure in prairie voles as a model of Neurodevelopmental disorders.

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Introduction: Neurodevelopmental disorders (NDDs) are a class of lifelong incurable disorders with few treatments are biomarkers. They share common comorbidities including deficits in communication and learning, repetitive behavior, and hyperactivity such as attention deficit hyperactivity disorder (ADHD), Autism, and Developmental disability. The incidence of NDDs has been rapidly rising affecting almost 17% of children in the US. Heritability is still a crucial factor in the etiology of NDDs, however large meta-analysis data now consider environmental impact as a major contributor. Recent epidemiological studies have shown the effect of pyrethroid pesticides on pregnant women and risk factors associated with the proper brain development in the children. Pyrethroids are common household insecticides widely used in the US and considered relatively “safe” by the Environmental Protection Agency (EPA). Despite growing evidence of the complex gene-environment interaction in the etiology of NDDs, very few environmental factors have been studied.

Methods: Previously, our lab has studied the effects of deltamethrin (pyrethroid) in mice. Metabolomic analysis on whole male brain samples provided suggested a disruption in folate metabolism pathway as a result of developmental pesticide exposure. Our research aims to look at the effect of developmental pyrethroid exposure (DPE) in prairie voles as a model of neurodevelopmental disorders, and folic acid supplementation as a potential therapeutic strategy.

Results: We exposed pregnant vole dams to 3mg/kg of deltamethrin two weeks prior to pregnancy, during pregnancy and throughout lactation. A subset of pesticide exposed voles were supplemented with 5’MTHF (folate vitamer). Following weaning, the offspring grow to adulthood and are subjected to a battery of behavioral tests to look at deficits in five different domains namely communication, cognition, social interaction, repetitive behavior, and locomotion. After concluding behavioral tests their brains are harvested along with other tissues for further studies. In order to understand molecular disruptions in the folate metabolic enzymatic pathway, we conducted western blot analysis in five participating enzymes in the folate metabolic pathway, namely Dihydrofolate Reductase (DHFR), Methyltetrahydrofolate Reductase (MTHFR), Serine

hydroxymethyl transferase (SHMT), Methyl tetrahydrofolate dehydrogenase (MTHFD), Methionine synthase (MTRR), and Folate receptor alpha (FOLR1).

Conclusions: Our results show that developmentally exposed prairie voles had deficits in communication, cognition, repetitive behavior, and locomotion (hyperactivity). Previously harvested brains from the mice study were used to look at disruptions in molecular mechanisms because of DPE. Our data also suggest that folate supplementation alleviates some of the behavioral deficits. Disruptions in enzymatic expression was observed in FOLR1, MTHFR, and SHMT1. Such disruptions could be an effect of treatment or a compensatory mechanism for changes caused by developmental pesticide exposure.

Purinergic System Perturbations in Schizophrenia

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Introduction: Schizophrenia is a devastating neuropsychiatric disorder characterized by hallucinations, delusions, and disordered thought processes. Dysregulation of the glutamate and dopamine neurotransmitter systems are implicated in the pathophysiology of schizophrenia. The adenosine system is an important neuroregulatory system in the brain that modulates glutamate and dopamine signaling via adenosine receptors; however, the gene expression of the high affinity adenosine A1 and A2A receptors (A1R and A2AR) is not well characterized in neurons in frontal cortical brain regions implicated in this disorder.

Methods: In the present study, we analyzed A1R and A2AR mRNA expression via qPCR in enriched populations of pyramidal neurons, isolated from postmortem anterior cingulate cortex (ACC) tissue from schizophrenia (n=20) and age and sex-matched non-psychiatrically ill control (n=20) subjects, using laser capture microdissection.

Results: A2AR mRNA expression was significantly increased in schizophrenia subjects who were off antipsychotic medication (ANCOVA: $F(1,12)=6.444$, $p=0.026$), suggesting that A2AR expression may be normalized by chronic antipsychotic treatment. A1R expression was significantly increased in female schizophrenia subjects compared to female control subjects ($t(13)=-4.008$, $p=0.001$). A1R expression was also significantly decreased in female controls compared to male control subjects ($t(17)=2.137$, $p=0.047$). We also identified a significant positive association between dementia severity and A2AR mRNA expression (Spearman's $r=0.424$, $p=0.009$).

Conclusion: Overall, these results provide novel insights into the pyramidal neuron specific expression of adenosine receptors in the ACC in schizophrenia and suggest that changes in receptor mRNA expression may be sex-dependent and associated with dementia in these subjects.

Role Of Heat Shock Factor 1 In Pancreatic Cancer Ferroptosis

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Introduction: Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer, with a low 5-year survival rate of less than 13%, which will be the second leading cause of cancer-related death by 2030. Hyperactivation of Heat Shock Factor 1 (HSF1), a pro-oncogenic transcriptional factor in regulating proteome homeostasis, is a molecular biomarker in PDAC tumorigenesis and a factor contributing to chemoresistance. Ferroptosis, an iron-dependent cell death is characterized by lipid peroxidation and provides unique avenue as therapeutic intervention. While recent studies have revealed the involvement of HSF1 in regulating ferroptosis, the precise mechanisms by which HSF1 regulates ferroptosis in the context of pancreatic cancer are not fully understood. To address this gap, we aim to investigate the biological and molecular mechanisms governing HSF1-mediated pancreatic cancer ferroptosis.

Methods: Ferroptosis marker Glutathione Peroxidase 4 (GPX4), phosphorylation of HSF1 at Ser326, and HSF1 protein were detected in MIA Paca-2 cells by Western blotting. HSF1 activity was inhibited by phase I clinical trial HSF1 inhibitor NXP800 and HSF1 small molecule inhibitor DTHIB. Erastin was used as a ferroptosis inducer. The mitochondrial mass was detected by MitoviewTM mitochondrial dye, and lipid peroxidation was measured by BODIPYTM 581/591 C11, a lipid peroxidation sensor, and observed by fluorescence microscopy. The ultrastructure of mitochondria was detected by transmission electron microscope. Reactive Oxygen Species (ROS) were detected by DCFDA Cellular ROS Assay Kit.

Results: Inhibition of HSF1 by NXP800 or DTHIB dose-dependently suppressed the expression of GPX4 in MIA PaCa-2 cells. Additionally, HSF1 inhibition altered mitochondrial morphology, reduced mitochondrial mass, induced lipid peroxidation, and enhanced Erastin-induced ROS production in MIA PaCa-2 cells.

Conclusion: We have found that inhibiting HSF1 promotes ferroptosis in pancreatic cancer cells. Targeting HSF1 to induce ferroptosis could be a novel therapeutic strategy for pancreatic cancer.

Pro-inflammatory response in MC-LR exposed Human Primary Airway Epithelial cells through increased NF-κB activity

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Introduction: The global rise in cyanobacterial harmful algal blooms (cHABs) had raised microcystin production, known for adverse effects on human and animal health. Among 300 congeners of microcystins, MC-LR is one of the most abundant with potent toxicity. The effects of aerosolized MC-LR exposure and their toxicity mechanisms remain unclear. The known mechanism identifies the cellular uptake through OATPs transporter and inhibition of PP1 and PP2A phosphatases which can affect the several downstream signaling pathways and their mediators involved in the inflammation and tissue injury. PP2A is known to dephosphorylate IKK and thus inhibits NF-κB. Therefore, we hypothesize that microcystin-induced PP2A inhibition results in NF-κB activation.

Objectives: We aim to delineate the inflammation mechanism to MC-LR exposure in primary human airway epithelial cells.

Methods: Top upregulated genes were determined by transcriptomic analysis of the healthy and asthmatic donor human primary airway epithelium cells with or without MC-LR exposure. The interactions of select upregulated genes with PP2A (PP2RA) were assessed using STRING. In addition, we evaluated MC-LR mediated NF-κB activity in A549-Dual reporter cells, a human airway epithelial cell line with or without pro-inflammatory stimuli (IL-1β).

Results: The interaction of select upregulated genes (TLR4, CxCL9, and CXCL11) after MC-LR exposure with specific proteins (IKBKG, IRAK1, TRAF6, and MYD88) suggests NF-κB involvement as a crucial mediator in how epithelial cells respond to MC-LR. The MC-LR pretreatment on A549 cells yielded no detectable change in NF-κB in the vehicle-exposed cells. However, the MC-LR exposure resulted in a slight, but significant increase in NF-κB activity irrespective of prior pretreatment ($P<0.001$). Moreover, exposing the cells to IL-1β resulted in a notable increase in NF-κB activity ($P<0.05$), further elevated with MC-LR pretreatment ($P<0.0001$).

Conclusion: MC-LR exposure heightens pro-inflammatory signaling by activating NF-κB in human airway epithelial cells, that is enhanced by prior IL-1β induction.

An Integrative Analysis of Kinomic and Proteomic Profiling in Chronic Mild Stress Mice

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Introduction: Major Depressive Disorder (MDD) is a severe mental health condition characterized by the DSM-5 as persistent feelings of sadness, hopelessness, and a lack of interest or pleasure in daily activities. n this study, we focus on modeling MDD in mice through Chronic Mild Stress (CMS), a well-established paradigm that mimics the chronic stressors contributing to the development and exacerbation of depressive symptoms. Kinomics, the study of protein kinases and their signaling pathways, and proteomics, the comprehensive analysis of proteins expressed in a biological system, offer a holistic perspective on the molecular alterations associated with MDD. By combining these two high-throughput techniques, we aim to unravel the intricate molecular landscape underlying depressive phenotypes induced by chronic stress.

Methods: We induced Chronic Mild Stress (CMS) in a mouse model to mimic Major Depressive Disorder (MDD) and collected brain tissue samples for analysis. Bioinformatic tools were employed to interpret the functional significance of differentially expressed proteins and identify kinase targets. The integrative analysis of kinomic and proteomic data unveiled intricate molecular changes associated with CMS-induced depressive phenotypes.

Results: Proteomic analysis revealed significant changes in protein expression patterns, indicating a broad impact on cellular processes in response to the experimental conditions. Kinomic profiling identified alterations in kinase activity, suggesting potential modulation of signaling pathways. Integrative analysis and the observed overlap between proteomic and kinomic changes hinted at complex regulatory networks affected by the experimental manipulations.

Conclusions: In summary, our study employing a mouse model subjected to Chronic Mild Stress (CMS) successfully illuminated significant alterations in protein expression and kinase activity, providing valuable insights into the molecular landscape associated with Major Depressive Disorder (MDD). The integrative analysis of kinomic and proteomic data unveiled intricate regulatory networks, underscoring the complexity of molecular changes induced by chronic stress and offering potential avenues for understanding and addressing depressive phenotypes.

Nutritional epigenetic rescue of metabolic syndrome

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Introduction: Metabolic syndrome (MetS) is a cluster of metabolic dysregulations including high blood pressure (BP), central obesity, and hyperglycemia. Regular exercise is known to prevent MetS by the generation of ketone bodies. Precisely, how ketone bodies operate to lower MetS, is largely unknown. Previously, we demonstrated that the ketone body, β-hydroxybutyrate, is a potent anti-hypertensive metabolite. β-hydroxybutyrate has been recently recognized to epigenetically modify histones by β-hydroxybutyrylation. We also found that histone β-hydroxybutyrylation contributes to chromatin remodeling and exposes regions for transcription of genes for β-oxidation of fatty acids. Therefore, we hypothesized that β-hydroxybutyrate alleviates MetS via histone β-hydroxybutyrylation-mediated transcriptional upregulation of β-oxidation of fatty acids.

Methods: To test this hypothesis, we examined the epigenetic effect of increasing systemic β-hydroxybutyrate in a genetic rat model of MetS inbred in our laboratory, the low-capacity runner (LCR/Tol) rats. Randomly selected two groups of six months old female LCR/Tol rats (n=5/group) were surgically implanted with radiotelemetry transmitters for BP measurement. The control group was given water, whereas the experimental group was given water containing 20%v/v of 1,3-butanediol (1,3-BD), precursor of β-hydroxybutyrate, for 10 weeks. Following euthanasia, sera and tissues were harvested. Histones were isolated and subjected to western blotting for quantitation of β-hydroxybutyrylation.

Results: Compared to the control group, 1,3-BD group had a significantly increased circulating β-hydroxybutyrate ($p<0.05$), lower body weight ($p<0.05$), lower fasting glucose ($p<0.05$), and lower 24-hour average systolic BP ($p<0.05$). Interestingly, histones isolated from 1,3-BD group also displayed a marked increase in β-hydroxybutyrylation ($p<0.01$). Further, the target transcriptional genes of histone β-hydroxybutyrylation, Hmgcs2 and Cyp2d4 were both significantly upregulated in 1,3-BD group ($p<0.01$).

Conclusion: This is the first study to demonstrate that enhanced systemic β-hydroxybutyrate attenuates MetS by histone β-hydroxybutyrylation-mediated chromatin remodeling to upregulate the transcription of Hmgcs2 and Cyp2d4 to promote β-oxidation of fatty acids.

Modulation of Endothelial Cell Mitochondrial Phenotype and Angiogenesis by TRPV4

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Introduction: Transient receptor potential vanilloid type 4 (TRPV4) is a mechanically activated ion channel implicated in the regulation of endothelial cell (EC) proliferation, migration, and angiogenesis. However, the molecular mechanism(s) by which TRPV4 regulates EC functions remain underexplored. Here, we investigated if TRPV4 channels mediate endothelial function via modulation of mitochondria.

Methods: Cell culture, Calcium imaging, Immunocytochemistry, Microscopy (Confocal and Transmission Electron Microscopy), Cell spreading on flexible substrates, Western blot, Metabolic flux assay, Boyden chamber migration assay, XTT proliferation assay, Ex vivo aortic ring assay

Results: Confocal microscopy showed a peri-nuclear localization of mitochondria in normal EC (NEC), while they were localized throughout the cell in TRPV4 knockout EC (KOEC). Further, transmission electron microscopy confirmed clear round mitochondria in NEC compared to elongated mitochondria with distinct cristae in KOEC. Importantly, we found increased distribution of mitochondria in KOEC with increasing stiffness, when cultured on extracellular matrix (ECM) gels of varying stiffness that mimic the stiffness of matrix in pathophysiological conditions such as tumor or heart failure (0.2, 8, and 50 kPa). Mechanistically, western blot analysis showed increased fusion/fission protein ratio (Optic Atrophy 1 (OPA1)/mitochondrial fission factor (MFF)), in KOEC compared to NEC. Seahorse flux analyzer analysis demonstrated increased basal oxygen consumption rate (OCR), maximal OCR, ATP-linked OCR, and spare capacity in KOEC compared to NEC, which were significantly attenuated by a small molecule inhibitor of OPA1, MYLS22. Finally, MYLS22 normalized TRPV4-knockout mediated abnormal proliferation, migration, and angiogenesis ex vivo.

Conclusion: Taken together, these findings indicate that mechanosensitive TRPV4 channels regulate mitochondrial phenotype and function during angiogenesis through OPA1.

Probing protein dynamics and conformational transitions of 14-3-3-sigma for the treatment of pancreatic cancer

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Introduction: The overexpression of 14-3-3-sigma in pancreatic cancer patients is a well-established marker linked to poor prognosis and treatment resistance. Consequently, it has emerged as a promising therapeutic target for pancreatic cancer treatment. However, there's a significant challenge in developing selective inhibitors for 14-3-3-sigma due to the high conservation and structural similarity among the seven isoforms of the 14-3-3 protein family. These inhibitors often lack the necessary specificity. Proteins, including 14-3-3 proteins, can exhibit dynamic behavior, with the ability to undergo structural transitions and adopt various conformational states. Hence, our research aims to unravel the dynamic properties of 14-3-3-sigma and leverage this understanding to discover targeted inhibitors that specifically target 14-3-3-sigma in pancreatic cancer. This undertaking holds the potential to revolutionize the strategy for targeted therapy in the treatment of pancreatic cancer by addressing the intricate dynamics of this protein family.

Methods: We conducted Molecular Dynamics (MD) simulations employing the Amber force field. Our laboratory-developed computational tools enabled the identification and characterization of key residue pairs in 14-3-3-sigma. To corroborate in-silico MD analyses, we conducted in vitro BioSAXS experiments, and high-throughput Fluorescence Polarization assays, both utilizing purified recombinant protein.

Results: Unbound 14-3-3 proteins exhibit dynamic transitions between “open” and “closed” conformational states. Conversely, the 14-3-3 protein-ligand complex displayed a stable “closed” conformational state. Notably, among the isoforms, 14-3-3-sigma displays more distinct conformational changes. We have identified key residues, and underlying mechanisms that likely account for the differences in the dynamics of 14-3-3 proteins. These residues will serve as prime targets for in-silico drug screening, aiming to discover sigma isoform-specific inhibitors for the treatment of pancreatic cancer.

Conclusions: Gaining insight into the dynamic properties of proteins holds the potential to unlock new avenues for the development of highly specific and potent inhibitors in the realm of targeted therapy for pancreatic cancer.

Evidence of viral DNA created during a flavivirus infection in vector mosquito cells

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Introduction: Flaviviruses include major pathogens such as dengue, yellow fever, Zika and West Nile virus (strain Kunjin, or KUNV). They are vector-borne positive-strand RNA viruses that cause viral hemorrhagic fevers and encephalitides and sicken 400 million individuals annually. Flaviviruses are transmitted by Anopheles, Aedes, and Culex mosquitoes, without substantially impairing mosquito health, in vivo or in in vitro cell lines. A fundamental gap in knowledge is the ability of these viruses to infect mosquito cells and establish a persistent infection without diminishing mosquito populations. Others have reported that host integration of viral DNA (vDNA) from RNA viruses lacking reverse transcriptase capability may be a critical step towards establishment of persistent infections. Here, we investigated if KUNV viral DNA is created in multiple mosquito cell lines, as well as mammalian cell lines (African green monkey). Further, we evaluated vDNA creation and maintenance in persistently-infected mosquito cells.

Methods: We infected cells in culture with KUNV (MOI 0.1-10) and collected total host DNA at various times post infection. Total DNA includes genomic, integrated chromosomal and short,episomal DNA. We performed PCR using virus-specific primers corresponding to regions within the KUNV genome. PCR products were run on a 2% agarose gel and imaged with Genesys. Results: Imaging revealed a PCR products of appropriate size and subsequent sequencing of PCR products further validated the presence of KUNV viral genome. Next, we investigated if vDNA creation is mediated by the host reverse transcriptase (RT) machinery. We administered an RT inhibitor, Zidovudine, prior to KUNV infection and then performed DNA extraction and PCR as indicated above. Zidovudine prevented vDNA creation when compared to control treated KUNV infections.

Conclusion: Our results show that mosquito cells use RT to convert KUNV RNA genomes into DNA. We will use this model to explore the role of vDNA in mosquito persistence and innate immunity during KUNV infections.

The herbicide glyphosate promotes hypertension via gut microbiota and vascular dysfunction

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Introduction: Glyphosate is a widely used potent herbicide developed by Monsanto under the trade name Roundup®. Glyphosate exposure can occur through the consumption of genetically modified crops tolerant to glyphosate. Glyphosate targets the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, which is only present in plants and microbes. However, humans are holobionts with symbiotic gut microbiota, which can be targeted by glyphosate to result in gut dysbiosis. Because our previous studies demonstrate that gut microbiota dysbiosis contributes to hypertension, we hypothesized that exposure to glyphosate aggravates gut dysbiosis-mediated hypertension.

Methods: Adult (8–10-week-old) genetically hypertensive Dahl salt-sensitive (S) male rats were surgically implanted with radiotelemetry transmitters to monitor blood pressure (BP) and administered with or without glyphosate (175 mg/kg body weight per day) in drinking water for 3 weeks. Fecal 16S rRNA gene and whole genome sequencing were performed for microbiota analyses. Vascular function was examined ex vivo in isolated mesenteric arteries using wire myography.

Results: As early as 1 week of exposure to glyphosate was sufficient to significantly increase mean arterial pressure (MAP) (148 mmHg vs. 141 mmHg, $p<0.0002$) in S rats. At week 3, the differences in BP were further increased (MAP; 168 mmHg vs. 155 mmHg, $p<0.0001$). This elevation in hypertension was accompanied by increased contractility of isolated mesenteric resistance arteries, as determined by their increased vasocontractile response to phenylephrine, and decreased vasorelaxant response to acetylcholine. Metagenomics data revealed distinct rearrangement of gut microbiota composition in the glyphosate-treated group compared to control. Significant shifts in both alpha diversity and β-diversity were observed in the glyphosate-treated group. Bacterial genera such as Candidatus, and Erysipelatoclostridium were more abundant in the glyphosate group, whereas Bifidobacterium that produces short-chain fatty acids, was depleted.

Conclusion: This is the first proof-of-concept study to demonstrate that glyphosate contributes to increased vascular contractility, altered gut microbiota composition and the escalation of hypertension.

Hypertensive mice are more susceptible to experimental malaria

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Introduction: Global prevalence of hypertension is on the rise, especially in developing countries where infectious diseases, such as malaria, are also rampant. Whether hypertension could predispose or increase susceptibility to malaria, however, has not been extensively explored. Previously, we reported that hypertension is associated with abnormal erythrocyte physiology and anemia. Since erythrocytes are target host cells for malarial parasite, *Plasmodium*, we hypothesized that hypertensive patients with abnormal erythrocytes physiology are at greater risk or susceptibility to *Plasmodium* infection.

Methods: To test this hypothesis, eight weeks old normotensive (BPN/3J) and hypertensive (BPH/2J) mice were characterized for their erythrocyte physiology and subsequently infected with green fluorescent protein-tagged *Plasmodium yoelii* (*P. yoelii*), a murine-specific non-lethal strain.

Results: When compared to BPN mice, BPH mice displayed microcytic anemia and their erythrocytes were highly resistant to osmotic hemolysis. Further, BPH erythrocytes exhibited an increase in membrane rigidity and an altered lipid composition, as evidenced by higher levels of phospholipids and saturated fatty acid, such as stearate (C18:0), along with lower levels of polyunsaturated fatty acid like arachidonate (C20:4). Moreover, BPH mice had significantly greater circulating $Ter119^{+}$ $CD71^{+}$ reticulocytes, or immature erythrocytes. Upon infection with *P. yoelii*, BPH mice experienced significant body weight loss accompanied by sustained parasitemia, indices of anemia, and substantial increase in systemic pro-inflammatory mediators, compared to BPN mice, indicating that BPH mice were incompetent in clearing *P. yoelii* infection.

Conclusions: Collectively, these data demonstrate that aberrant erythrocyte physiology observed in hypertensive BPH mice contributes to an increased susceptibility to *P. yoelii* infection and malaria-associated pathology.

Use of a *Borrelia burgdorferi* Mutant as an Attenuated Vaccine

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Introduction: Lyme disease is the most common tick-borne disease in the United States, with nearly 500,000 estimated infections per year. *Borrelia burgdorferi*, the bacterial cause of Lyme disease, possesses numerous motility and chemotaxis genes vital for its ability to infect and persist inside a host. Infections are unable to be cleared without antibiotics, and no vaccine is currently available. However a chemotaxis mutant of *Borrelia* is able to be cleared rapidly and possesses qualities which suggest it could be a candidate for an attenuated vaccine.

Methods: Multiple immunization doses consisting of wild-type *Borrelia*, chemotaxis mutant *Borrelia*, heat-killed *Borrelia*, or growth media as negative controls were given to groups of C57BL/6 mice (IACUC protocol 103714), representing a natural reservoir for *Borrelia*. Quantitative PCR was performed to ensure bacterial clearance from multiple tissues after challenges. Antibody levels against *B. burgdorferi* antigens were assessed via ELISA. Lymph nodes were collected and stained to assess disruption of healthy developing germinal centers.

Results: *Borrelia* were unable to be detected in any of the tested Lyme disease-associated tissues infected with the chemotaxis mutant or negative controls, even at doses exceeding 1000-fold of wild-type; alternatively, infection with wild-type *B. burgdorferi* developed significant bacterial loads in all tested tissues. Increasing dose sizes of the chemotaxis mutant led to step-wise increases in IgG serum titers, matching those seen with continual wild-type infection. Germinal centers of chemotaxis mutant-infected mice remained intact, suggesting proper memory cell generation for an adaptive immune response.

Conclusions: These results confirm the suitability of the mutant as a vaccine candidate, and future protection studies will determine its effectiveness.

Cysteinyl leukotriene receptors promote melanoma progression and metastasis

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Introduction: Cysteinyl leukotrienes (cys-LTs; LTC4, LTD4, LTE4) are pro-inflammatory mediators mainly produced by hematopoietic cells, which enhance inflammation through their receptors, CysLT1R and CysLT2R. They are crucial in causing chronic asthma in humans. The link between inflammation and cancer has sparked interest in the role of cys-LTs in cancer progression and metastasis. Yet, the precise molecular mechanisms through which these inflammatory mediators regulate tumor proliferation and metastasis are still unexplored. A thorough investigation of these molecular mechanisms, and identification of the specific receptor/s responsible, can aid in understanding their therapeutic potential in various cancers like melanoma. Since the inhibitors of these receptors are already FDA approved for asthma treatment, we aim to repurpose these drugs to treat melanoma progression, thereby achieving maximum efficacy with minimal side effects.

Objective: To determine the mechanistic aspects of how CysLTRs regulate melanoma tumor initiation, progression, and metastasis.

Methods: Protein expression by western blotting and ELISA, transcript expression by qPCR, viability and proliferation by XTT and BrDU, migration using trans-well assay, tumor growth and metastasis examined through in vivo experiments.

Results: B16F10 melanoma cells express high CysLT1R compared to CysLT2R. Further, cys-LTs mediated the activation of major signaling proteins such as ERK and p38 that are important for melanoma survival and proliferation. Moreover, treatment with CysLTR antagonists significantly reduced melanoma cell proliferation, survival, and migration in vitro. Accordingly, we observed a significant reduction in the melanoma tumor volume in vivo in both Cysltr1-/- and Cysltr2-/- mice compared to the WT mice. Interestingly, angiogenesis was significantly reduced in Cysltr2-/- mice but not in Cysltr1-/-.

Conclusions: Therefore, we speculate that while both receptors play a crucial role in tumor proliferation in vivo, CysLT2R is the main driver of angiogenesis and metastasis. Therefore,

targeting both these receptors using their specific antagonists can offer effective therapy for melanoma progression.

Discovery of a new gut bacterial biomarker of developing hypertension with anti-hypertensive properties

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Introduction: The gut microbiome is home to complex and diverse microbial communities that play a crucial role in host homeostasis. Gut dysbiosis has been linked with hypertension (HTN), but the precise mechanisms of host-microbiota interactions that contribute to HTN are unclear. Here, we investigated temporal changes in the gut bacteria and their metabolites in spontaneously hypertensive rat (SHR), a rodent model of age dependent HTN associated with gut dysbiosis, to identify potential biomarkers of developing HTN in rodents that may be used as future therapeutics.

Methods: Fecal samples were collected from male and female SHR and their normotensive genetic controls the Wistar Kyoto (WKY) rats at 4, 8, and 13 weeks old (wo), and 16S bacterial sequencing and metabolomics were performed in all samples (N=7-10/group). Following metabolomics analyses, a newly discovered bacterial metabolite found to be associated with age-dependent HTN in the SHR (3-amino-4,6-diol, 1µg/ml) was supplemented in drinking water of adult male SHR and WKY rats for 2 days (N=4/group) ad libitum, blood pressure (BP) and heart rate (HR) were measured and compared to baseline recordings.

Results: Metabolomics analysis showed significantly lower levels of 3-amino-4,6-diol in both male and female SHR starting from weaning (i.e., 4wo, at pre-HTN stage) and continuing through 8wo (early HTN) to 13wo SHR (with established HTN) compared to sex- and age-matched WKY. Supplementation with 3-amino-4,6-diol in drinking water produced a significant decrease in BP (>10mmHg) and HR (~25bpm) in male adult SHR but not the WKY rats. Correlation analysis showed a positive correlation between fecal 3-amino-4,6-diol levels and two bacterial families*:* *Lachnospiraceae FCS020* (R^2=0.7512) and *[Eubacterium] nodatum* (R^2=0.7477) in 13wo WKY males.

Conclusion: We discover a new gut bacterial metabolite biomarker of developing HTN with anti-hypertensive properties. Future studies should elucidate select bacterial species producers of 3-amino-4,6-diol to fully explore its potential as an innovative therapeutic target.

Uncovering biologically relevant Autism subtypes using advanced machine learning techniques

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Introduction: Autism spectrum disorder (ASD) is characterized by main deficits in social interaction and social communication. This vague definition of ASD does not encompass the wide heterogeneity of its phenotypical presentation. It is critical to identify ASD subtypes that are biologically relevant and that respond to personalized treatment.

Objectives: The objective of this study is to use a multimodal approach to create biologically relevant subtypes.

Results: 114 adult men (18-45 years old), including 74 neurotypical (NT) and 40 ASD were recruited and completed a series of behavioral tests such the NEO-PI-R, reading the mind in the eyes (RMET), Symptom Checklist 90-revised questionnaire (SCL-90), intelligence quotient (IQ), and Broader Autism Phenotype Questionnaire (BAPQ). Clinical measurements and fMRI data was collected from ASD subjects for validation. We used a random forest tree algorithm to classify ASD and NT. We included NEO-PI-R and RMET in the main classifier. The random forest tree model classified ASD and NT with an average accuracy of 80%. Top features included personality domains such as extraversion and neuroticism. K-means clustering was used to derive ASD subtypes based on the shapely values; this created 3 subtypes (Subtypes 1, 2, 3). T-tests indicated significant differences in the following measures: ADI-R repetitive behaviors, BAPQ, IQ, SCL-90, neuroticism, extraversion, RMET and rs-FC between the superior temporal sulcus, anterior cingulate, and insula.

Conclusions: Our results suggest that ASD subtype 1 is characterized by high neuroticism, lower warmth, higher scores on RMET, higher IQ, and higher rsFC between STS and salience network. Subtype 2 was found to be close to neurotypicals. Subtype 3 is characterized by high neuroticism, high repetitive behaviors, and lower rsFC between STS and salience network. These results are very promising, and the next step is to examine whether these putative subtypes are biologically relevant and whether specific subtypes respond better to certain pharmacological treatments.

Investigation of TRIM2 as an antiviral factor during dengue virus infection

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Introduction: Dengue virus (DENV) is a hemorrhagic flavivirus and the most-common mosquito-borne virus responsible for human infections. Each year, up to 400 million people get infected with DENV and 22,000 die from severe infection. Unfortunately, although the severity and spread of dengue infections is increasing, there is no licensed anti-DENV antivirals or immuno-therapeutics. The nonstructural 5 (NS5) protein is encoded by the DENV genome and contains the RNA polymerase and methyltransferase enzymes required for viral replication. NS5 also functions as a major inhibitor of interferon (IFN) signaling, well known as the first line antiviral defense. That's why NS5 is a potent target for antiviral drug development.

Methods: Virus titration by immunofocus assay, transfection, western blot, confocal microscopy, site directed mutagenesis, molecular cloning.

Results: By studying virus-host protein interactions, we identified that the human tripartite motif (TRIM)-2 protein acts as an anti-DENV restriction factor. DENV NS5 interacts with TRIM2, a host E3 ubiquitin ligase predominantly expressed in the brain, where it performs neuroprotective functions. By confocal microscopy, we found TRIM2 colocalizes with DENV NS5 and co-immunoprecipitations demonstrated physical interaction between the proteins. DENV replication is reduced selectively in human cell lines expressing high levels of TRIM2 compared to control cell lines. Co-transfection of NS5 and increasing amount of TRIM2 reduces NS5 protein level significantly. Thus, TRIM2 is a virus-specific restriction factor that targets DENV through binding to NS5. The mechanism of NS5 degradation by TRIM2 is under investigation.

Conclusions: Completion of these studies will help identify a mechanism of DENV restriction in brain tissue and will potentially provide insight into the design of therapeutics, such as a TRIM2 mimetic, that prevents DENV disease in non-neuronal tissue (e.g., liver).