43rd Graduate Research Annual Forum

March 19-20, 2025

Abstract Booklet

Presented By

Council of Biomedical Graduate Students (CBGS)

COLLEGE OF MEDICINE AND LIFE SCIENCES
THE UNIVERSITY OF TOLEDO

BIOMEDICAL SCIENCE GRADUATE PROGRAM

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Thank you to our Judges and Moderators!

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Rejina Shrestha
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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 Pharmacology and Experimental Therapeutics at the University of Toledo. This includes the Biomedical Science Graduate Program (BMSP) and related graduate program 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 a month to discuss any issues that need to be addressed and to plan and organize various events throughout the year. 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 Welcome 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, postdocs, and fellow students

Please contact us at councilgraduatestudents@utoledo.edu if you have any questions or let us know how we can help you. Visit us at http://www.utoledo.edu/med/grad/biomedical/cbgs/ for more information.

Dean's Message

Welcome to the 2025 Graduate Research Annual Forum (GRAF)!

For more than four decades, this student-led forum – organized by the Council of Biomedical Graduate Students – has been a cornerstone of collaboration, discovery and professional growth at The University of Toledo. Since 1979, GRAF has provided an invaluable opportunity for our graduate students to showcase their research, refine their presentation skills and engage in meaningful scientific discussions with peers and faculty.

This year, GRAF will take place on March 19-20, 2025, featuring an exciting lineup of oral presentations, poster sessions, and a data blitz competition. Around 40 students will participate, sharing their innovative research with our academic community. Faculty and postdoctoral fellows will serve as judges, selecting the Top 3 winners, who will be awarded cash prizes for their outstanding work.

A highlight of this year's forum is our distinguished keynote speaker, Dr. Priya Shah, Associate Professor at UC Davis, whose expertise and insights will inspire and challenge our students as they advance in their academic and professional journeys.

We appreciate your participation and wish you a rewarding and inspiring event!

Imran Ali, M.D., FAAN, FAES
Dean, College of Medicine & Life Sciences
Professor, Department of Neurology
The University of Toledo

Welcome to the Graduate Research Annual Forum 2025

I am delighted to welcome all students, faculty, postdocs, and visitors to the **43rd Graduate Research Annual Forum (GRAF 2025).** For over four decades, the **Council of Biomedical Graduate Students (CBGS)** has proudly organized this event, providing an invaluable platform for students to showcase their research and receive insightful feedback from expert postdocs and faculty. Beyond presentations, GRAF serves as a unique opportunity for all tracks to engage with one another and foster interdisciplinary collaborations.

Our students are very excited for this year's forum, including 37 posters and oral presentations. We have 20 volunteer judges to evaluate the presentations. 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 being delivered by **Dr. Priya Shah**. Dr. Shah is an Associate Professor of Chemical Engineering and Microbiology & Molecular Genetics at the University of California, Davis. Her research bridges the fields of chemical engineering and microbiology, where her lab employs quantitative systems biology tools to investigate virus-host interactions. With over 40 publications on topics ranging from protein behavior and aggregation to virus-host protein interactions, Dr. Shah is a leading voice in her field. In 2024, she authored **"Pride and Prejudice (and Ph.D.s)"** which connects themes from Jane Austen's classic novel "Pride and Prejudice" with experiences in academic research.

I would like to take this opportunity to extend my heartfelt gratitude to my executive team members and track representatives for their dedication in planning this event. A special thank you to **Dr. Kandace Williams** and **Dr. David R. Giovannucci** for their invaluable guidance as Faculty Advisors to the Council. I am especially grateful to **Allison Spencer** and **Paige McVay** from the Graduate Program Office for their unwavering support. This event would not have been possible without the generous funding from the **UTCOM&LS Foundation, the COMLS Alumni Board of Trustees, the Student Allocation Committee, the COM&LS Graduate Program Office, and the departments within our program,** we truly appreciate their contributions. A sincere thank you to **Margaret Hoogland, Dr. Robert Smith,** and the journal *Translation* for collaborating with us on the abstract booklet and the publication of abstracts. Last but certainly not least, I deeply appreciate the assistance of all **judges, moderators, and volunteers**. I hope all attendees find this forum to be a valuable and enriching experience.

Sachin Aryal
President
Council of Biomedical Graduate Students

Schedule

Wednesday, March 19, 2025

Data-Blitz: 8:30 am – 9:30 am

Location: Collier Building Room 1210

Poster Presentations: 10:30 am – 1:30 pm

Location: Collier Building Room 2409

Lunch: 1:30 pm – 2:15 pm

Location: Collier Building Room 2409

Oral Presentations Group 1: 2:30 pm – 3:30 pm

Oral Presentations Group 2: 3:30 pm – 4:30 pm

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

Location: Health Education Building 103

Thursday, March 20, 2025

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

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 Lobby

Keynote Lecture, Daniella Gamboa Pabon Award for Research Dedication Announcement, and GRAF Winner Announcement: 3:30 pm – 5:00 pm

Dr. Priya Shah, PhD: "*Harder, Better, Faster, Stronger: Integrating Virology and Engineering to Drive Discovery*"

Location: Health Education building Room 110

Keynote Speaker: Priya Shah, PhD

Dr. Priya S. Shah is an Associate Professor of Chemical Engineering and Microbiology & Molecular Genetics at the University of California, Davis. Her research bridges the fields of chemical engineering and microbiology, where her lab employs quantitative systems biology tools to investigate virus-host interactions.

A Toledo native, Dr. Shah began her research career in high school at the University of Toledo. She earned a B.S. in Chemical Engineering from MIT, focusing on microchip drug delivery, and a Ph.D. from UC Berkeley, researching HIV resistance to RNAi therapies. She completed her postdoctoral fellowship at UC San Francisco, studying flavivirus-host protein interactions.

Dr. Shah joined UC Davis as an Assistant professor, earning several prestigious awards, including the Outstanding Teaching Award, the Chancellor's Award for Creativity and Innovation, and the Lois K. Miller Lectureship from American Society of Virology. In 2024, she was promoted to Associate professor and was recognized with Joe and Essie Smith Endowed Chair of Chemical Engineering.

At UC Davis, Dr. Shah's lab explores two main research areas: virus-host interactions and synthetic biology. In the virus-host interaction domain, her team combines comparative proteomics with high-throughput animal models to investigate how Zika virus-host protein interactions disrupt host proteins and impair brain development. She also examines how flaviviruses like dengue and Zika manipulate host gene expressions to evade immune responses.

In synthetic biology, her lab addresses challenges in viral protein production for applications such as vaccines and diagnostics, using biomolecular engineering and biomanufacturing techniques to enhance SARS-CoV-2 protein production. Additionally, they are developing innovative methods to control gene expression using light and nutrients, with applications in tissue engineering and cultivated meat production.

With over 40 publications on topics ranging from protein behavior and aggregation to virus-host protein interactions, Dr. Shah is a leading voice in her field. In 2024, she authored "Pride and Prejudice (and Ph.D.s)" which connects themes from Jane Austen's classic novel "Pride and Prejudice" with experiences in academic research.

The Council of Biomedical Graduate Students is honored to welcome Dr. Shah as the Keynote Speaker for the 43rd Graduate Research Annual Forum, celebrating her remarkable career and contributions to science.

Data-Blitz, 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 poster and oral group.

For the Data Blitz session, we will directly select Top 3 Winners from the first session.

Final Session:

Finalists will be presented to a special panel of judges, consisting of:

- Dr. Priya Shah, 2025 Keynote Speaker
- Dr. Kandace Williams, Senior Associate Dean, College of Medicine & Life Sciences Graduate Programs
- Dr. David R. Giovannucci, Professor & Chair, Department of Medical Education; Professor, Department of Neurosciences & Neurological Disorders

Prizes:

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

Data-Blitz Presentations: March 19, 2025

COB 1210, 8:30 – 9:30am **Moderators**: Mrunmayee Kandalgaonkar Dipesh Pokharel	Ahmad Hegazi Oluwatosin Mautin Akinola Matthew Hathaway Emma Elizabeth Sabu Kattuman Tania Akter Jhuma Upasana Shrestha Ishan Manandhar Kesha Dalal Ali Imami

Poster Presentations: March 19, 2025

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

Group 1 COB 2409, 10:30am – 12:00pm **Moderators**: Tania Akter Jhuma Prisca C. Njoku	Ahmad Hegazi Rahul Verma Oluwatosin Mautin Akinola Bennett Allison Matthew Hathaway Emma Elizabeth Sabu Kattuman Ryan Allan Harris Joanna Stuck John B. Presloid
Group 2 COB 2409, 12:00 – 1:30pm **Moderator**: Oluwatosin Mautin Akinola	Hunter Eby Tania Akter Jhuma Prisca C. Njoku Austin Kunch Tanisha Chaudhary Dipesh Pokharel Mir Himayet Kabir Jennifer H. Nguyen Upasana Shrestha
Group 3 COB 2409, 12:00 – 1:30pm **Moderator**: Rejina Shrestha	Caroline C. Swain Arturo Grano de Oro Jessica M. Jiron Wisdom Ahlidja Ishan Manandhar Kesha Dalal Ali Imami Gabby Vento Breanna Coffman Sushma Khatri

Oral Presentations: March 19, 2025

Group 1 HEB 103, 2:30 – 3:30pm **Moderator**: Benjamin French	Aliu Olalekan Olatunji Augustine Kwabil Sanjana Kumariya
Group 2 HEB 103, 3:30 – 4:30pm **Moderator**: Kesha Dalal	Syed Abdil-Moiz Hasan Somayeh Darzi Rejina Shrestha
Group 3 HEB 103, 4:30 – 5:30pm **Moderator**: Wisdom Ahlidja	Azeezat Osikoya Bivek Timalsina Shubhra Kanti Dey

Role of heat shock factor 1 in nab-paclitaxel sensitization in pancreatic ductal adenocarcinoma

Ahmad Hegazi[1], Kuo-Hui Su[1], Shi-He Liu[1*]

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Background: Pancreatic ductal adenocarcinoma (PDAC) is primarily treated with chemotherapy, with first-line options including gemcitabine, 5-fluorouracil, and nab-paclitaxel (NP). However, drug resistance is a frequent occurrence and poses a significant challenge. Heat shock factor 1 (HSF1) is a key regulator of the proteotoxic stress response (PSR), and this HSF1-mediated PSR promotes tumor survival and contributes to drug resistance. Despite these established roles, the impact of HSF1 on the reaction to antimicrotubule agents such as NP in PDAC remains unexplored. It has been reported that HSF1 prolongs mitotic arrest under the antimicrotubule agent nocodazole through delaying Cyclin B1 degradation in lung and bone cancer, a process key in increasing antimicrotubule cytotoxicity. The objective of this study is to determine whether HSF1 modulates sensitivity to NP through prolonging NP-induced mitotic arrest in PDAC.

Methods: Drug sensitivity (IC50) to NP was evaluated in PDAC cell lines (PANC-1, MIA PaCa-2, PDCL-5, HPAFII, and Capan-2) using CellTiter-Blue® Cell Viability Assay. Relative HSF1 protein expression levels were measured via immunoblotting. The level of Cyclin B1, cleaved PARP, and phosphorylated HSF1 at Ser326 were analyzed following NP treatment using immunoblotting. HSF1 was knocked down using siRNA or lentiviral shRNA and rescued with exogenous lentiviral Flag-HSF1 full-length plasmids.

Results: In a panel of five human PDAC cell lines (PANC-1, MIA PaCa-2, PDCL-5, HPAFII, and Capan-2), relatively higher HSF1 expression was associated with reduced NP IC50 ($p=0.008$). NP IC50 was increased in PANC-1 cells following HSF1 siRNA knockdown. NP treatment induced HSF1 phosphorylation at Ser326, which correlated with Cyclin B1 expression and mitotic arrest at 12 hours in PANC-1 cells.

Conclusion: PDAC cell lines with higher basal HSF1 expression exhibit greater sensitivity to NP. The following studies will validate these findings in vivo and investigate the detailed mechanism by which HSF1 regulates antimicrotubular-induced mitotic arrest in PDAC.

Keywords: Pancreatic Cancer, Chemotherapy, Heat Shock Factor 1

HSP70 and CHIP mediate PZ39-induced ABCG2 ubiquitination and degradation

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Background: Multidrug resistance (MDR) is a major clinical challenge in cancer chemotherapy. Overexpression of ATP-binding cassette (ABC) transporters, such as ABCG2, is thought to contribute to MDR by actively pumping chemotherapeutic out of cancer cells, reducing their efficacy. Successful discovery and development of inhibitors targeting ABC transporters is expected to help overcome MDR. Previously, we identified a potent dynamic ABCG2 inhibitor, PZ39, that not only inhibits ABCG2 function but also induces ABCG2 degradation in the lysosome. Understanding the mechanism of PZ39-induced ABCG2 degradation will not only facilitate the development of PZ39 as a therapeutic to overcome ABCG2-mediated MDR but will also promote the future discovery of membrane protein degraders for the development of novel membrane PROTACs (proteolysis-targeting chimeras).

Methods and Results: We tested the hypothesis that PZ39 induces a conformational change in ABCG2, which inactivates the transporter and recruits HSP70 and CHIP (C-terminal Hsc70-Interacting Protein) complex, which facilitates ABCG2 endocytosis and subsequent degradation in the lysosome through a ubiquitination-dependent pathway. We conducted a ubiquitin pull-down assay to assess the effect of PZ39 on ABCG2 ubiquitination and found that PZ39 treatment significantly enhanced ABCG2 ubiquitination. Western blot analysis revealed that inhibiting HSP70 with YM08, a known HSP70 inhibitor, effectively rescued ABCG2 from PZ39-induced degradation, providing direct evidence of HSP70's role in this process. Additionally, silencing either HSP70 or CHIP using siRNAs similarly rescued ABCG2 from PZ39-induced degradation, further supporting their involvement in the PZ39-induced degradation pathway.

Conclusions: These findings suggest that PZ39 induces a conformational change in ABCG2, leading to its inactivation and recruitment of the HSP70-CHIP complex, which facilitates ubiquitination and subsequent degradation. This mechanism could serve as a blueprint for the future development of membrane-targeting degraders and the potential application of PROTAC strategies in overcoming MDR in cancer chemotherapy.

Keywords: Proteasomal degradation, Protein Dynamics, Drug

Empagliflozin-Metformin in hypertension therapy synergistically upregulate 3-hydroxy-3-methylglutaryl-coenzyme A synthase 2 and Acetyl-coenzyme A acyltransferase 1B

Oluwatosin Mautin Akinola[1], Saroj Chakraborty[1], Wisdom Ahlidja[1], Sachin Aryal[1], Ishan Manandhar[1], Blair Mell[1], Bina Joe[1*]

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Background: Combination therapy with Empagliflozin (a Sodium Glucose Transporter 2 Inhibitor) and Metformin (a biguanide) effectively lowers blood pressure (BP) in type 2 diabetes mellitus patients with hypertension. Both drugs enhance lipid catabolism, although the underlying mechanisms remain unclear. Empagliflozin facilitates ketosis by increasing the levels of the ketone body β-hydroxybutyrate (βOHB), a metabolite previously reported by our laboratory to lower hypertension. Our unpublished data suggest βOHB upregulates key lipid catabolic genes, Hmgcs2 and Acaa1b, in kidneys, facilitating energy metabolism. Given that Metformin also supports lipid catabolism, we hypothesize that combination therapy synergistically upregulates these genes, mitigating hypertension.

Methods: Male Dahl Salt-Sensitive rats were assigned to four groups: Control, Metformin, Empagliflozin, or a combination of Empagliflozin+Metformin (n = 7–9/group). Treatment was administered via drinking water for seven weeks. BP was measured continuously using radiotelemetry. Serum βOHB levels were analyzed colorimetrically, and renal expression of Hmgcs2 and Acaa1b was assessed by quantitative PCR.

Results: Combination therapy significantly reduced systolic and diastolic BP compared to controls (systolic: 168.7 vs. 217.9 mmHg, diastolic: 122.2 vs. 162.5 mmHg, $p<0.0001$), outperforming individual treatments ($p<0.0001$). Serum βOHB levels were highest in the combination group ($p<0.05$), correlating with synergistic ketosis. Hmgcs2 and Acaa1b were upregulated in the combination group ($p<0.01$ and $p<0.05$, respectively). Hmgcs2 upregulation was exclusive to combination therapy, whereas Acaa1b also increased in the Metformin group ($p<0.01$), highlighting Metformin's role in lipid oxidation.

Conclusions: Combination therapy with Empagliflozin and Metformin synergistically enhances βOHB levels and upregulates lipid catabolic genes Hmgcs2 and Acaa1b, promoting lipid utilization and reducing metabolic stress. This novel mechanism significantly reduces salt-sensitive hypertension, offering dual benefits of BP reduction and improved lipid metabolism for patients with hypertension and diabetes.

Keywords: Hypertension, Energy metabolism, Diabetes

Targeting solute carrier transporters in enzalutamide-resistant prostate cancer

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Introduction: After initial therapy fails, castration-resistant prostate cancer patients are treated with androgen receptor-targeted agents such as enzalutamide. However, many patients develop resistance to enzalutamide within 9-15 months. Advanced prostate cancer cells rely on upregulated glycolysis through a metabolic rewiring process, which can lead to therapy resistance. Previous work from our lab and others have shown that several transporters belonging to the solute carrier (SLC) family are key in enabling a therapy-resistant phenotype including monocarboxylate transporters (MCTs) and amino acid transporters. Our previous results found that inhibiting MCTs with specific inhibitors re-sensitizes resistant prostate cancer cells to enzalutamide. We also found that enzalutamide-resistant prostate cancer cells treated with MCT inhibitors upregulate the amino acid transport pathway. Based on these findings, we hypothesize that inhibition of transporters will lead to apoptosis and a significant decrease in glycolytic metabolism.

Methods: To analyze transporter inhibition efficacy, we will treat enzalutamide-sensitive C4-2B and enzalutamide-resistant C4-2B-MDVR cells with MCT or amino acid transporter inhibitors AR-C155858, AZD3965, syrosingopine, V9302, or JPH203, either singly or in combination with Enzalutamide. We will assess apoptosis markers such as cleaved caspase 3 and cleaved PARP. We will analyze whether the inhibition of SLCs affects AR signaling using qPCR. We will use the Agilent Seahorse xFe96 Analyzer to examine the glycolytic capacity of treated cells.

Results: We anticipate that resistant cells will exhibit higher levels of apoptosis and lower glycolytic activity when treated with combinations of enzalutamide and transporter inhibitors compared with parental cells. We also expect that AR signaling may be downregulated upon transporter inhibition.

Conclusions: These experiments would help establish the importance of solute carrier transporters in enzalutamide resistance and identify the combinations that are most effective in overcoming such resistance. These findings will also provide a better understanding of the metabolic process involved in prostate cancer therapy resistance.

Keywords: Prostate Cancer, Enzalutamide Resistance, Glycolytic Metabolism, Solute Carrier Transporters

Characterizing tick-borne orthoflavivirus mutations in viral protease

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Background: The increasing incidence of orthoflavivirus infections have exacted an enormous burden on healthcare systems worldwide. This is exacerbated by the lack of therapeutics available and limited availability of vaccines. One of the most promising candidates is the NS3 viral protease. Our lab has published on its ability to interact with TRAF6 in a proviral manner that is specifically conserved tick borne flaviviruses (TBFV). TRAF6 is an E3 ubiquitin ligase that plays a central role in Toll-like receptor (TLR) and RIG-I-Like receptor (RLR) signaling cascades. From this interaction, we began to study the role of ubiquitination in the functionality of the NS3 protease and how it may affect viral replication.

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 to determine phenotypic changes. We also performed luciferase using an NF-κB reporter to measure changes in NF-κB activation in the presence of NS3 and the various mutants.

Results: These results revealed that modifying lysine residues on the NS3 protease led to changes in total levels of total ubiquitination.

Conclusions: We have demonstrated that removing lysine residues led to changes in ubiquitination which was also found to influence viral replication. Future studies will work to identify the critical residues involved and may identify potential therapeutic sites.

Keywords: Flavivirus, Ubiquitination

Repurposing of cysteinyl leukotriene receptor antagonists in melanoma therapy

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Background: Cysteinyl leukotrienes (Cys-LTs) are pro-inflammatory mediators primarily produced by hematopoietic cells. They amplify inflammation through ubiquitously expressed G protein-coupled receptors, CysLT1R and CysLT2R, and are key players in chronic asthma. Given the link between inflammation and cancer, recent studies have focused on the role of Cys-LTs in cancer progression, although the underlying mechanisms remain unclear, especially in melanoma. Considering that CysLT antagonists are FDA-approved for asthma, our study explores their potential repurposing for melanoma treatment. Objective: To elucidate the precise molecular mechanisms through which CysLTRs mediate melanoma initiation, progression, and metastasis, and evaluate their therapeutic potential.

Methods: Protein expression by western blotting, transcript expression by qPCR, viability and proliferation by XTT and BrDU, migration using scratch-wound assay, in vivo tumor growth by s.c. injection of melanoma cells under the flank of WT, *Cysltr1-/-* and *Cysltr2-/-* mice, for in vivo therapeutic evaluation, CysLTR antagonists were injected (i.p.) post tumor development, tumor volume measured using vernier caliper, angiogenesis using immunostaining of tumor sections with endothelial marker.

Results & Conclusion: Both murine melanoma cell lines (B16F10 and YUMM1.7) express CysLT1R and CysLT2R at both transcript and protein level. Further, Cys-LTs triggered the activation of key survival and proliferation proteins, including ERK and p38 in both cell lines. Additionally, CysLT1R and CysLT2R antagonists (MK571 and BayCysLT2 respectively) significantly inhibited melanoma cell proliferation, survival, and migration *in vitro*. Accordingly, we observed a significant reduction in the melanoma tumor growth *in vivo* in both *Cysltr1-/-* and *Cysltr2-/-* mice compared to the WT mice. Interestingly, tumor angiogenesis was significantly reduced in *Cysltr2-/-* mice but not in *Cysltr1-/-* mice. Furthermore, CysLT1R and CysLT2R antagonists (MK571 and BayCysLT2) in WT mice significantly reduced tumor growth, highlighting their therapeutic potential for melanoma. Thus, our study highlights a promising new strategy of repurposing anti-asthma drugs with minimal side effects for melanoma.

Keywords: Drug repurposing, Melanoma, Cysteinyl leukotrienes

Reservoir host restricts tick-borne flavivirus replication through enhanced antioxidant activity

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Background: 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 disease 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 and Results: 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 the 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. 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.

Keywords: Flavivirus, Virus, Tickborne diseases, Nrf2, Infectious Clone

A novel role of HSF1 in vascular smooth muscle cells

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Introduction: Vascular smooth muscle cells (VSMCs) exhibit plasticity that allows them to dedifferentiate from a quiescent, contractile phenotype into a synthetic, proliferative, and migratory phenotype depending on environmental cues. This ability to switch phenotypes is essential for vascular remodeling and wound healing but has also been implicated in many vascular pathologies such as atherosclerosis and neointimal hyperplasia. Heat shock factor 1 (HSF1) is a transcription factor with well-described effects in the heat shock response and tumorigenesis. However, its role in VSMCs has not been explored.

Methods: Human coronary artery smooth muscle cells (HCASMCs) were transduced with HSF1 loss-of-function or control shRNA lentiviral vectors. Cell signaling was analyzed at the protein level utilizing western blot analysis. Gross changes in cell confluency and morphology were analyzed by live cell imaging. A nuclear and cytoplasmic extraction Kit was used to analyze HSF1 abundance in cytoplasmic and nuclear protein fractions.

Results: Silencing HSF1 in VSMCs led to decreased activation of proliferative signaling including pERK½, pAKT, pS6, and pP90S6K, downregulation of proliferation marker expression (cyclin D1), and inhibition of cell proliferation. Conversely, silencing HSF1 led to upregulation of VSMC differentiation markers (SM α-actin and calponin). Culturing HCASMCs in complete versus serum free media led increased abundance of HSF1 in the nuclear fraction, suggesting activation.

Conclusions: Our findings demonstrate, for the first time, that HSF1 may be a novel master regulator of VSMC phenotypic switching from a differentiated into proliferative and migratory phenotype. Ongoing experiments will further examine the effects of HSF1 loss- and gain-of-function on VSMC phenotypic switching, will identify the potential intermediary transcriptional mechanisms, and will test the effects of HSF1 deficiency in mouse models of vascular remodeling in vivo. In summary, these findings suggest a novel role of HSF1 in VSMC phenotypic switching and may provide future therapeutic targets for vascular wall diseases.

Keywords: HSF1, VSMC - Vascular smooth muscle cells

Use of a *Borrelia burgdorferi* mutant as an attenuated Vaccine

John B. Presloid[1], R. Mark Wooten[1*]

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Background: 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 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*. After 6 months immunized mice were challenged with *Borrelia*-carrying ticks to mimic natural infection. One month later mouse tissues were collected and assessed. Quantitative PCR was performed to quantitate bacterial burden in multiple tissues. Antibody levels against *B. burgdorferi* antigens were assessed via ELISA.

Results: *Borrelia* were unable to be detected in any of the pilot-tested Lyme disease-associated tissues immunized with the chemotaxis mutant or negative controls, even at doses exceeding 1000-fold 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 stepwise increases in IgG serum titers, matching those seen with wild-type infection. After 6 months, high immunization doses were protective against natural acquisition of a homologous strain but were less protective against heterologous challenge.

Conclusions: These results confirm the suitability of the mutant as a vaccine candidate and offers some measure of protection after 6 months.

Keywords: Lyme Disease, Borrelia, Vaccine

Sex-specific insights into renal transplant rejection: potential role of atorvastatin as a repurposed therapy

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Background: Renal transplant rejection occurs in approximately 20% of recipients, reducing graft lifespan by up to 50%, often necessitating re-listing for transplantation and dialysis. Limited research has explored sex-based differences in rejection mechanisms. This study aimed to investigate sex-specific differences in renal transplant rejection and identify FDA-approved drugs that could be repurposed to mitigate rejection events.

Methods: We analyzed publicly available data (GSE50084) from 54 patients categorized as follows: 12 DSA-, 14 DSA+/AMR-, and 28 DSA+/AMR+. Differentially expressed genes (DEGs) were identified for each group. DEGs specific to AMR+ samples were analyzed in iLINCS to generate discordant signatures for FDA-approved drugs. Drugs with high discordant scores (< -0.5) were shortlisted. Sig2Lead was used to analyze the chemical structures of identified drugs to find structurally similar therapeutic candidates. Additionally, we plan to analyze outcomes in post-transplant patients using the TriNetX database, comparing those on atorvastatin versus no statin and atorvastatin versus other statins. Data will be stratified by sex to assess whether males experience better outcomes on atorvastatin than females.

Results: Atorvastatin showed a strong discordant signature against rejection when pooled across sexes, with a higher score in males compared to females. This suggests potential sex-specific activity, with atorvastatin demonstrating stronger protective effects in males. Structurally, atorvastatin differs from other statins but shares similarities with the -nib family of drugs, indicating unique therapeutic properties. Ongoing analyses include bioinformatic and epidemiological studies, with findings to be presented at the conference. This research highlights the potential of atorvastatin as a repurposable drug for renal transplant rejection and underscores the importance of considering sex-specific differences in drug efficacy.

Keywords: Kidney Transplant, Bioinformatics, Drug Repurposing, Sex Differences

Akkermansia muciniphila induces sex specific blood pressure changes and RAS activation

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Background: *Akkermansia muciniphila* (Akk), an intestinal mucin degrading bacterium has been associated with hypertension, a key risk factor for cardiovascular disease. Still, there's no clear evidence showing how Akk directly affects blood pressure. Renin-angiotensin- system (RAS) is a well-known blood pressure regulator, activation of which leads to oxidative stress. Previous studies have reported that Akk is more prevalent in females comparing to males and blood pressure exhibits sexually dimorphic traits. Therefore, we hypothesize that Akk regulates both RAS and blood pressure in a sex-dependent manner.

Methods: Spontaneously hypertensive rats (SHR) of both sexes (10-13-week-old, N=5-6 rats/group) were maintained on a normal chow (Harlan Teklad, TD 7034). These rats were gavaged with either 10^8 CFU/ml of Akk or PBS as control every other day for four weeks. Blood pressure was continuously monitored for 24 hours every week by radiotelemetry. After four weeks, kidney tissues were collected for the analysis of RAS associated receptors and oxidative stress related genes by real time PCR.

Results: Oral administration of Akk significantly decreased systolic BP (176.7 vs. 170.6 mmHg, $p<0.0001$) and mean BP (148.0 vs. 144.7 mmHg, $p=0.0004$) in male SHR rats. In contrast, in female SHR rats, systolic BP (161.2 vs. 164.5 mmHg, $p=0.01$) and mean BP (137.3 vs. 139.6 mmHg, $p=0.0032$) was increased following oral ingestion of Akk. Treatment with Akk showed higher expression of angiotensin II type 1 receptor a (*at1ra*) ($p=0.0315$) in females, a receptor for vasoconstrictive angiotensin II. However, this difference in *at1ra* was not observed in males. No significant differences were observed for angiotensin II type 2 receptor (*at2r*), which has opposing effects and protective function. Oral administration of Akk resulted in a trend toward reduced expression of the vasodilatory receptor *mas1* in females compared to males ($p=0.0680$). In male SHR rats, Akk significantly increased the mRNA expression levels of the antioxidant enzyme superoxide dismutase 1 (*sod1*), which protects against oxidative stress.

Conclusion: We report sex specific effects of Akk, a promising next generation probiotic, on blood pressure, that is, Akk increases blood pressure in females and decreases it in males. These are associated with corresponding changes in the renal RAS of both sexes, suggesting that Akk may regulate blood pressure via its impacts on renal RAS and oxidative stress. Our study provides evidence supporting Akk-mediated precision medicine for hypertension based on sex. Protein and enzymatic activity of renal RAS component will be studied in the future.

Keywords: *Akkermansia muciniphila*, Renin-angiotensin- system (RAS), Hypertension

Claudins mediates osteoblast-induced prostate cancer dormancy

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Introduction: 10% of prostate cancer (PCa) patients were diagnosed, but 80% of patients died with bone metastases in developed countries. The early disseminated tumor cells (DTCs) in distant organs, including bones, are potential cells of origin for metastatic progression and recurrence after various curative therapies, including prostatectomy. These DTCs stay dormant to evade therapies and reactivate to cause fatal metastases. Previous studies suggested osteoblasts induced PCa cell dormancy in the bone cortex. We developed a mixed co-culture model, revealing that physical contact with osteoblasts induces PCa dormancy. RNA sequencing revealed a dormant PCa cell gene signature, in which claudin 11 (Cldn11) was increased. We also identified Cldn19 increased in osteoblasts relative to other bone stromal cells that cannot induce dormancy. Claudins form tight junctions between adjacent cells. Therefore, we hypothesized that osteoblasts form tight junctions with PCa cells and induce dormancy.

Methods: We analyzed public datasets to determine the clinical relevance of Cldn11. To assess its function, we overexpressed or knocked down (KD) Cldn11 or Cldn19 in PCa cells or osteoblasts. We visualized tight junction scaffold protein ZO-1 via immunofluorescence and confocal microscopy.

Results: We found that Cldn11 is positively associated with better patient survival. PCa cell dormancy was reduced in the mixed co-culture with Cldn11 KD C4-2B or Cldn19 KD MC3T3-E1 murine osteoblasts. ZO-1 is commonly recruited to tight junctions. Confocal imaging showed that ZO-1 expression spread across C4-2B cells but was highly enriched at PCa cell membranes in the mixed co-culture.

Conclusion: Cldn11 in C4-2B cells and Cldn19 in osteoblasts are indispensable for osteoblast-induced dormancy. Tight junctions are possibly induced in C4-2B cells co-cultured with osteoblasts. We will further validate tight junctions among cells in the co-culture using transmission electron microscopy. We will also determine how tight junctions induce PCa cell dormancy.

Keywords: Dormancy, Claudins, Prostate Cancer, Bone Microenvironment

A multi-vertex pattern and network analysis for structural MRI data

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Background: Broadly, structural MRI thickness data analyses consist of group difference association, or structural covariance network approaches, at region-of-interest and vertex-based levels. An integrative method that jointly analyzes structural focal differences and structural network differences at both ROI and vertex-based levels would allow quantification of relative importance of these different measures for predictiveness.

Methods: We developed a logistic regression model with penalty terms and coefficients for individual vertices, and interaction terms between ROIs. The model incorporates lasso penalty on all coefficients, and group lasso and fused lasso penalties on vertex coefficients, to account for ROI level groups of vertices and the spatial topology of the cortex, respectively. We tested the model on harmonized ENIGMA PTSD working group standardized sMRI data, that is harmonized to remove site, study and scanner effects of 2061 PTSD subjects and 8100 controls (age 18 to 65 years) aggregated from 38 laboratories globally.

Results: Our current preliminary analyses show AUCs of 0.79, 0.81, 0.83, and 0.82 for logistic regression, lasso regression, sparse group lasso, and fused sparse group lasso regression respectively. Thus, the model is more appropriate than standard regression modeling for analyzing differences between PTSD and control cortical thickness. Adding interaction terms did not affect AUC.

Conclusion: We report an integrated machine learning approach that jointly analyzes network and focal differences in structural MRI cortical thickness data. This approach performs better than more basic approaches, and we demonstrate that lasso and group lasso penalties may be added to other novel approaches to improve predictive performance, and result in outcomes that are more predictive of disease using cortical thickness data.

Keywords: Neuroimaging

Characterizing the role of SipA in *Vibrio cholerae*: Investigating the connection between SipA and other aspects of *V. cholerae* physiology

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Introduction: Stress response mechanisms are crucial for bacterial survival under varying environmental conditions. This study focuses on the highly conserved SipA protein in *Vibrio cholerae*, which is essential for stress tolerance and impacts bacterial physiology, including biofilm formation and carbon utilization. Despite significant preliminary findings, the regulatory mechanisms and functional roles of SipA remain poorly understood.

Methods: In past studies we employed genetic and biochemical approaches, including mutant strain construction, survival and β-galactosidase assays, and advanced techniques like immunoprecipitation and mass spectrometry. Localization studies confirmed SipA's cellular position, while regulatory pathways were investigated through transcriptomic analysis. Both Classical and El Tor biotypes of *V. cholerae* were studied to reveal biotype-specific roles of SipA.

Results: Previous research established that SipA is regulated by a two-component system (VC1638-VC1639), which influences its expression in response to environmental stress, particularly the presence of antimicrobial peptides (AMPs). SipA plays a critical role in AMP resistance by interacting with the outer membrane protein OmpA, suggesting that they work together to facilitate AMP export and alleviate stress. This cooperative mechanism likely involves SipA aiding in the binding or transport of AMPs through OmpA, thereby reducing their toxic accumulation in the periplasm. To better understand how the two proteins relieve AMP stress, we are using ompA deletion strains and cellular fractionation assays to assess accumulation of AMPs in the periplasm. Additionally, we have observed that sipA-deficient strains exhibited increased biofilm production and growth defects on specific carbon sources, indicating its broader physiological roles beyond stress resistance.

Conclusions: SipA is a multifaceted protein central to *V. cholerae* stress responses and physiology. Its role in AMP resistance highlights a conserved mechanism with potential applications in understanding bacterial stress survival strategies.

Keywords: sipA, ompA, Vibrio cholerae

Gastrointestinal dysmotility in the P+L rat model of Parkinson's disease

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Introduction: Parkinson's disease (PD) is a chronic, progressive neurodegenerative disorder primarily caused by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). PD is characterized by motor manifestations such as bradykinesia, resting tremor, and rigidity, which is frequently preceded by gastrointestinal (GI) dysfunction, particularly constipation for decades (1). Braak, et. Al. hypothesized that PD pathology originates in the GI tract and then travels retrograde via the vagus to the brain (2). We showed the Braak hypothesis can be modeled in the rat by administration of subthreshold doses of Paraquat (P) and Lectin (L) to cause aggregation of misfolded α-synuclein in the GI tract that ascends retrograde via the vagal nerves into the medulla and then via the nigro-vagal pathway to initiate PD-related pathology in the SNpc (2-4).

Objectives: This study aimed to investigate the effects on total oro-anal transit time (TOTT) in the P+L rat model of PD and to explore the temporal course of the onset of GI dysfunction and motor symptoms in this model. Methods: Carmine, red dye solution (75 mg/mL in 0.9% carboxymethyl cellulose) was administered via oral gavage to rats (n=16) (5). TOTT was recorded as the interval in minutes between gavage and the first appearance of carmine, red in the feces over 24 hours with video recording at baseline, 1-, 2- and 4 weeks post P+L exposure (5). All rats were exposed to oral P+L for 7 days as we have previously described causing parkinsonism as evidenced in a rodent behavioral battery of tests (4).

Results: All rats exhibited a significant increase in TOTT at 1- (p-value 0.142,12.4%), 2- (p=0.27, 9.4%), and 4 weeks (p=0.003, 24.3%) post-exposure compared to baseline. Parkinsonism as expected began at 2 weeks and was robustly established at 4 weeks in all rats that were levodopa responsive. Histological analysis revealed nigrostriatal neurodegeneration and pathologically misfolded alpha-synuclein in the SNpc neurons.

Conclusions: TOTT is delayed in the P+L rat model of PD and GI dysmotility appears to precede the onset of motor manifestations mimicking human disease. Therefore, the P+L rat model may be suitable for experimental therapeutics that target the GI tract to mitigate PD pathology in the gut and the brain.

Keywords: Parkinson's Disease (PD), Gastrointestinal dysfunction, GI symptoms, Paraquat, Nigro-Vagal impairment, alpha-synuclein, Gut and Brain, GI Dysmotility

TRIM2 targets NS5 for binding and autophagy depended degradation to inhibit dengue virus replication

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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 around 22,000 dies from severe infection. Unfortunately, although the severity and spread of 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, and is therefore a potent target for antiviral drug development.

Methods and 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 human brain, where it is known to perform neuroprotective functions. By confocal microscopy, we found TRIM2 colocalizes with DENV NS5 and co-immunoprecipitations demonstrated physical interaction between the proteins. DENV replication is significantly reduced in TRIM2 over expressing human cells line compared to control cell lines. Co-transfection of NS5 and increasing amount of TRIM2 reduces NS5 protein level significantly. Thus, TRIM2 is a dengue virus-specific restriction factor that targets DENV through binding to NS5 for autophagy dependent degradation. Among the four canonical domains of TRIM2, RING domain alone is enough to degrade the NS5.

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 TRIM2 mimetic, that prevents DENV disease in non-neuronal tissue.

Keywords: TRIM2, Dengue virus, NSF, Autophagy, Antiviral Response, Innate Immunity

Developmental pyrethroid exposure disrupts molecular pathways for circadian rhythms and synaptic plasticity in mouse brain.

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Introduction: Neurodevelopmental disorders (NDDs) are a group of conditions that impact the developing nervous system, often with few or no clear biomarkers. Environmental factors play a significant role in the risk of NDDs, including attention deficit hyperactivity disorder (ADHD). One such environmental risk is exposure to pyrethroid pesticides during pregnancy, which has been linked to an increased risk of NDDs in the developing fetus. Our recent research showed that low-dose exposure to the pyrethroid pesticide deltamethrin during development in mice results in male-biased changes in ADHD- and NDD-related behaviors, as well as alterations in the striatal dopamine system.

Objective: This study utilized an integrated multiomics approach to comprehensively identify biological changes in the mouse brain caused by developmental pyrethroid exposure (DPE). Methods: In a litter-based, split-sample design, we exposed pregnant and lactating mice to deltamethrin (3 mg/kg) or a vehicle every three days at a dose significantly lower than the EPA's benchmark for regulatory guidelines. Male offspring were raised to adulthood, euthanized, and their brains collected. Whole brain samples were pulverized and analyzed through split-sample methods, including transcriptomics, kinomics, metabolomics, and multiomics integration.

Results: Transcriptomic analysis revealed changes in several key clock genes, while kinomic analysis showed altered activity in kinases linked to synaptic plasticity. Metabolomic profiling identified alterations in folate biosynthesis, which is crucial for preventing neural tube defects. Multiomics integration highlighted a disrupted protein-protein interaction network, emphasizing key clusters related to mitogen-activated protein (MAP) kinase pathways, apoptosis regulation, and synaptic function.

Conclusions: These findings indicate that developmental pyrethroid exposure (DPE) leads to a multi-modal biophenotype in the brain associated with ADHD and suggests new potential mechanisms underlying this effect.

Keywords: Neurodevelopmental Disorders; Autism, Pyrethroids

Synergistic effects of microcystin and microplastics on cytotoxic and pro-inflammatory effects on human airway epithelial cells

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Background: Harmful algal blooms (HABS) release potent cyanotoxins into water bodies, posing serious threats to human and animal health due to their toxic effects on vital organs, including liver, kidneys, and lungs. Lake Erie HABs are dominated by microcystin (MC) -producing cyanobacteria, with microcystin-leucine arginine (MC-LR) being one of the most prevalent and toxic variants. In addition to health risks posed by MC, there is a growing concern about microplastic (MP) in and around water bodies. Humans can be exposed to MCs and MPs through ingestion, skin contact, and inhalation. While most research have focused on oral exposure, emerging evidence highlights dangers of inhaling MCs and MPs, both of which can cause lung injury. Furthermore, aerosols contaminated with these contaminants can travel over 30 km from affected areas, potentially exposing large population to inhalation risks. Given these concerns, we aim to investigate how these contaminants amplify their harmful effects and impact the health of individuals exposed to them.

Methods: A 3D model of cultured primary human airway epithelial cells was exposed to either polystyrene MPs and MC-LR (alone and in combination), or a vehicle control (saline) using the SCIREQ *ExpoCube in vitro* System. Exposures were performed for 10 minutes daily over a 3 day-period. Conditioned media were collected 24 hours after the final exposure to evaluate cytotoxicity. RNA isolated from the cells were subjected to Real-time PCR, and fold change was calculated using ΔΔCt method.

Results: Using ex vivo experiments, we observed that co-exposure with MC-LR and MPs significantly increased cytotoxicity. Further, there were significant changes in the expression of genes associated with inflammation (e.g., *IL-6, TNF-α*) and oxidative stress (*SOD1*) as determined by RT-PCR analysis.

Conclusions: Co-exposure with microcystin and microplastics have a significant negative impact on cellular toxicity and inflammatory responses. Future studies involve investigating the combined damaging effects of microcystin and microplastics *in vivo*.

Keywords: Microcystin, Microplastics, Airway Inflammation, Human Airway Epithelium, Environmental Health

Paraquat-induced rodent models of Parkinson's disease: a PRISMA compliant systematic review and meta-analysis

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Introduction: Sporadic Parkinson's disease is one of the most common neurodegenerative diseases, yet etiology is unknown. Exposure to the herbicide paraquat has been linked with parkinsonism due to its ability to directly lead to dopaminergic degeneration within the substantia nigra pars compacta (SNpc). Numerous preclinical animal models have been developed where paraquat is used to induce parkinsonism. The aim of this systematic review and meta-analysis is to evaluate the face validity of paraquat-induced rodent models of Parkinson's disease and perform meta-analyses on the observed neuronal loss in the SNpc and observed motor deficit via behavioral motor tests.

Methods: An electronic search in three databases was performed, and studies of all rodent species, strain, age, and sex, by which paraquat was administered singularly or co-administered to induce parkinsonism were included. Studies which had quantitative analysis of both motor behavior and SNpc neuronal loss were included. SYRCLE risk of bias tool was used to evaluate bias of each study. A random effects model was used with continuous data to evaluate mean difference of SNpc neuronal loss across all studies and of motor deficit score across studies that analyzed via various behavioral motor tests.

Results: A total of 54 studies that utilized paraquat to induce parkinsonism were included. The pooled standardized mean difference in SNpc neuronal count across all studies was -2.63 (95% CI -3.12, -2.13). Between study heterogeneity was high ($I2= 74.0\%$, $p<0.0001$). Sub-analyses based on moderators and meta-analyses of motor behavior tests are ongoing.

Conclusions: The pooled standardized mean difference in SNpc neuronal count across studies indicate a strong effect, although heterogeneity was high. This may be due varying study characteristics, and additional sub-analyses are necessary and ongoing. Meta-analyses of motor behavior deficit is also ongoing to further evaluate the face validity of paraquat-induced rodent models of Parkinson's disease.

Keywords: Paraquat, Parkinson's, Animal Model

Gut microbiota-derived metabolite, shikimic acid, is a novel regulator of vascular tone

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Introduction: Gut dysbiosis is linked to vascular wall disease, yet the mechanisms through which gut microbiota interact with host cells remain largely unexplored. Shikimic acid (SA) is a biochemical intermediate synthesized in plants and microorganisms, not mammals. We recently identified SA as a novel gut microbiota-derived metabolite that is readily detectable in human and murine blood. Since vascular cells are the first to encounter circulating metabolites, in this study, we examined for the first time the potential effects of SA on vascular tone, utilizing conduit and resistance arteries isolated from WT C57BL/6 mice.

Methods: Vascular reactivity was evaluated *ex vivo* by wire myography, using dorsal aortic rings and mesenteric resistance arteries isolated from adult male and female WT C57BL/6 mice.

Results: Our results demonstrate that while SA alone does not alter basal vascular tone, it triggers substantial, concentration-dependent vasorelaxation in dorsal aortic rings preconstricted with Gq-coupled pathway agonists, such as phenylephrine (PE) or serotonin (5-HT). Time course assays demonstrated that treatment with a single concentration of SA (10 mM) induces a marked relaxation that persists for at least 20 minutes after treatment. Consistently, we found that pretreatment of vessels with SA attenuates sensitivity (-log EC_{50}) to both PE and 5-HT but does not alter maximum response (Emax). Notably, these observations were reproducible in mesenteric arteries and did not exhibit differences between male versus female mice. Ongoing experiments aim to elucidate the potential underlying mechanisms mediating the effects of SA on vascular reactivity.

Conclusion: This study provides the first evidence of the substantial vasorelaxant effects of the microbiota-derived metabolite SA, establishing a foundation for future research into its potential therapeutic role as a postbiotic in mitigating arterial hypertension.

Keywords: Shikimic Acid (SA), Gut Microbiota, Blood Pressure, Cardiovascular Disease

Diverging replication dynamics in mammalian and mosquito cells: acute decline vs. persistent infection by Orthoflavivirus Kunjin

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Background: Orthoflaviviruses, including dengue, yellow fever, Zika, and West Nile virus (Kunjin strain, KUNV), are positive-strand RNA viruses that cause viral hemorrhagic fevers and encephalitides, affecting 400 million individuals annually. These viruses establish persistent infections in Aedes, and Culex mosquito cells without causing significant cytopathic effects, whereas mammalian cells typically undergo acute infection immediately followed by cell death. A key unanswered question remains: how do mosquito cells evade apoptosis and sustain a persistent infection, while mammalian cells succumb to viral cytopathic effects?

Methods: We infected mosquito cells (C6/36 and U4.4) and mammalian cells (HEK293T and Vero) in parallel with KUNV MOI:10. Viral replication dynamics and cell viability were monitored over time. Growth curves were generated by plaque assays to quantify viral replication at various time points. To assess apoptosis, we anticipate performing Annexin V/PI flow cytometry and caspase activity assays, comparing apoptotic responses during virus infection. Additionally, total host genomic DNA will be extracted at various time points to evaluate KUNV viral DNA (vDNA) integration via PCR with virus-specific primers.

Results: Growth curves show KUNV is capable of infecting both mammalian and mosquito cells, with both cells achieving equal viral titer between 48-72 hours. An inflection point, where the curves intersect, was observed at 48-72 hours in mammalian cells, followed by a decline in viral titers that correlated with significant cell death, suggestive of apoptosis. In contrast, mosquito cells sustained increased viral production without significant cell death. Virus infection suggests that mosquito cells either suppress or evade canonical apoptotic pathways, thereby supporting long-term viral persistence.

Conclusion: Our findings suggest that mosquito cells suppress apoptosis during KUNV infection, facilitating persistent infection. Future studies will focus on identifying apoptotic regulators involved and the role of vDNA in modulating host antiviral responses. Understanding these mechanisms may reveal novel strategies for controlling orthoflaviviruses.

Keywords: Orthoflavivirus, Persistent Infection, Acute Infection, Apoptosis

Microbiota adapt to high-salt diet by downregulating the phosphotransferase system

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Introduction: Previous studies have demonstrated that a high dietary salt intake imposes a high demand for ATP and dampens host energy. Since the host is inhabited by trillions of energy-dependent microbiota, we reasoned that a high-salt diet lowering the energy of the host will also impose stress on the gut microbiota energy metabolism. Microbiota use the phosphotransferase system as an energy-efficient sugar transport mechanism by using phosphoenopyruvate, a high-energy molecule. This allows bacteria to conserve ATP for other processes. Accordingly, we hypothesized that the phosphotransferase system of gut microbiota is jeopardized by a high-salt diet.

Methods: To test this hypothesis, six weeks old male S rats (n=8/group) were fed with high (2%) and low (0.3%) NaCl containing diet for 30 days. On day 31, all the animals were euthanized, and their fecal samples and sera were collected for gut microbiota profiling using 16S RNA sequencing and Gas Chromatography time-of-flight Mass spectrometry-based untargeted metabolomic analysis, respectively. Ingenuity Pathway Analysis was used for pathway analysis of the metabolomic data.

Results: A large-scale remodeling of gut microbiota was noted in the high salt-fed rats. Specifically, Proteobacteria were depleted (45.54%) while *Prevotella* were repleted (18.39%) in the rats on a high-salt diet compared to the low-salt group. Notably, metagenomic analysis of functional pathways revealed that the phosphotransferase system was the highest-ranking pathway significantly downregulated (2.3-fold) in the high-salt fed rats compared to the low-salt fed rats. Additionally, Ingenuity Pathway Analysis indicated metabolic disease pathway as one of the major pathways impacted by a high salt diet.

Conclusion: This study is the first to reveal that a high-salt diet, which imposes a high demand for energy on the host, also triggers an adaptive response by gut microbiota. Specifically, in response to a high-salt diet, microbiota succumbs by downregulating its phosphotransferase system for conserving bacterial energy.

Keywords: Gut Microbiota, Phosphotransferase system, Energy metabolism

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Farnesoid X receptor deletion protects against hypertension, remodels gut microbiome, and improves cardiovascular function

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Introduction: Bile acids, synthesized by hepatocytes, are metabolized by gut microbiota within the gut-liver axis. Although bile acid receptors are known to regulate various physiological processes, their role in blood pressure (BP) regulation remains unknown. Farnesoid X receptor (FXR), a key nuclear receptor for bile acids, is a negative regulator of bile acid synthesis and influences gut microbiota composition. We hypothesized that genetic ablation of FXR lowers BP by increasing bile acid levels and remodeling gut microbiota.

Methods: A novel FXR knockout (*Fxr*KO) rat model was generated on the Dahl Salt-Sensitive (S) genetic background using CRISPR/Cas9 technology. Adult male S (n=8) and *Fxr*KO (n=9) rats were studied, with BP measured via radiotelemetry. Gut microbiota analyses included 16S rRNA and Oxford Nanopore whole-genome sequencing. Microbial bile salt hydrolase (BSH) activity was assessed by ninhydrin colorimetry. Cardiac function was evaluated by echocardiography, vascular function by wire myography, and serum bile acids by colorimetric assay. Kidney function was assessed via urinary protein excretion (UPE).

Results: FXR deletion resulted in elevated serum bile acids and significantly reduced systolic, diastolic, and mean arterial BP ($p<0.0001$). *Fxr*KO rats showed improved cardiac function (higher ejection fraction and fractional shortening). Vascular function analysis in the dorsal aorta of *Fxr*KO rats further revealed increased sensitivity to both endothelium-dependent, (acetylcholine) and endothelium-independent vasodilators (sodium nitroprusside), with both exhibiting lower EC50 values. This suggests improved vascular relaxation. Renal function was improved in *Fxr*KO rats indicated by reduced UPE. Moreover, microbiota analysis revealed significant rearrangement of gut microbiota composition in *Fxr*KO rats compared to S rats (alpha-diversity, $p<0.01$; beta-diversity, $p<0.001$)., with higher *Akkermansia muciniphila* abundance. *Fxr*KO rats exhibited increased BSH activity, negatively correlating with BP.

Conclusions: The novel *Fxr*KO rat model demonstrates that FXR deletion protects against hypertension by elevating bile acids, enhancing cardiac and vascular function, and remodeling gut microbiota.

Keywords: Microbiome, Hypertension, Bile acids, Farnesoid X receptor

TRPV4-mediated mitochondrial dynamics: a novel mechanism regulating endothelial function and angiogenesis

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Introduction: Transient receptor potential vanilloid type 4 (TRPV4) is a mechanosensitive ion channel involved in the regulation of endothelial cell (EC) functions, including proliferation, migration, and angiogenesis. However, the molecular mechanisms underlying TRPV4-mediated regulation of EC activity remain inadequately understood. In this study, we explored whether TRPV4 channels influence endothelial function through modulation of mitochondrial dynamics.

Methods: Cell culture, Immunocytochemistry, 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 revealed a peri-nuclear localization of mitochondria in normal EC (NEC), whereas mitochondria in TRPV4 knockout EC (KOEC) were diffusely distributed throughout the cytoplasm. Transmission electron microscopy further confirmed the presence of rounded mitochondria in NEC, in contrast to elongated mitochondria with distinct cristae in KOEC. Notably, the distribution of mitochondria in KOEC increased in response to varying matrix stiffness, as observed when cells were cultured on extracellular matrix gels with stiffness values mimicking pathological conditions (0.2, 8, and 50 kPa). At the molecular level, western blot analysis revealed an elevated ratio of fusion to fission proteins (Optic Atrophy 1 (OPA1)/mitochondrial fission factor (MFF)) in KOEC compared to NEC. Furthermore, seahorse flux analysis indicated significantly higher basal oxygen consumption rate (OCR), maximal OCR, ATP-linked OCR, and spare capacity in KOEC relative to NEC, all of which were notably reduced upon treatment with the small molecule OPA1 inhibitor MYLS22. Importantly, MYLS22 restored TRPV4 knockout-associated defects in proliferation, migration, and angiogenesis in ex vivo models. In vivo, MYLS22 treatment significantly impaired tumor angiogenesis and growth in endothelial-specific TRPV4 knockout mice.

Conclusion: Collectively, these findings demonstrate that TRPV4 channels regulate angiogenesis by modulating mitochondrial dynamics through OPA1 and highlight endothelial TRPV4 as a novel therapeutic target for the regulation of mitochondrial function in EC.

Keywords: TRPV4, TRPV4mitochondria, mitochondria dynamics, dynamics mechanical signals, mechanical signals morphology, morphology OCR, OCRMYLS22

Connecting the dots: understanding the relationship between social determinants and schizophrenia

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Background: Schizophrenia is a debilitating mental illness that affects a significant portion of the population. The disease has far-reaching consequences that often go beyond the patient themselves, involving their families and the community at large. However, the diagnosis and treatment of schizophrenia are not equally distributed across different socioeconomic groups, leading to significant health inequalities. This poses a significant roadblock in achieving two of the UN's Sustainable Development Goals of Good Health and Well Being (SDG3) and Reduced Inequalities (SDG10).

Methods: The current project utilizes Bayesian Network Modeling to identify significant factors that can impact a person receiving a diagnosis of schizophrenia. The study utilizes the Health Care Utilization Project (HCUP) annual National Inpatient Sample datasets for years 2016 through 2020. The analysis methodology proceeds from identifying patients with schizophrenia and then matching them by age and sex to non-psychiatric controls. Individual Bayesian network models are then fit using the tabu algorithm for structure learning, followed by fitting posterior probability maps from the data.

Results and Conclusions: Our preliminary analysis of the HCUP data has shown that race and socioeconomic status are the biggest factors in someone receiving a diagnosis of schizophrenia. By identifying the social determinants of schizophrenia, we can better understand the underlying causes of health inequalities and work towards reducing them.

Keywords: Schizophrenia, Social determinants of health, Healthcare Utilization Project (HCUP), Bayesian Network Modeling, Health inequalities

Using iLINCS to mine for Oxytocin signature to find new drug candidates or molecular pathways that can be used for future treatment for autism

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Introduction: There are currently no pharmacological treatments available to treat social affective symptoms in Autism. It has been proposed that the social-bonding hormone oxytocin could be administered to promote social affective ability in those with ASD 1–3. The problem with simply administering Oxytocin as a neuropeptide is that its half-life is only around three minutes, thus greatly limiting its clinical usefulness 2. Identifying molecular pathways of oxytocin and mechanisms of action (MOA) of targets that are similar to oxytocin's action, using transcriptomics available datasets, can be promising in identifying genes and pathways that could be further investigated in future translational autism research.

Methods: Three RNA-SEQ studies were identified on the NCBI database that examined Oxytocin exposure to nerve cells in these data sets: GSE210528, GSE24666, and GSE199427, with the first two studies from rat tissue samples and the last one from human stem cells in a laboratory setting 4–6. The data was analyzed with the Library of Integrated Network-Based Cellular Signatures – or ILINCS, to find molecular pathways of oxytocin and mechanisms of action of genes that have similar transcriptomics signature to oxytocin.

Results: Oxytocin had a significant effect on neural activity and signaling pathways, including enrichment of MAPK signaling. Oxytocin exposure led to positive regulation of protein phosphorylation and to Phosphatase Binding. Among the top concordant mechanisms of actions were shown to be HDAC Inhibitor, NF-kB Pathway Inhibitor, Dopamine Receptor and Serotonin Receptor Antagonist.

Conclusions: This analysis indicates that Oxytocin does enrich molecular pathways, and has similar mechanisms of action to genes, that are directly involved in autism psychopathology. A combination of oxytocin and SSRIs or anti-inflammatory markers can be further investigated in laboratory settings under control conditions to better understand its mechanisms in the context of autism research.

Keywords: Autism, ASD, Aspergers, Oxytocin, Psychoneuroendocrinology

Defining the role of Synaptotagmin-7 in insulin secretion and glucose metabolism

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Background: More than 500 million people worldwide are currently affected by diabetes mellitus, with Type 2 diabetes comprising well over 90% of cases. Ultimately in Type 2 diabetes, there is not sufficient insulin secretion from pancreatic beta cells to match the body's sensitivity to insulin. Although the process of insulin secretion is calcium-mediated, the specific roles of the calcium sensors that facilitate this process remain unclear. Based on qPCR of FACS-sorted beta cells, we have identified Synaptotagmin-7 (Syt7) as a highly abundant calcium-sensing synaptotagmin in beta cells, making it a likely candidate to be involved in this process. As such, the primary objective of this study focuses on defining the role of Syt7 in insulin secretion and glucose metabolism, in vivo and in vitro.

Methods and Results: Our experiments utilize a beta cell-specific Syt7 knockout (KO) mouse model that was generated by crossing Ins1-Cre heterozygous animals to Syt7 Floxed animals. Metabolic phenotyping of male animals, which were subjected to a high-fat diet, revealed insulin secretion impairments at times ≥ 30 minutes - a result consistent with a deficit in "second-phase insulin release". A unique feature of our animal model is that the beta cells also selectively express a human insulin transgene bearing a superfolder-GFP (sfGFP)-tagged C-peptide. TIRF microscopy was employed to evaluate the secretory phenotype of Syt7-KO islet beta cells in vitro. Preliminary findings indicate impaired glucose-stimulated insulin secretion in the Syt7-KO cells.

Conclusion: By linking to the biophysical origins of insulin secretion, our studies offer new insights into future strategies to enhance insulin secretion for the prevention and/or treatment of type 2 diabetes.

Keywords: Insulin, Fluorescence microscopy

Understanding the role of small-molecule inhibitors in mediating Survivin degradation

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Background: Survivin, also known as BIRC5, is a member of the Inhibitor of Apoptosis Protein (IAP) family and plays a dual role in regulating the cell cycle and inhibiting apoptosis. Survivin is overexpressed in various cancers but is absent in most normal cells except for some proliferating cells and early stages of fetal development, making it a potential tumor target. Although survivin has long been recognized as a therapeutic target, its lack of enzymatic activity has made effective targeting challenging. Therefore, alternative approaches beyond traditional strategies are needed. Our lab has identified some small molecules that can effectively inhibit survivin's function and target survivin for degradation, demonstrating cytotoxicity for cancer cells. In this study, we show that newly synthesized compounds have improved potency and our efforts to elucidate the precise mechanism by which these small molecule inhibitors mediate survivin degradation.

Methods: The potency of the newly synthesized compounds was assessed using a Cell Viability Assay. A Proteasome Rescue Assay was conducted to identify the pathway through which these compounds induce survivin degradation. A protocol for purifying histidine-tagged survivin from BL21 E. coli cells was established, and protein purity was confirmed by SDS-PAGE. A YFP-survivin and CFP-survivin co-expression plasmid was constructed to assess the binding parameters of small molecule inhibitors.

Results: The newly synthesized compounds were found to have high potency, inducing proteasomal degradation of survivin. A His-survivin construct was generated and expressed in BL21 E. coli cells, with protein purity confirmed by SDS-PAGE. Sequencing and SDS-PAGE confirmed the successful construction of a YFP-survivin and CFP-survivin co-expression plasmid.

Conclusions: The newly synthesized compounds effectively induce proteasomal degradation of survivin. The successful expression and purification of His-survivin, along with the co-expression of YFP-survivin and CFP-survivin, provide a solid foundation for further dissection on how these small molecules target survivin for degradation.

Keywords: Survivin, Small Molecule Inhibitors, Recombinant protein purification, Proteasomal degradation

The role of platelets in neutrophil apoptosis during Sendai virus infection

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Background: Platelets are small, anucleate cells traditionally recognized for their crucial role in hemostasis. Recent studies, however, have established their role as key mediators of immune responses, particularly during viral infections. Platelets interact with viruses through specific receptors, leading to activation. Importantly, during thrombocytopenia, mice infected with Sendai virus show increased inflammation and poor outcome. Of note, increased neutrophil presence in lungs of thrombocytopenic mice was observed suggesting that platelets may modulate neutrophil apoptosis.

Methods: In this study, we used Sendai virus (SeV) as a model respiratory pathogen to investigate the role of platelets in neutrophil apoptosis. Platelets were isolated from mouse blood, and neutrophils were harvested from bone marrow using density gradient centrifugation. Neutrophil counts and viability were assessed using trypan blue and Ly6G staining, respectively. Apoptosis and necrosis were quantified using Annexin V and Propidium Iodide staining, analyzed by flow cytometry.

Results: Our results demonstrate a 40% increase in neutrophil apoptosis upon SeV infection in the presence of platelets compared to no platelet controls. Moreover, we found that direct contact between platelets and neutrophils is not necessary for inducing apoptosis.

Conclusion: These findings highlight the protective role of platelets in modulating the immune response during viral infections by promoting neutrophil apoptosis, thereby potentially reducing inflammation.

Keywords: Platelets, Neutrophils, Apoptosis, Sendai Virus

Role of FAK in prostate cancer cell dormancy and tumor dissemination

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Background: Up to 30% of prostate cancer (PCa) patients relapsed and progressed to bone metastases despite various curative treatments. These progressed bone metastases are possibly due to early dissemination to distant organs such as the bones. These early disseminated tumor cells (DTCs) remain dormant and evade therapies. Previous studies demonstrated that osteoblasts induced PCa cell dormancy (i.e., decreased cell proliferation and increased dormancy markers such as NR2F1). Furthermore, PF-562271 (PF-271), a focal adhesion kinase (FAK) small molecule inhibitor, was predicted to mimic most of the dormant PCa cell gene signature and was shown to increase NR2F1 and decrease proliferation markers cyclin D1 and MKi67 in PCa cells, suggesting a potential role of FAK in PCa cell dormancy. This study aims to determine the role of FAK and PF-271 in PCa cell dormancy.

Methodology: We generated FAK knockdown in C4-2B and PC3 PCa cells and compared the expression of cell proliferation and NR2F1 in these cells relative to their respective parental cells using qRT-PCR. In vivo, we treated C4-2B xenografted mice daily with PF-271 or vehicle for a week or two after the treatment did not impact the tumor growth. We then harvested the bones, separated the bone marrow and cortex, and quantified the DTCs using species-specific PCR.

Results: FAK knockdown decreased PC3, but not C4-2B, cell proliferation. FAK knockdown increased NR2F1 but not Ki67 or cyclin D1 in C4-2B cells. Furthermore, in vivo studies showed that PF-271 reduced bone marrow DTCs without affecting DTCs in the bone cortex.

Conclusion: These discrepancies (between cells, and between drug treatment and the FAK gene) suggested the cell-specific and partial role of the FAK gene in PCa cell dormancy. We will further explore the cell-specific regulations between FAK and NR2F1 and cell proliferation and the other significant factors in PCa cell dormancy regulated by PF-271.

Keywords: FAK

A novel link between YAP1 and AMPK/mTORC1 signaling in vascular smooth muscle cells

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Introduction: Cardiovascular disease remains the leading cause of preventable mortality in the US and worldwide. Vascular smooth muscle cell (VSMC) phenotypic modulation from a contractile to a synthetic state is central to the etiologies of multiple vascular wall diseases. We have previously demonstrated that Yes-associated protein 1 (YAP1), a transcriptional co-activator, induces VSMC phenotypic switching and neointima formation. However, the underlying mechanisms remain largely unknown.

Methods: *In vitro* loss- and gain-of-function studies utilizing human coronary artery SMCs. Cell proliferation: WST-1, EdU incorporation, and CyQUANT assays. Cell migration: Scratch wound-healing assays. Cell signaling: Immunoblotting and qRT-PCR. Arterial injury: Left femoral artery wire injury in mice.

Results: Loss- and gain-of-function studies demonstrate, for the first time, that YAP1 is required and is sufficient to activate mTORC1 in VSMCs, a key regulator of VSMC phenotypic switching. Consistently, we found that YAP1 is induced following arterial injury and correlated with mTORC1 activation and VSMC phenotypic switching *in vivo*. Importantly, mTORC1 inhibition with rapamycin markedly abolished YAP1-induced VSMC proliferation and migration. Next, we examined the potential upstream regulatory mechanisms by which YAP1 may activate mTORC1. We found that YAP1 inhibits AMPK signaling, a key negative upstream regulator of mTORC1, suggesting an intermediary role of AMPK in mediating the effects of YAP1 on mTORC1, which was validated by co-transfection studies. Next, we examined the potential upstream regulatory mechanism(s) by which YAP1 may regulate AMPK. ChIPseq data analysis identified the catalytic subunit (PPP2CB) and the regulatory subunit (PPP2R1B) of the protein phosphatase PP2A as potential YAP1 transcriptional targets that may mediate the effects of YAP1 on AMPK signaling. Consistently, we found that YAP1 induces PPP2CB and PPP2R1B at both the mRNA and protein levels.

Conclusion: This study identifies a novel signaling pathway linking YAP1 to AMPK/mTORC1 signaling, which plays a key role in VSMC phenotypic switching.

Keywords: AMPK, YAP1, VSMC-Vaascular Smooth Muscle Cells

Identifying and comparing learning and memory deficits in two different models of repetitive traumatic brain injury

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Background: Many pastimes and sporting events today involve regular or repetitive hits to the head, resulting in traumatic brain injury (rTBI) and, chronic traumatic encephalopathy (CTE). These sports include American Football, boxing, martial arts, hockey, lacrosse, basketball, and many more. It has been reported that players who play these sports will undergo a significant behavioral change over time as well as molecular abnormalities such as increased risk of neuroinflammation and tauopathy. Understanding these changes and potentially developing a treatment for them remains to be a crucial mystery researchers could solve. Our lab has investigated the relationship between adverse behavior in the form of memory and learning which is associated with repetitive traumatic brain injury. While there are many different studies associated with rTBI, there seems to be no agreement on optimal experimental conditions.

Methods: In this study, two different methodologies will be compared to optimize the effects and safety of experimental subjects. The first method is a repetitive TBI with 48 hours in between each TBI for one week. The second comparative method involves one weekly TBI for four weeks. As a comparison, both methods have two groups: a surgery group where the TBI will be administered and a sham surgery group mimicking the surgery group in all conditions, except TBI. Memory and learning were determined through a T-maze and Open Field task.

Results: in the 48-hour repetitive TBI, the surgery group alternated significantly less (30% ± 12.25 vs 90% ± 6.124) than the sham group implying a significant difference in cognition. Further the open field heat map showed that the surgery group would cluster towards one side of the open field, whereas the sham group adequately explored more sides of the open field implying again a large difference in learning between the rTBI group and the sham surgery group.

Keywords: Repetitive TBI, Neuroinflammation, Open Field, T-Maze, Memory and Learning

Cysteinyl leukotriene receptors mediate macrophage-endothelial cell crosstalk and contributing to atherosclerosis progression

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Introduction: Atherosclerosis a leading cause of cardiovascular disease, occurs when cholesterol-rich plaques accumulate in arteries, narrowing their lumen. This chronic inflammatory disease involves dysfunction of endothelial cells (ECs) and macrophages (MΦs), which can lead to increased uptake of oxidized low-density lipoprotein (ox-LDL) by MΦ. Cysteinyl leukotrienes (cys-LTs; LTC_4, LTD_4, LTE_4) are inflammatory molecules that act through their receptors, $CysLT_1R$ and $CysLT_2R$. Few studies have examined cys-LTs in ECs or MΦs individually, and their effects on EC-MΦ interactions are unclear. Therefore, we investigated the role of CysLTR signaling in EC-MΦ interactions using an in vitro co-culture system and an in vivo PCSK9-induced atherosclerosis model employing CysLTR knockout mice.

Objectives: To uncover how CysLTRs modulate EC-MΦ function in atherosclerosis progression.

Methods: In vivo, atherosclerosis was induced in WT, *Cysltr1*$^{-/-}$ and *Cysltr2*$^{-/-}$ mice by ip injections of PCSK9 followed by high-fat diet (HFD) for 12 weeks. Aortic plaques were examined to analyze plaques and their composition using Oil-red O staining and immunofluorescence. In vitro, mouse ECs were co-cultured with BMDMs from WT, *Cysltr1*$^{-/-}$ and *Cysltr2*$^{-/-}$ mice in transwell system for 6 hours, analyzed by ELISA and qPCR.

Results: In EC-BMDM co-cultures, BMDMs showed increased expression of $CysLT_1R$ and an inflammatory profile, with higher levels of IL-6, IL-1β, GM-CSF, and scavenger receptors like OLR-1, compared to isolated cultures. Interestingly, *Cysltr1*$^{-/-}$ BMDMs co-cultured with ECs exhibited significantly reduced levels of inflammatory cytokines and scavenger receptors compared to WT BMDMs. Importantly, PCSK9 + HFD *Cysltr1*$^{-/-}$ mice exhibited reduced plaque formation, despite higher lipid profiles compared to PCSK9 + HFD WT mice. Further, we found reduced macrophage infiltration and smooth muscle cell migration in plaque areas of *Cysltr1*$^{-/-}$ mice compared to WT mice.

Conclusion: Our study underscores the vital role of CysLT receptors in regulating EC-MΦ interactions and driving atherosclerosis progression.

Keywords: Cysteinyl leukotrienes, Atherosclerosis, Inflammation, Macrophage, Endothelial cells

HSF1 mitigates copper-induced DLAT protein aggregation

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Background: Cuproptosis, a non-apoptotic copper-induced cell death, is characterized by the aggregation of dihydrolipoamide S-acetyltransferase (DLAT), an essential enzyme involved in maintaining mitochondrial function and has been known to induce proteotoxic stress response (PSR). Heat shock factor 1 (HSF1), a master transcription regulator of PSR, is highly expressed in various cancer types and plays a chaperone role in protein folding and refolding to maintain protein stability. Cancer cells rely on this function to sustain their growth and proliferation. HSF1's ability to preserve proteomic stability is a key factor in its cytoprotective role, suggesting that HSF1 may contribute to the mechanisms that allow cancer cells to tolerate elevated copper accumulation. We aim to investigate the role of HSF1 in sustaining tumor viability in pancreatic ductal adenocarcinoma (PDAC) by mitigating copper-induced cytotoxicity.

Methods: In the human PDAC cell model, we used western blotting to analyze protein poly-ubiquitination, HSF1, and DLAT expression levels. Amyloid fibril formation was detected using a sandwich ELISA with an anti-OC antibody. Aggresomes were visualized using a fluorescence microscopy-based aggresome detection kit. The CellTiter-Blue® Cell Viability Assay was used to evaluate the cell viability. Non-reducing SDS-PAGE was performed to examine protein post-translational modifications.

Results: HSF1 overexpression effectively mitigates copper-induced protein ubiquitination and aggresome formation. Conversely, inhibiting HSF1 through knockdown or HSF1 inhibitor exacerbated the copper-increased amyloid fibril formation. Excess copper triggered DLAT aggregation and reduced its lipoylation, while HSF1 overexpression restored DLAT integrity and lipoylation levels. HSF1 inhibition enhanced the decrease in cell viability induced by copper-ionophore treatment in PDAC cells.

Conclusion: Our study highlights the protective role of HSF1 in mitigating copper-induced cellular toxicity in PDAC cells. Combining HSF1 inhibition with copper treatment presents a promising therapeutic strategy for targeting PDAC.

Keywords: HSF1, Copper, DLAT, Aggregation

Role of the mRNA helicase eIF4A1 in cancer stemness program

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Background: Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer (BC), characterized by poor prognosis and high mortality rate. The standard first-line treatment is neoadjuvant chemotherapy (NACT) such as taxanes. Despite initial treatment response, the tumors develop chemoresistance and relapse ensues, highlighting the need for more efficacious therapeutic strategies. Drug resistance is mostly due to a small population of cells residing within the tumor known as breast cancer stem-like cells (BCSCs) or tumor initiating cells (TICs). This leads to minimal residual disease and subsequent repopulation of aggressive and highly metastatic tumors. BCSCs contribute significantly to primary tumor progression, chemoresistance, and metastasis due to their self-renewal capability and expression of pluripotency transcription factors (PTFs) including SOX2, OCT4, NANOG and KLF. Previously, we demonstrated that the eukaryotic translation initiation factor 4A1 (eIF4A1) is associated with resistance to paclitaxel in TNBC cell lines in vitro. Here, we will employ both in vitro and in vivo models to definitively identify the causative role for eIF4A1's helicase activity in mediating BC stemness.

Methods: To definitively identify that the helicase activity of eIF4A1 contributes to BC stemness, we ectopically expressed wild type (WT) eIF4A1 and helicase-defective mutants with differential helicase activity (0%, 10%, and 50%) in the eIF4A1-KO background. An immunoblotting approach was initially employed to ascertain that the restoration of the helicase activity of eIF4A1 was proportional to the level of dysfunction of the helicase activity in the eIF4A1 mutants. The protein and mRNA levels were evaluated for PTFs. Additionally, we evaluated the functional impact of eIF4A1 helicase activity on the self-renewal capacity of BCSCs.

Results: Immunoblot analysis confirmed the successful rescue of WT eIF4A1 phenotype in the knockout background as compared to the CRISPR control. Interestingly, cells expressing helicase-defective mutants (0%, 10%, and 50% activity) exhibited a significant downregulation of PTFs demonstrating that the helicase activity of eIF4A1 is essential for maintaining the expression of these key regulators of BC stemness. The degree of helicase activity correlated with the expression levels of the PTF factors, further underscoring the direct role of eIF4A1 in maintaining the stem-like properties in TNBC cells. The number and size of colonies formed by WT eIF4A1, and helicase-defective mutants were compared to determine the influence of differential helicase activity on BCSC properties.

Conclusion: Our findings suggest that eIF4A1 helicase activity plays a key role in sustenance of the pluripotent state by regulating PTFs involved in self-renewal and pluripotency. Targeting the helicase activity of eIF4A1 might be a promising therapeutic strategy to eliminate BCSCs and bulk tumor cells, potentially overcoming plastic interconversion and chemoresistance thus reducing relapse in TNBC. In the future, we will examine the mammosphere formation efficiency and perform preclinical murine studies to ascertain tumor initiation by eIF4A1 as well as primary tumor progression and metastasis.

Keywords: Breast Cancer stemness, protein translation, eIF4A1

Inflammatory impact of aerosolized microcystin on healthy and asthmatic human airway epithelial cells

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Background: Cyanobacterial harmful algal blooms (cHABs) are increasing, leading to higher levels of toxic microcystins. MC-LR, MC-RR and MC-LA congeners are also reported in significant concentration in cHABs aerosols. While these toxins induce inflammatory responses, the differences in how they affect healthy individuals versus those with pre-existing conditions remain unclear.

Objectives: We aimed to evaluate the physiological and inflammatory impacts of aerosolized microcystins in lung airway epithelium. Methods 3D Air-liquid interface cultured human primary airway epithelial cells (hAEC/B) from healthy and asthmatic donors were exposed to aerosolized microcystins and vehicle saline control. Various inflammatory gene expressions were determined using qRT-PCR. The Microcystin-induced NF-kB activity of 1μM MC-LR, MC-LA or vehicle was evaluated in A549_Dual reporter cells on the background of IL-1B stimulation (0.5 ng/ml).

Results: The fully differentiated hAEC/B cells exhibited cilia beating and mucociliary clearance with minimal changes in cellular morphology with 10μM MC-LR exposure. Exposure to 1 μM MC-LR led to a 1.8-fold increase in TNF-alpha, and a 1.9-fold increase in IL-6 in healthy group ($p<0.001$). These increments were significantly higher in asthmatic group of airway epithelial cells, with a 3.5-fold increase in TNF-alpha and a 2.6-fold increase in IL-6 ($p<0.001$). MC-LR and MC-LA affected NF-κB activity in A549 cells differently. While vehicle-exposed cells showed no change, MC-LR pretreatment followed by exposure led to a modest ~10% increase in NF-κB activity ($P<0.01$). In contrast, MC-LA pretreatment and exposure caused a robust ~50% increase ($P<0.01$) and was significantly higher (~40%) than MC-LR-induced activity ($P<0.001$). Furthermore, IL-1β-stimulated NF-κB activity was elevated by ~40% after MC-LR pretreatment ($P<0.0001$) and ~70% after MC-LA pretreatment ($P<0.0001$), highlighting a stronger pro-inflammatory effect of MC-LA.

Conclusion: Aerosolized MC-LR exposure significantly increases inflammatory genes in airway epithelial cells, with stronger effects in asthmatic cells. MC-LA exposure induces a significantly stronger pro-inflammatory response in A549 cells than MC-LR, with higher increases in NF-κB activity.

Keywords: Microcystin, Airway Inflammation, Environmental Exposure

Loss of PTH1R in osteoblasts inhibits prostate cancer metastases

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Background: Nearly 90% of patients who die from prostate cancer (PCa) have bone metastases. Parathyroid hormone-related protein (PTHrP) is shown to be one of the drivers of PCa bone metastases. PTHrP is secreted mainly by cancer cells and acts through binding with the parathyroid hormone type 1 receptor (PTH1R) on osteoblasts. Blocking PTHrP with monoclonal antibody showed palliative but not survival benefits in patients. PTH1R—a G protein-coupled receptor (GPCR)—remains mostly underexplored despite GPCRs accounting for 35% of FDA-approved drugs. We aim to investigate the role of PTH1R in osteoblasts in PCa metastasis using genetic-engineered mouse models.

Methods: Luciferase-labeled PCa cells, AR-positive 22Rv1 or AR-negative PC3 cells, were intracardially injected into the PTH1RColCreERT knockout (KO), and the PTH1RFloxE2, and PTH1RFloxE2 littermates served as control. Tamoxifen injection-induced Cre recombinase activation selectively deleted PTH1R in mesenchymal-lineage cells, including osteoblasts. We monitored cancer metastases weekly through longitudinal optical imaging. At the end time points, metastasized tissues were harvested for histology analyses and RNA sequencing to determine the mechanism of action.

Result: We found that PC3 cells metastasized various organs, such as the liver, kidneys, and bones. 22Rv1 cells metastasized exclusively to the bones. Compared to the PTH1RFloxE2 control mice, the PTH1RColCreERT KO mice showed significant reductions in metastatic progressions, measured by the luminescent signal intensities and bone lesion areas; decreased PCa cell proliferation, measured by immunohistochemistry (IHC) of the phospho-histone H3 (pHH3), and decrease osteoclast activation and differentiation, identified by Tartrate-Resistant Acid Phosphatase (TRAP) staining.

Conclusions: Loss of PTH1R in osteoblasts decreases PCa metastases and bone lesion development—this significant role of PTH1R warrants further delineating how loss of PTH1R inhibited the overall and bone metastases.

Keywords: PTH1R, Osteoblasts, Prostate Cancer Metastases