INTERACT

INSTITUTE FOR TRANSLATIONAL AND EMERGING RESEARCH IN ADVANCED COMPARATIVE THERAPY

2024 **Symposium**

One Health & **One Medicine**

ConocoPhillips OSU Alumni Center
Stillwater, Okla.
September 17-18

TABLE OF CONTENTS

ADVANCING GLOBAL HEALTH THROUGH ONE HEALTH AND ONE MEDICINE

Colleagues and guests,

Oklahoma State University's research impacts the world. It lies at the core of our modern land-grant mission. As we welcome delegates and speakers from several countries to Stillwater, we look forward to learning about scientific discoveries that have reshaped our approach to health and medicine.

By creating a collaborative, scientific environment that brings together various disciplines from across the university, INTERACT sets an example for other institutions and embodies the cooperative spirit among our most talented research leaders. It is advancing One Health and One Medicine for both human and veterinary medicine, which are crucial in addressing society's most urgent needs.

I commend each one of you for your pursuit of therapeutic and diagnostic advances. Congratulations also to our faculty and students for showcasing such meaningful and impactful research in this symposium.

Thank you, and Go Pokes!
Dr. Kayse Shrum
President, Oklahoma State University

CVM: SPEARHEADING INNOVATIVE APPROACHES THAT SEAMLESSLY INTEGRATE VETERINARY AND HUMAN MEDICINE

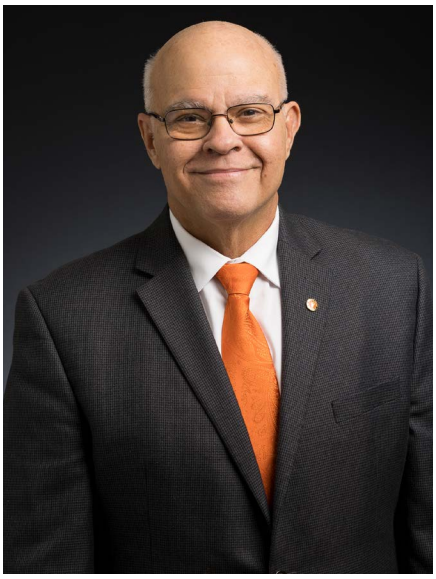
Greetings,

Thank you for attending the third international INTERACT Research Symposium on One Health and One Medicine. Through INTERACT, the college has created a university-wide network of investigators from different colleges, fostering a platform that supports interdisciplinary and translational research in a collaborative, team-oriented environment.

Members of INTERACT are conducting innovative research and clinical trials focused on developing new therapeutic approaches and diagnostic methods for treating various diseases in both large and small animals. This merging of basic and clinical sciences not only enhances research capabilities, but also cultivates specialized expertise in One Health and One Medicine related disciplines.

It is gratifying to see a diverse group of speakers and attendees from national and international universities. The CVM will continue to collaborate with other colleges and departments to strengthen the INTERACT program. I look forward to the opportunity to host more gatherings and the development of new collaborative research programs in the years to come.
I would like to express my sincere gratitude to all the faculty, staff and students for their unwavering dedication to conducting cutting-edge research that leads to the development of new technologies that will improve the health and well-being of people in Oklahoma and worldwide.

Sincerely,
Dr. Carlos Risco
Dean and Professor, College of Veterinary Medicine



TUESDAY, SEPTEMBER 17

7:30 a.m.	Breakfast
8:30 a.m.	Opening and welcome
9 a.m.	1.1 - Dr. Joy Scaria | Oklahoma State University | *Microbiota-Gut Interactions: A Systems Biology Lens on Gastrointestinal Diseases*
9:45 a.m.	1.2 - Dr. Purna Kashyap | Mayo Clinic | *TBA*
10:30 a.m.	Break
10:40 a.m.	1.3 - Dr. Crystal Johnson | Oklahoma State University | *OneHealth applications for Microbiome-based antimicrobials*
11:20 a.m.	1.4 - Dr. Alain Stintzi | University of Ottawa | *Unraveling the Hidden Players: Gut Bacteriome and Virome Insights in Chronic Diseases*
12 p.m.	1.5 - Dr. Reed Stubbendieck | Oklahoma State University | *Bacterial Competition in the Aerodigestive Tract: Antibiotics, Probiotics, and Beyond*
12:45 p.m.	Lunch - Student Posters
1:45 p.m.	2.1 - Dr. Lesley Smyth | University of West London, UK | *Extracellular vesicles in immunology: health and disease*
2:30 p.m.	2.2 - Dr. Charlotte Lawson | University of Central Lancashire, UK | *AdEVentures in EV Land: Do extracellular vesicles contribute to inter-species crosstalk?*
3:15 p.m.	Break
3:30 p.m.	2.3 - Dr. Liz McCullagh | Oklahoma State University | *Contribution of mitochondria to sound processing difficulties in autism*
4:15 p.m.	2.4 - Dr. Claire Thornton | Royal Veterinary College, UK | *Repairing damaged mitochondria to prevent neonatal brain injury*
5 p.m.	2.5 - Dr. Michael Davis | Oklahoma State University | *Mitochondrial Exercise Physiology*
5:45 p.m.	2.6 - Dr. Madhan Subramanian | Oklahoma State University | *Emerging Role of Glial Cells in Obesity-Induced Hypertension*
6:30 p.m.	Dinner - Student Posters
7 p.m.	2.6 - Mr. Lance Walker | Director, Human Performance & Nutrition Research Institute, Oklahoma State University | *HPNRI: An Intersection of Innovation, Prosperity, and Teamwork*

WEDNESDAY, SEPTEMBER 18

7:30 a.m.	Breakfast
8:30 a.m.	3.1 - Dr. Mana Mahapatra | Pirbright Institute, UK | *Foot-and-mouth disease control by vaccination*
9:15 a.m.	3.2 - Dr. Veerasak Punyapornwithaya | Chiang Mai Univ, Thailand | *Modelling in veterinary study: Insight from outbreak, surveillance and one-health data*
10 a.m.	3.3 - Dr. Saidu Oseni | OAU, Nigeria | *Digital Technologies for Broiler Health and Welfare Management in the Humid Tropics of Nigeria*
10:45 a.m.	Break
11 a.m.	3.5 - Dr. Rashmi Singh | DUVASU, India | *Rotavirus and One Health: Evolution/Epidemiology, Interspecies Transmission and Zoonoses*
11:40 a.m.	3.6 - Dr. Fernando Bauermann | Oklahoma State University | *Virome Surveillance and Strategies for Viral Control and Antiviral Testing*
12:30 p.m.	Lunch
1 p.m.	Student Awards
1:15 p.m.	4.1 - Dr. Gaëlle Kamdjo | National Veterinary Laboratory, Cameroon | *Brucella abortus, a neglected biothreat in Cameroon: seek and you shall find*
2 p.m.	4.2 - Dr. Anthony Onipede | OAU, Nigeria | *Nigeria: A One-Health Challenge of Zoonotic and Neglected Tropical Diseases*
2:45 p.m.	Break
3 p.m.	4.3 - Dr. Ashley Railey | Oklahoma State University | *Household control decisions for foot-and-mouth disease in Africa*
3:45 p.m.	4.4 - Dr. Adam Roth | Oklahoma State University | *Tracking Social Lives in Real Time: Methodological Innovations for Healthy Aging*
4 p.m.	4.5 - Dr. Amy Hagerman | Oklahoma State University | *Economic Dimensions of One Health: Emphasis on Animal Health and Agricultural Dependent Economies*
4:45 p.m.	4.6 - Panel Discussion | Social Dimension of Zoonotic Disease in Rural Communities
5:45 p.m.	Reception - Cash bar/Hors d'Oevres | Speaker Recognition
8:30 p.m.	Close

PLENARY SPEAKER

Dr. Purna Kashyap

Dr. Purna Kashyap is a professor of medicine and physiology, and the Bernard and Edith Waterman Director of the Microbiome program at Mayo Clinic, Rochester, MN. He leads a NIH-funded laboratory focused on delineating complex interactions between diet, gut microbiome, and host physiology using germ-free mouse models. In parallel, his laboratory uses a systems approach incorporating multi-omics, patient metadata, and human tissue physiologic responses, to identify novel microbial drivers of DGBI. Dr. Kashyap has published nearly 100 peer-reviewed articles including journals like Cell, Cell Host Microbe, Science Translational Medicine, Nature Communications, and Gastroenterology. He was inducted to ASCI in 2021 and has previously served on the ANMS council, NGM board of editors, and the scientific advisory board of AGA Gut Microbiome Center. He currently serves on the AGA council and research committee, in editorial roles at Gut Microbes and FASEB, and as an ad hoc reviewer on NIH study sections.

PLENARY SPEAKER

Lance Walker

Lance Walker is an internationally recognized expert in fusing human performance, sports science, and sports medicine for athletes of all ages, ability levels, and sports disciplines. He is the former Executive Vice President and Global Performance Director of Michael Johnson Performance. Walker has experience in both private business of sports performance and working in organizations within the NFL, NCAA, and High School levels. Walker is a published author, former university human performance lab coordinator, NIKE Performance Council Member, and exercise science researcher. Additionally, he is a performance coach, registered physical therapist, adjunct professor and clinical instructor with nearly three decades of sports performance training and sports medicine experience spanning over 50 professional, elite, collegiate, high school, and youth sports and thousands of current and past athletes supported, including First Round NFL/NBA/CHL/MLB draft choices, Olympic and Paralympic Gold medalists, World Champions, World Record Holders, Super Bowl MVPs, Champions League Stars, and NFL Hall of Famers.

PLENARY SPEAKER

Dr. Mana Mahapatra

Dr. Mana Mahapatra is trained as a veterinarian in India with a master's degree in Veterinary Biochemistry. With a Commonwealth Scholarship, she pursued her PhD in Molecular Virology at The University of London. She worked as a veterinary virologist at TPI working on the development of recombinant vaccines and associated diagnostic tests for two important viral diseases of domestic animals, Foot and Mouth Disease (FMD) and Peste des petits ruminants (PPR). She has over 70 numbers of publications to her credit. She was successful in receiving > £6.5 million in funding from various funding bodies. She has extensive experience in training and capacity building in third-world countries, mainly in Africa, the Middle East, Latin America, and Asia. At the Pirbright Institute, UK she was involved in conducting training courses for animal diseases and, was also part of the national emergency response team for animal diseases. Recently she worked on a project for WOAH where she prepared e-learning modules on PPR for the veterinarians, para-veterinarians, and lab scientists of Southeast Asian countries and, developed a risk register that documents possible risks for incursion of the disease into the region and how to prevent it.

PLENARY SPEAKER

Dr. V Punyapornwithaya

Dr. Veerasak Punyapornwithaya is an associate professor of veterinary epidemiology at the Faculty of Veterinary Medicine, Chiang Mai University, Thailand. He earned his DVM degree from Kasetsart University, Thailand, and his Ph.D. from Washington State University, USA. With over 15 years of experience in epidemiology, he specializes in applying statistical models and data science techniques to animal disease data. He served as the principal investigator for a study assessing the impact of lumpy skin disease (LSD) in Asia, funded by the World Organization for Animal Health (WOAH). Currently, he is on the advisory board for establishing LSD prevention and control strategies in Southeast Asia. Additionally, he continues to receive funding from WOAH for developing predictive models for livestock disease trends based on animal price fluctuations and related factors. He has collaborated with mathematicians, statisticians, data scientists, and public health professionals in various studies.

INVITED SPEAKERS

Dr. Fernando Bauermann

Assistant professor

Oklahoma State University

Dr. Michael Davis

Professor, Physiological Sciences; John Oxley Endowed Chair Equine Sports Medicine

Oklahoma State University

Dr. K. Gururaj

Senior Scientist, Division of Animal Health

ICAR-Central Institute for Research on Goats in Mathura, India

Dr. Amy Hagerman

Associate professor, Agricultural Economics

Oklahoma State University

Dr. Crystal Johnson

Assistant professor, Microbiology

Oklahoma State University's Center for Health Sciences

Dr. Charlotte Lawson

Associate dean, Business Development: School of Pharmacy and Biomedical Sciences,

University of Central Lancashire

Dr. Liz McCullagh

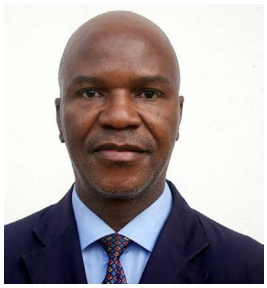
Assistant professor, Integrative Biology

Oklahoma State University

Dr. Anthony Onipede

Professor, Medical Biology

Obafemi Awolowo University, Ile-Ife, Nigeria



Dr. Saidu Oseni

Professor, Animal Sciences

Obafemi Awolowo University, Ile-Ife, Nigeria

Dr. Ashley Railey

Assistant professor, Sociology

Oklahoma State University

Dr. Adam Roth

Assistant professor, Sociology

Oklahoma State University

Dr. Joy Scaria

Associate professor; Walter R. Sitlington Endowed Chair, Infectious Diseases

Oklahoma State University

Dr. Rashmi Singh

Professor; head, Veterinary Microbiology; dean, College of Biotechnology

DUVASU, Mathura (India)

Dr. Lesley Smyth

Associate professor, Immunology

University of West London

Dr. Alain Stintzi

Professor; director, School of Pharmaceutical Sciences

University of Ottawa

Dr. Reed Stubbendieck

Assistant professor, Microbiology & Molecular Genetics

Oklahoma State University

Dr. Madhan Subramanian

Associate professor

Oklahoma State University

Dr. Claire Thornton

Senior lecturer, Cell Biology

Royal Veterinary College

STUDENT ABSTRACTS

FUS PLAYS A ROLE IN FIBROBLAST ACTIVATION AND circCOL1A1 BIOGENESIS

Akshaya Surendran, Chaoqun Huang, Lin Liu

The Lundberg-Kienlen Lung Biology and Toxicology Laboratory,
Department of Physiological Sciences,
College of Veterinary Medicine, Oklahoma State University

Abstract

Idiopathic Pulmonary Fibrosis (IPF) is a deadly fibrotic interstitial lung disease which mostly affects the aged. This illness significantly affects the patient's quality of life, has poor prognosis due to diagnostic challenges and lack effective treatments. Fused in sarcoma (FUS) is an RNA-binding protein which has been implicated in circular RNA biogenesis, genomic integrity, and regulation of gene expression. Defects in this gene are associated with the development of amyotrophic lateral sclerosis type 6. However, the involvement of FUS in IPF is still unclear. In this study, we investigated the role of FUS in lung fibroblast activation and possible mechanisms. Genotype tissue expression (GTEx) database revealed that FUS is widely expressed in various organs including lungs. Single cell RNA sequencing dataset showed that FUS was upregulated in fibroblasts and myofibroblasts of IPF lungs compared to those of healthy lungs. Western blot and Immunofluorescence showed that FUS is located in the nucleus of human pulmonary fibroblasts (HPFs). Transforming Growth Factor- β1 (TGFβ1) treatment of HPFs did not change the nuclear localization or mRNA levels of FUS. Knockdown of FUS in HPFs led to a significant reduction in the expression of fibroblast activation markers including α-smooth muscle actin (α-SMA), fibronectin and type 1 Collagen A1 (COL1A1). As FUS is involved in circular RNA biogenesis, we examined the effect of FUS knockdown on circCOL1A1 expre sion. We observed that the relative expression of circCOL1A1 in HPFs was adversely impacted by the knockdown of FUS. CircCOL1A1 silencing suppressed TGFβ1-induced HPF activation as demonstrated by the decreased expression of α-SMA and COL1A1. Our results suggest that FUS is a pro-fibrotic gene that activates pulmonary fibroblasts likely by promoting circCOL1A1 biogenesis.

Development of a Multi-Polyphenol Metabolizing Probiotic Consortium through Genistein Enrichment in Pig Gut Microbiota Using a Mini Bioreactor Model

Amal Cheemadan, Theresah Amponsah, Binta Varghese, Vishnu Thayil Valappil, Prabhjot Sekhon Kaur, Achuthan Ambat, Joy Scaria

Department of Veterinary Pathobiology,
College of Veterinary Medicine, Oklahoma State University

Background

Intensive pig farming often disrupts homeostasis and raises the risk of infectious disease outbreaks, harming livestock health and productivity. To address these issues, enhancing dietary to harmonize gut microbes and promote overall health. Understanding the effect of polyphenolgenistein, an isoflavone abundant in soybean-based pig diets, on the gut microbial community is essential, and Identifying bacteria capable of degrading polyphenols is crucial for developing a probiotic consortium to improve gut health and enhance resistance to infections by metabolizing various polyphenol compounds.

Methods

Fecal samples from five healthy pigs were pooled and used for genistein enrichment in an in vitro mini bioreactor under anaerobic conditions for 21 days. Samples were taken weekly for microbial analysis via 16S rRNA sequencing and SCFA quantification by gas chromatography. Bacterial species were isolated using a micro-cultivation array and anaerobic culture with modified BHI media. The safety of the bacterial library was confirmed by hemolysis and invasion assays, and the genistein biotransformation ability of the species was validated using a DPH assay. Finally, the distribution of different flavonoid-metabolizing enzymes in the genistein-enriched library of bacterial species was illustrated using in silico method.

Results

Genistein supplementation enhances the gut microbiota structure in a bioreactor model by increasing the abundance of beneficial bacteria, such as lactic acid bacteria, and reducing the abundance of Proteobacteria, which includes several pathogens responsible for conditions characterized by inflammation and diarrhea. SCFA analysis revealed a significant increase in acetate levels. The majority of bacterial species from the genistein-enriched microbiota can serve as a potential probiotic consortium, capable of metabolizing multiple polyphenolic compounds by participating in various flavonoid-metabolizing pathways such as polyphenol metabolizing pathways and benzoic acid pathways.

Conclusion

This study demonstrates that genistein enrichment in pig gut microbiota fosters the development of a probiotic consortium capable of metabolizing multiple polyphenols, potentially enhancing gut health and infection resistance. The effective metabolism by these isolates indicates potential for broader flavonoid metabolism studies, optimizing their application in therapeutic and nutritional contexts, and contributing to overall health improvements in animals.

Bridging Laboratory Findings to Aquatic Toxicology at Grand Lake, Oklahoma: Significance of Cadmium & Copper Toxicity in Fish Gill Cells.

Aryanna Carr, Stacey Herriage, Dr. Matteo Minghetti

Abstract

The impact of metal chemical speciation on metal absorption, and toxicity in metal mixture exposures remains poorly understood. In laboratory settings, cells are typically exposed to individual metals, rather than metal mixtures. However, in natural environments, metals often interact with one another and with surrounding inorganic and organic matter. For example, the Tar Creek Superfund site, which spans northeastern Oklahoma, southeastern Kansas, and southwestern Missouri, is contaminated with high levels of cadmium, copper, arsenic, and other heavy metals. To investigate the health effects of metal toxicity, we used RTgill-W1 fish cells derived from rainbow trout. These cells were seeded at a density of 150,000 cells/cm^2 in 24-well plates, incubated for 48 hours at 19°C, and then exposed to various concentrations of copper, cadmium, and copper-cadmium mixtures for 24 hours in a synthetic medium mimicking fresh water but compatible with cell culturing. After exposure, a multiple endpoint viability assay was used to measure metabolic activity, cell membrane integrity, and lysosomal membrane integrity. This approach allowed the determination of dose-response curve and calculation of the effective concentration inhibit cell viability by 50% (EC50) for cadmium and copper. A result from the cytoxicity assay resulted in the concentrations of each metal that inhibited half of the cell's standard metabolic activity for copper and cadmium, 6.32 uM and 87.63 uM. Both metals are toxic to intracellular mechanisms such as cell metabolic activity and membrane integrity, with copper being particularly harmful at higher concentrations despite its role as an essential element. Each metal and metal combination were tested in triplicate to ensure statistical significance. The next step will be to use the single metal EC50s to determine the concentration to add metals in mixture.

Targeting Colorectal Cancer Stemness Through Epigenetic Regulation by TIP60

Asad Mohammad and Sudhakar Jha

Department of Physiological Sciences,
College of Veterinary Medicine, Oklahoma State University

Objective

To develop strategies to target colorectal cancer (CRC) stemness for cancer treatment.

Background and Objective

Colorectal cancer (CRC) is the second-leading cause of cancerrelated deaths in the United States. Despite new targeted medicines and multiple therapeutic combinations, tumor relapse and drug resistance persist. Colorectal cancer stem cells (CRCSCs) are regarded as the primary drivers of cancer development, recurrence, and dissemination. Cancer stem cells (CSCs), within the tumor bulk, self-renew and differentiate, leading to tumor recurrence, metastasis, and multidrug resistance. Therefore, the call for a better translational approach focusing on cancer stem cells geared toward personalized therapeutic options is mandatory.

Materials and Methods

The HCT116 CRC cell lines were cultivated in DMEM (high glucose) supplemented with 10% FBS. Lentiviral vectors were employed to accomplish shRNA-induced suppression of TIP60. The effectiveness of TIP60 knockdown and CD44 expression levels were confirmed by qPCR. For the colony formation experiment, 1000 HCT116 shLuc control and ShTIP60 cells were seeded in 6-well plates and cultured for 11 days. Cells were cultivated on coverslips in 6-well plates for immunofluorescence labeling, fixed with 100% methanol for 20 minutes, and blocked with 3% BSA. The spheroid culture was conducted on 96-well ultra-low attachment plates employing 100 cells per well.

Findings

This study demonstrates the critical role of HIV Tat-interactive protein (TIP60) in regulating colorectal cancer (CRC) stemness. TIP60 depletion resulted in a significant decrease in cellular proliferation, highlighting the importance of TIP60 in CRC development. Furthermore, TIP60 depletion altered colony formation, transitioning from densely packed formations to scattered spindle networks, suggesting its involvement in the epithelial-mesenchymal transition (EMT). 3D culture models demonstrated that TIP60 is essential for spheroids formation, emphasizing its significance in promoting cancer stemness. TIP60-depleted cells exhibited enhanced invasion in a 3D basement membrane extract (BME) invasion matrix, accentuating its crucial contribution to cellular invasiveness. Furthermore, we have identified that TIP60 undergoes liquid-liquid phase separation (LLPS) and forms biomolecular condensates. TIP60 harbors an intrinsically disordered region (IDR) essential for inducing LLPS in CRC. We have also discovered that TIP60 IDR regulates colorectal cancer stemness and epithelial-mesenchymal transition (EMT) in CRC. In addition, the reduction of TIP60 resulted in a decrease in the expression of CD44, which is a crucial indicator for cancer stem cells (CSCs), and CD44 overexpression reinstated the efficiency of spheroid formation and reversed the epithelialmesenchymal
transition (EMT) phenotype.

Conclusions and Clincial Implications

Our findings suggest that TIP60 has cancer stem-cell characteristics in CRC, and targeting TIP60 will offer a novel therapeutic approach against colorectal cancer stem cells.

Dasatinib and Quercetin Mitigate Palmitic Acid-Induced Microglial Activation: Potential Therapeutics for Obesity-Induced Sympathoexcitation

Bhuvana Plakkot and Madhan Subramanian

Department of Physiological Sciences,
College of Veterinary Medicine, Oklahoma State University

Background

Glial cell dysfunction has been implicated in obesity-induced oxidative stress and DNA damage, which increases the risk for cellular senescence, a state of irreversible growth arrest and neuroinflammation. This is associated with increased sympathetic nerve activity in high-fat diet-induced obese mice causing cardiovascular disease risk. This study hypothesized that a senolytic combination of Dasatinib and Quercetin (D+Q) would reverse glial cell dysfunction in an obese microenvironment.

Methods

Palmitic Acid (PA), a major saturated fatty acid in high-fat diets, can cross the blood-brain barrier and promote inflammation. Human microglial cells (n=6 wells/group/experiment) were treated with 35 µM PA for 24 hours. Subsequently, cells were treated with Dasatinib (250 nM) and Quercetin (100 µM) for 48 hours. Real-time PCR, lipid staining, and Senescence-Associated βeta-Galactosidase (SA-β-GAL) were performed to evaluate changes in microglia due to PA and D+Q treatments. Immunofluorescence staining assessed microglial activation and Vesicular Glutamate Transporter (VGLUT1) expression. One-way ANOVA was performed and $P \leq 0.05$ considered statistically significant.

Results

Microglial activation was confirmed by the upregulation of Iba1 and CD11b in PA-treated cells. There was significant upregulation of senescence genes (p16 and p21), senescence-associated secretory phenotypes (TNFα and MMP3), and SA-β-GAL staining in the PA treatment group, indicating cellular senescence. PA treatment significantly upregulated glutamate transporters EAAT1 and EAAT2, suggesting an adaptive response to counteract excitotoxicity. BODIPY and Oil-Red-O (ORO) staining revealed increased lipid accumulation in the PA group, indicating altered lipid metabolism. The D+Q group showed a significant decrease in p16 and p21 and SA-β-GAL staining, along with a reduction in lipid markers (perilipin 1 and 2) and decreases in Iba1 and CD11b. A significant decrease in EAAT1 expression was also observed. Immunofluorescence showed a significant decrease in VGLUT1 expression in the D+Q compared to the PA-treated group.

Conclusion

These findings suggest that palmitic acid treatment of human microglial cells could lead to cellular senescence and inflammation, affecting the glutamate clearance pathway and potentially causing excitotoxicity and sympathetic overactivity in obesity. The senolytic combination (D+Q) reversed PA-induced lipid accumulation, senescence, and inflammation in microglial cells, indicating a promising therapeutic strategy for managing glial cell dysfunction in obesity.

Redefining the gut microbiome estimate in human population: The Significance of Low Abundance Bacteria

Binta Varghese, Achuthan Ambat, Vishnu Thayil Valappil, Shalabh Mishra, Tanim Islam, Amal Cheemadan, Joy Scaria

College of Veterinary Medicine, Oklahoma State University

Background

Human gut microbiome studies have predominantly focused on highly abundant species, often neglecting low-abundant bacteria. Despite their exclusion in scientific studies, these bacteria may play crucial ecological roles. This study leverages 25,000 global human gut metagenome samples, integrating metabolic modeling and community dynamic experiments to highlight the contributions of low-abundance species.

Methods

We performed shotgun metagenomic analysis on 25,000 human fecal metagenomic datasets to determine global species patterns. To experimentally determine conditions in which rare species are impacted, we used 384 unique nutrients to enrich fecal bacteria from healthy human donors. Species composition following nutrient selection was determined by long-read sequencing on the PacBio platform. Genome-scale metabolic modeling with flux balance analysis was used to contrast the metabolic potential of low-abundance species against high-abundance species. Extended bigdata and statistical analysis was performed with R and Python.

Results

Contrary to the prevailing belief that low-abundance taxa are not culturable, we successfully enriched species with abundances as low as 0.00001% from healthy humans, challenging the traditional 0.01% cutoff used to minimize contamination and sequencing errors in metagenomic studies on human gut microbiome. This discovery, supported by similar trends in existing microbial culturomics data, underscores the biological relevance of low-abundance microbes. Single nutrient screening using 384 conditions revealed that even minor nutrient changes can significantly impact the growth and diversity of low-abundance bacteria, causing proliferation of extremely low-abundance microbes several fold higher. Nearly 500 species identified from 25000 population analysis have more than 50% prevalence with abundance very low to high. Our genome-scale metabolic modeling on this species demonstrated clear metabolic potential separations and interactions between low- and high-abundance bacteria. These results suggest that low-abundance bacteria play crucial ecological roles, potentially influencing both health and disease states through their interactions with high-abundance species.

Conclusion

This study challenges the conventional approach of excluding low-abundant gut bacteria in gut health and disease studies. By demonstrating that extremely rare gut bacteria are nearly universally present across human populations and that these species have distinct nutritional capabilities, our research advocates for a broader inclusion of low-abundance species in future microbial studies and therapeutic strategies.

Nox2 activation promotes SARS-CoV-2-induced lung inflammation and severe pneumonia.

Cody Whitley, Roshan Ghimire, Paige Johnson, Rakshya Shresth, Sunil More, Thota Ganesh, Rudra Channappanavar

Department of Veterinary Pathobiology,
College of Veterinary Medicine, Oklahoma State University
Department of Pharmacology and Chemical Biology,
Emory School of Medicine, Atlanta, GA
Oklahoma Center for Respiratory and Infectious Diseases,
Oklahoma State University

Abstract

Severe COVID-19 caused by SARS-CoV-2 continues to cause morbidity and mortality worldwide. However, the mechanistic basis for the SARS-CoV-2-induced lethal pneumonia is not well understood. Violi and his colleagues reported significantly higher levels of NADPH Oxidase Enzyme 2 (Nox2) in the blood of patients with COVID-19, and even higher levels of Nox2 in patients that were submitted to the ICU. Thus, implicating Nox2 as a possible cause of severe disease in SARS-CoV-2 infections. Nox2 is a reactive oxygen species (ROS) generating enzyme that is activated in response to various stimuli, including virus infections. Nox2 promotes inflammation in response to a pathogen insult by converting molecular oxygen (O2) into hydrogen peroxide (H2O2) and other toxic intermediates. High levels of Nox2 have been reported in myeloid cell populations, with studies linking an exuberant amount of ROS generated by NOX enzymes with excessive inflammation and tissue damage. In our preliminary studies, we observed a significant increase in lung Nox2 levels in SARS-CoV-2 infected mice compared to naïve cohorts. Using murine bone marrow derived macrophages (BMDM) from wild type (WT) mice, we blocked Nox2 with a variety of different inhibitors. Afterwards, we stimulated with different TLR agonists and found inhibitor-specific suppression and enhancement of pro-inflammatory cytokines, while also identifying a significant increase in anti-viral cytokines. Additionally, we observed results that coincided with an inhibitor of Nox2, GSK, in mice that were deficient in Nox2 knockout (KO) mice. We then further explored the ability of the Nox2 inhibitors to suppress viral replication in-vitro. This led to an in-vivo study in which WT and Nox2 KO mice were infected with a mouse-adapted-SARS-CoV-2 (MA-CoV-2). While 50% of the WT mice died, none of the KO mice succumbed to the infection, and were able to clear the virus significantly faster than WT mice. Collectively, these results demonstrate a critical role for Nox2 signaling in SARS-CoV-2-induced dysregulated immunity. Our future work will focus on a deeper understanding of the mechanistic basis for the immunomodulatory and compensatory functions of Nox2 and its isoforms, as well as exploring the effects of Nox2 inhibitors on SARS-CoV-2 infections in-vivo.

SARS-CoV-2 infection generates a broad antigen specific T-cell response in mice

Debarati Chanda and Rudragouda Channappanavar

Department of Veterinary Pathobiology,
College of Veterinary Medicine, Oklahoma State University

Abstract

The global outbreak of COVID-19 has led to development of several vaccines that primarily target SARS-CoV-2 spike protein through generation of neutralizing antibodies in host. However, the spike protein is prone to mutations causing these vaccines to be ineffective against variants. Therefore, a broad-spectrum response against different viral proteins needs to be identified to address the gap in vaccine efficacy. T-cell based immune response is long-lasting and can recognize a variety of viral epitopes. Recognition of these viral epitopes by CD4 and CD8 T-cells leads to the generation of polyfunctional cytokines comprising of interferon gamma (IFNγ) and tumor necrosis factor (TNF). This response is vital for viral clearance from host cells. In this study, we evaluated the response generated by T-cell subsets in mouse-adapted SARS-CoV-2 (MA-CoV-2) infected mice. We found that upon stimulation of infected lung and spleen cells with peptides encoding the spike, membrane and Orf3a proteins, there was a significant induction of IFNγ and TNF by CD4 and CD8 T-cells. Furthermore, we identified high levels of spike-specific CD8 tetramer (S538-546,VNFNFNGL) T-cells in infected mice lungs in comparison to uninfected. Overall, a broad T-cell based immune response will be useful in developing vaccines for long-lasting immunity against SARS-CoV-2 variants.

Manipulating a viral virulence factor to improve systemic and mucosal immune responses by a live-attenuated Respiratory Syncytial Virus vaccine

Jeeviya Murugesan, Pramila Lamichhane, Tom Oomens

Department of Veterinary Pathobiology,
College of Veterinary Medicine, Oklahoma State University

Background

Respiratory Syncytial Virus (RSV) is a negative sense RNA virus responsible for more than 100,000 deaths/year in infants worldwide, and a pediatric vaccine is not available. A formalin-inactivated vaccine developed in 1967 not only failed to protect the infants who received it, but it also worsened the disease when exposed to RSV naturally. Hence, it is crucial to develop an RSV vaccine with safety as its utmost priority. We previously generated a prototype live-attenuated RSV vaccine (RSV-NS1@1) that was safe and protective in mice. To ready the vaccine for future human application, the long-term goal of the current project is to further enhance vaccine efficacy. The short-term goal is to reduce the expression of virulence factor and interferon antagonist non-structural protein 1 (NS1), to examine if this can improve antiviral antibody levels and make the vaccine more efficacious.

Materials and Methods

Two experimental vaccines were generated, one with the NS1 gene at the original position in the genome (RSV-NS1@1), which results in a high expression level, the other with the NS1 gene moved to the 8th position (RSV-NS1@8), which lowers expression level. First, NS1 levels were determined in vitro. Next, both vaccines were tested intranasally in BALB/c mice by prime/boost vaccination regimes. Blood samples were collected three weeks after the boost and examined for systemic IgG levels. Additionally, perfused lung samples were collected two weeks post-boost and analyzed for IgA levels by ELISA.

Results

Serum and lung samples were analyzed for antibody levels against the two major viral antigens, fusion protein F and attachment protein G. Analyses of serum from vaccinated mice revealed that RSV-NS1@8 induced similar levels of serum antibodies than RSV-NS1@1. However, vaccination with RSV-NS1@8 raised anti-F mucosal IgA antibody levels relative to RSV-NS1@1.

Conclusion

Reducing expression levels of viral virulence factor NS1 enhanced mucosal anti-viral antibody levels. Since mucosal IgA plays an important role in preventing RSV infection in the upper respiratory tract, manipulation of NS1 should contribute to improving vaccine efficacy.

Exploiting liquid-liquid phase separation properties of HBx to treat HBV-driven hepatocellular carcinoma

Kainat Ahmed, Asad Mohammad, Nan Chaiyariti, Udhav Ramachandran, Danya Sankaranarayanan, Sudhakar Jha

Department of Physiological Sciences,
College of Veterinary Medicine, Oklahoma State University

Background

Liver cancer's global death burden is over 800,000 annually, which makes it 4th most common cause of death worldwide. Hepatocellular carcinoma (HCC) accounts for 90% of primary liver cancer, with approximately 50% of HCC induced by chronic hepatitis involving the pathogenic factor Hepatitis B Virus (HBV). According to current research, HBV induces tumor formation by causing persistent infection-related immune evasion or coding for oncoproteins, thereby causing genome instability and hijacking key cell signaling pathways. HBV codes for a non-structural oncoprotein known as Hepatitis B X protein (HBx). HBx promotes HBV replication by interacting with various proteins. However, despite the current knowledge of HBx interactome and its related pathways, there is still a gap in understanding the exact mechanism behind its tumorigenicity. We wanted to target HBx oncoprotein and its various protein interactomes with the help of a newer approach known as liquid-liquid phase separation (LLPS). Some viral proteins form factories undergoing multiple processes, such as replication and viral protein packaging. These viral factories are an essential manifestation of LLPS, commonly known as biomolecular condensates.

Methodology

We hypothesized that HBx forms condensate and wanted to find the exact region of HBx responsible for forming condensate. To support this hypothesis, we made various deletion (54-154, Δ24-52, 1-52, 24-52) and substitution mutant (C61A, C69A, C137A, H139A) HBx-eGFP plasmid constructs based on known condensate-promoting feature like intrinsically disordered region (IDR) and zinc domain using in-vivo assembly and site-directed mutagenesis and finally transfecting them in HEPG2 cells to visualize condensate formation. HBX IDR was identified using a bioinformatic prediction tool called prediction of natural disordered region (PONDR).

Results

Experimental validation showed HBx form biomolecular condensates in HEPG2 cells. We found that HBx consists of an IDR from 22-52 amino acids using PONDR. However, transfecting the deletion of HBx-eGFP constructs revealed that HBx forms condensate with the transactivation domain, indicating that IDR is not required to form biomolecular condensates. Furthermore, zinc domain-related mutants in the transactivation domain did not disrupt condensate formation.

Conclusion

This study will provide future therapeutic targets for HBV-associated HCC by targeting the exact region of HBx responsible for condensate-driven HCC.

Active tick surveillance on cattle farm in central Oklahoma determines vegetation type alters tick risk

Mary Neff, Rachel Smith, Kellee Sundstrom, Kathryn Duncan

College of Veterinary Medicine, Oklahoma State University

Abstract

In central Oklahoma, ticks and tick-borne pathogens pose a significant threat to livestock and their handlers. The presence of ideal tick habitat affects tick abundance and species richness; therefore, variation of terrain across cattle farms in Oklahoma would be expected to alter tick risk. To better understand this, ticks were collected every 7-10 days for 9 weeks by targeting two different terrains on an approximately 30-acre cattle plot in Payne County, Oklahoma. Drags and dry ice traps were used on predominately grassy areas while flags and dry ice traps were utilized in woods and high brush areas. Ticks were identified using morphologic keys and microscopy. Given the tick-borne pathogen concern on cattle farms in this area, PCR of a partial gene fragment (rrs) was performed on nucleic acids of Dermacentor variabilis to amplify any species of Anaplasma, and Sanger sequencing with GenBank comparison were used for species identification. In total, 538 ticks were collected. Amblyomma americanum was most abundant (n=523) followed by D. variabilis (n=14) and a single A. maculatum. Significantly more ticks (506/538; p-value<0.0001) were collected in wooded areas compared to grassy areas. Results for Anaplasma spp. testing are pending. Together, these results show a greater risk for tick parasitism for cattle and their handlers
when terrain contains high brush and woods, and revealed A. americanum, a vector of zoonotic pathogens, as the predominant tick species on this farm. This information can be used to direct environmental control efforts given the limited tick control options for cattle; however, further research is warranted to explore the direct impact of environmental control on tick abundance on cattle farms.

A User-Centered Smart Inhaler Algorithm for Targeted Drug Delivery in JORRP Treatment Integrating Computational Fluid Particle Dynamics and Machine Learning

Mohammad Rashedul Islam and Yu Feng

School of Chemical Engineering,
Oklahoma State University

Abstract

Recurrent respiratory papillomatosis (RRP) is a chronic condition caused by viral infection, commonly seen as Juvenile Onset RRP (JORRP) in children. It poses severe risks due to its tendency to spread throughout the respiratory tract. The primary treatment for JORRP is surgical removal, but antiviral medications are also necessarily employed to reduce surgical needs, control papillomata growth, and prevent disease spread. Therefore, effective targeted drug delivery (TDD) is crucial to ensure that aerosolized drugs reach critical areas like the larynx and glottis without depositing on healthy tissues and causing side effects. This study (1) analyzed how drug properties and human factors influence TDD efficacy for treating JORRP and (2) innovatively developed personalized inhalation therapies through a machine learning (ML)-enhanced smart inhaler that optimizes the inhaler nozzle position and diameter based on drug-specific and patient-specific inputs. An experimentally validated Computational Fluid Particle Dynamics (CFPD) model quantified the effects of peak inhalation flow rate, particle release time, and particle diameter on drug delivery efficiency in a 6-year-old mouth-to-trachea airway geometry. A Classification and Regression Trees (CART) machine learning model was developed to provide an interpretable framework for TDD adjustments. The training dataset comprised 35 selectively chosen datasets from an initial pool of 315 CFPD simulations, filtered by maximum nozzle diameter and delivery efficiency. CFPD results indicated that inhaled particle size significantly affects deposition in the upper airway; specifically, larger particles (about 10 µm) mainly settle in the oral cavity and pharynx, leading to minimal deposition in the larynx and glottis. Additionally, optimal nozzle diameter and targeted delivery are impacted by factors like particle size, inhalation rate, and release timing, with a proper inlet Reynolds number being crucial for achieving precise delivery to the larynx and glottis. Despite challenges from the limited dataset, the ML-based TDD approach showed an 80% improvement over traditional inhalation therapy, enhancing drug delivery efficiency to critical regions. The introduction of the ML-powered smart inhaler based on this TDD strategy marks a significant step towards leveraging AI in building personalized and precise pulmonary healthcare solutions through inhalation therapy.

Unraveling bacterial colonization dynamics and associated factors in germ-free chicks with defined microbial communities

Phurt Harnvoravongchai, Supapit Wongkuna, Vishnu Thayil Valappil, Achuthan Ambat, Samara Paula Mattiello, Joy Scaria

Department of Biology,
Mahidol University, Bangkok, Thailand
Department of Veterinary Pathobiology,
College of Veterinary Medicine, Oklahoma State University
Department of Veterinary and Biomedical Sciences,
South Dakota State University

Background

Host-Microbe interactions are considered to be a mutualistic relationship where both sides are working together to provide each other benefits. The influence of microbes on their hosts has been extensively reviewed; however, the factors determining the selection of microbial species for colonization are still not well understood.

Methods

The gut contents of healthy feral chickens were pooled for bacterial isolation, and species were identified using MALDI-TOF and 16S rRNA gene sequencing. Functional categories of the defined chicken gut microbial community were predicted with BlastKOALA. Each species was tested on plates containing 95 different carbon sources to determine the carbon utilization pattern. The defined chicken gut microbial community or feral chicken cecal content was orally administered to germ-free chickens, and fecal samples were collected for 21 days to study microbial dynamics. Cecum were collected from euthanized chicks for transcriptome analysis. Bacterial network, random forest prediction, and correlation were analyzed using R-statistical software.

Results

In this study, we successfully constructed a defined chicken gut microbial community (Mix42) comprising 42 species from 6 different phyla isolated from the chicken intestine. Shotgun metagenome sequencing demonstrated that Mix42 shared similar functional capacity with the natural chicken intestinal microbiome. The diversity of Mix42 is evidenced by the ability of the species to utilize a broad range of carbon sources. Dynamic changes of bacterial colonization of Mix42 were observed in germ-free chicks, with 17 stably colonizing the chick intestine by day 21. Our network correlation and machine learning analyses demonstrated that bacteria-bacteria interactions and the ability to utilize specific substrates could be key factors in determining community composition. Additionally, host-transcriptomic data also revealed that colonized species stabilized the chick's cellular response, particularly mechanisms related to host defense.

Conclusion

Our study successfully established Mix42, a defined microbial community representing the functional capacity of the natural chicken gut microbiome. The stable colonization of specific bacterial species in germ-free chicks and the observed bacteria-bacteria interactions underscore the complexity of microbial community dynamics. Furthermore, the positive impact on host cellular responses highlights the factors for studying host-microbiome interactions and enhancing poultry health.

Functional Significance of RD3 in Cancer Cell Metabolic rewiring

Poorvi Subramanian and Natarajan Aravindan

Department of Physiological Sciences,
College of Veterinary Medicine, Oklahoma State University

Abstract

Metabolic reprogramming in cancer cells is crucial for tumor pathogenesis and disease progression. The nutrient deprivations in tumor microenvironment (TME) dictates cancer cells' metabolic rewiring to maintain cellular growth and proliferation. Mechanistically, such triggers prompt altered flux in cancer cells through various metabolic pathways. Our studies uniquely unveiled the function of RD3 in regulating tumor pathogenesis, TME and, recognized RD3 as a good prognostic factor. With our findings that the loss of RD3 drives tumor evolution in solid cancers, herein we investigated the significance of RD3 in regulating cancer metabolism. Data from three RD3-reverse engineered clones (RD3-, compared to corresponding parental RD3+ clones) identified 575 gene modifications. Imputing this 'cell line-independent' candidates in Metscape3.1 bioinformatics platform recognized 23 genetic determinants in cancer cell metabolism. Of these, 11 genes (QPCT, PRODH, NMNAT3, HS3ST3A1, EPHA8, MAPK15, ATP4A, ATP5L, USP6, PDE1-1A, POLA2) were significantly downregulated and another 13 genes (PLA2G4C, USP18, PAH, ACOX2, NEK8, MLKL, TGFBR2, PTPRB, EIF4EBP3, HAS2, TPK1, ASS1, A4GALT) were upregulated upon RD3 loss. In depth metabolic pathway analysis identified the intricate function of these molecules in 20 crucial pathways including fatty acids and phospholipids. Together, outcomes crucially identified a RD3-dependent 23-gene signature metabolic panel, and further imply that pro-survival metabolic flux in progressive cancer cells is harnessed by RD3 loss.

Genome-scale Identification of lncRNAs that Protect against Influenza Virus Infection using a CRISPR Activation Screen

Prince Jhandai, Kishore Vaddadi, Chaoqun Huang, Quanjin Dang, Lin Liu

The Lundberg-Kienlen Lung Biology and Toxicology Laboratory,
Department of Physiological Sciences,
College of Veterinary Medicine, Oklahoma State University

Abstract

Influenza is a global disease caused by influenza A virus (IAV), responsible for millions of infections and deaths worldwide each year. Devoid of any locomotion and metabolic activities, IAV, like other viruses, relies on host cells at each stage of its life cycle. Long noncoding RNAs (lncRNAs) are non-coding RNAs with more than 200 nucleotides in length and have an important role in many cellular processes including virus-host interactions. Here, we performed a genome-scale CRISPR activation screen to identify lncRNAs having antiviral properties against influenza virus. We first established a monoclonal dCas9-VP64 -expressing human lung epithelial A549 cell line and amplified the whole genome lncRNA library containing pooled 96,458 sgRNA targeting 10,504 lncRNAs. Pooled gene edited human A549 lung cells were challenged with influenza A virus, WSN strain (H1N1). Surviving cells were isolated and next genome sequencing was performed. gRNA counting was performed by using CRISPRAnaLyzeR software. Candidate genes were identified and ranked using the MAGeCK and Z-score analysis. We identified 118 positive lncRNAs with a Z-score cutoff of over 1.96. 24 of these lncRNAs were in the top 50 enriched lncRNAs from MAGeCK analysis. RHPN1-AS1, CTAGE7P, ABCC13, PRORSD1P, PDXP-DT, LINC01257, LINC01501, and CRNDE were selected for further studies. Next, we examine the changes in expression levels of these lncRNAs during influenza virus infection. A549 cells were infected with IAV-WSN strain at a multiplicity of infections (MOI) of 0.01 for 48 hours. The expression levels of lncRNAs were determined using real time PCR. The results demonstrated that RHPN1-AS1 and PROSD1P expression levels increased in IAV-infected cells compared to mock uninfected cells. CTAGE-7P and CRNDE showed no change in expression levels between infected and uninfected cells. The expression levels of PDXP-DT, ABCC13, LINC01257, and LINC01501 were undetectable in both infected and uninfected cells. Our results identified two anti-viral lncRNAs, RHPN1-AS1 and PROSD1P as influenza virus upregulated lncRNAs. These studies provide potential targets for developing antiviral therapeutics against Influenza A virus.

SARS-CoV-2-induced TLR-ERK1/2 activation promotes dysregulated immunity.

Rakshya Shresthaa (graduate student), Roshan Ghimirea, Titus Pattona, Rudra Channappanavara

Department of Veterinary Pathobiology,
College of Veterinary Medicine, Oklahoma State University

Abstract

Emerging and re-emerging human coronaviruses (hCoVs) cause severe respiratory illness in humans and continue to remain a persistent challenge to global public health. These highly pathogenic hCoVs evade host anti-viral immunity and replicate to high titers causing excessive inflammation, acute lung injury (ALI), acute respiratory distress syndrome (ARDS), and fatal pneumonia. However, the key PAMPs and host factors that facilitate impaired antiviral response and excessive inflammation (also known as dysregulated immunity) leading to severe lung pathology and fatal pneumonia are not well known. Therefore, in this study, using murine bone-marrow derived macrophages, we first evaluated the mechanistic basis of SARS-CoV-2-induced dysregulated immunity. In novel findings, we show that toll-like receptor (TLR)-extracellular regulated kinase (ERK)1/2 activity promotes SARS-CoV-2 and viral-RNA-induced dysregulated lung immunity in macrophages. Notably, blocking ERK1/2 activity not only reduced SARS-CoV-2-induced inflammatory cytokine and chemokine production in viral RNA mimic and SARS-CoV-2 structural proteins-stimulated macrophages but also enhanced antiviral IFN-I response. However, blocking other known inflammatory pathways such as p-38 MAPK, NF-kB, and JNK was less efficient in suppressing inflammation compared to ERK1/2 inhibition. Additionally, unlike the loss of ERK1/2 signaling, blocking p-38 MAPK, NF-kB and JNK failed to enhance anti-viral IFN/ISG response, suggesting that ERK1/2 activation uniquely facilitates hCoV-induced dysregulated immunity. Our results further show that inhibiting ERK1/2 activity in SARS-CoV-2 infected human airway epithelial cells (Calu-3) cells reduced viral titers which correlated with high interferon and interferon stimulated gene (ISG) expression. Collectively, we demonstrate that ERK1/2 signaling is a key host factor that drives SARS-CoV-2-induced dysregulated immunity. Our data also provides a strong foundation for evaluating the therapeutic potential of ERK1/2 inhibitors to moderate pathogenic lung inflammation while enhancing anti-viral response, which in combination with anti-virals, can be used to provide superior protection compared to the current COVID-19 therapies.

Toxoplasma gondii and rabies. Is the parasite, the virus, or both?

Ragan Wilson, Ryan Carson, Shannon Caseltine, Edith Will, Jeremiah Saliki, Ruth C. Scimeca

Department of Veterinary Pathobiology,
College of Veterinary Medicine, Oklahoma State University
Oklahoma Animal Diseases Diagnostic Laboratory, Oklahoma State University

Abstract

Toxoplasma gondii is an intracellular protist parasite that infects a wide range of vertebrates including humans. Although cats are the only definitive hosts any warm-blooded animal can act as paratenic host. Throughout the years this apicomplexan parasite has been studied due to its wide prevalence, zoonotic potential, and host behavioral alterations. The objective of this study was to evaluate the prevalence of T. gondii DNA in brain tissue collected for rabies testing. Because rabies is a zoonotic virus that alters host behavior, brain tissue from diverse animals is often submitted for testing. Between the months of May 2021 and April 2024, we tested 903 brain tissue samples from 22 animal species submitted for rabies testing to the Oklahoma Animal Diagnostic Disease Laboratory. Overall T. gondii prevalence was 3.96%, with 1.8% cats (Felis catus), 1.7% dogs (Canis familiaris), 0.3% skunks (Mephitis mephitis) and 0.2 % cattle (Bos taurus) infected. Analysis among T. gondii positive hosts revealed a statistically significant difference in dogs, when comparing neutered vs. intact males, with 7.94% (5/63) T. gondii positive neutered males and 1.61% (3/186) T. gondii positive intact males ($\chi 2 = 6.05$, $df = 1$, $P = 0.01$). All the T. gondii positive samples were negative for rabies. Anamnesis on some of the T. gondii positive samples included ataxia, aggression, muscle rigidity, lethargy, and seizures, with the last one also described in dogs and aggression in the positive bovine sample. The clinical signs described in the T. gondii infected hosts can be mistaken with rabies infection; therefore, we highlight here the importance of considering T. gondii as differential diagnosis in suspected rabies cases.

Fragile X syndrome disrupts gut microbiota, bioelemental makeup, and gut barrier integrity in mice

Sabiha Alam, Puni Jeyasingh, Babu Fathepure, Edralin Lucas, Elizabeth McCullagh

Department of Integrative Biology; Department of Molecular Genetics and Microbiology;
Department of Nutritional Sciences; Department of Integrative Biology
Oklahoma State University

Abstract

Fragile X Syndrome (FXS) is the leading genetic cause of autism, resulting from mutations in the Fmr1 gene. In addition to the well-known cognitive and behavioral abnormalities, FXS patients experience gastrointestinal (GI) dysfunction with poorly understood mechanisms. The gut microbiota influences the host metabolism and absorption of bioelements, thus regulates the elemental bioavailability in the host. Here, we tested the hypothesis that FXS alters the intestinal microbiota profile and bioelemental composition along with impairing the intestinal barrier integrity in mammals. To test this hypothesis, we used a Fmr1 knockout (KO) mouse model that recapitulates FXS disease phenotypes and wild-type (WT) littermate control. To test whether FXS causes alteration in the gut microbiota profile, we performed 16S ribosomal RNA sequencing in the gut samples of both WT-type and KO mice. This study revealed that the KO had a distinct gut flora than WT mice, demonstrating a causal relationship between the mutated Fmr1 gene and gut microbiota composition. We next assessed gene expression on the intestinal samples using the RT-qPCR (real-time polymerase chain reaction) technique. We found that the expression of gut-barrier-maintaining genes (OCLN1, CLDN2, MUC2, ALPi, PLVAP, REG3G, and TJP1) was significantly downregulated in KO mice than their WT littermates, suggesting impaired gut barrier integrity in FXS. Finally, we measured the elemental distribution of gut and brain tissues using ICP-MS (mass spectrometry) technology. We found that Fmr1 KO mice had the higher level of elemental shifts (Ca, Cu, Li, Mn, Mg, Na, P, S, Zn) compared to WT mice. Further correlational analysis of the elemental compositional network with intestinal microorganisms may reveal the elemental and microbial parameters as the indicators of gut pathogenesis in FXS. Collectively, the results of this study indicate that the onset of FXS triggers altered gut microbiota and bioelemental profile along with disrupted gut barrier function in FXS. Future research is warranted to establish the potential probiotic treatments to ameliorate GI disturbances in FXS. Thus, the results from this study hold promises for establishing potential biomarkers and alternative treatments to tackle GI illnesses in FXS.

A Revolutionary Genetic Discovery in Secondary Muscle Invasive Bladder Cancer

Sabir Salim, Sreenidhi Mohanvelu, Poorvi Subramanian, Natarajan Aravindan

Department of Physiological Sciences,
College of Veterinary Medicine, Oklahoma State University

Abstract

Bladder cancer (BLCA) is the 10th most common cancer globally and the fourth most common in men, accounting for 3% of all cancer deaths. About 75% of patients have non-muscle invasive BLCA (NMIBC) and the rest have muscle-invasive BLCA (MIBC) at diagnosis. About one-third of high-risk NMIBC progress to deadly MIBC (secondary MIBC) and the knowledge about such evolution is thus far unrealized. The Retinal Degeneration Protein 3 (RD3) is a 195 amino acid protein that was initially thought to be present only in the retina. We showed that RD3 is present in all tissues beyond the retina and is significantly reduced in aggressive tumors, suggesting its potential tumor-protective role. In this study, we have shown a transcriptional (QPCR) and translational (ELISA) loss of RD3 in the MIBC cells (TCCSUP, J82) compared to the NMIBC cells (RT4). In a cohort of 55 NMIBC patients, we compared the association of RD3 loss to the advanced disease stage, progression, therapy resistance, metastasis, tumor recurrence, and clinical outcomes including overall (OS), progression-free (PFS), and relapse-free (RFS) survival. RD3 Immunohistochemistry in TMAs disclosed less RD3 expression in BLCA samples compared to normal muscle tissue. RD3 loss significantly correlated with an increase in risk, stage, disease dissemination, therapy, tumor recurrence, and a decrease in OS, PFS, and RFS. Investigating in a large patient cohort (n=2724, Caris Precision Oncology Alliance database) bolstered the relevance of RD3 loss in metastatic BLCA. Irradiating (2Gy/day for 5 days) patient-derived MIBC clones crucially recognized the acquisition of RD3 loss with therapy pressure. Co-culturing parental cells with FIR exposed cells mimic the IR response in bystander cells and a corresponding increase in NFkB (EMSA) and NFkB-transcriptional signaling (qPCR profiling). Together, these findings strongly suggest that RD3 loss has a substantial role in BLCA development and its progression to secondary MIBC. Further investigation into this discovery could greatly improve the accuracy of disease staging and lead to more effective personalized treatment plans.

Association of Neutrophil Kinetics with Disease Severity in a Translational Feline Model for COVID-19

Sachithra Gunasekara, Miruthula Tamil Selvan, Chelsea Murphy, Shannon Cowan, Jerry Ritchey, Craig Miller, Jennifer Rudd

Department of Veterinary Pathobiology,
College of Veterinary Medicine, Oklahoma State University
Department of Mathematical Sciences,
College of Arts and Sciences, Oklahoma State University

Background

Neutrophil hyperactivation and neutrophil extracellular traps (NETs) formation are linked to exacerbated disease severity in acute SARS-CoV-2 infection, notably in hospitalized patients. Hence, understanding neutrophil kinetics is essential for developing targeted COVID-19 therapies, requiring reliable, translational animal models. Utilizing our previously established feline model that mimics human COVID-19, inducing significant clinical signs and inflammatory lesions, we hypothesize that neutrophil dysregulation and NETs release, worsen pulmonary damage, correlating with clinical severity in domestic cats.

Materials and Methods

Specific-pathogen-free cats (n=12) were inoculated with SARS CoV-2 (B.1.617.2) or vehicle (n=6) and plasma (0-days post-infection (dpi), 4dpi, 8dpi, and 12dpi), bronchoalveolar lavage fluid (BALF), and lung tissues (4dpi and 12dpi) were collected. Myeloperoxidase (MPO)-DNA ELISA, Quant-iT PicoGreen® dsDNA assay, multiplex immunoassay, RNA sequencing, and bioinformatics analysis were conducted to evaluate neutrophil recruitment and NET-specific markers.

Results

Cytokine profiling exhibited a significant increase in indicators of neutrophil activation (IL-8, CXCL1, SDF-1) in the BALF and plasma of infected cats compared to uninfected controls. Infected cats showed significantly elevated NETs-specific markers; MPO-DNA complexes and cell-free dsDNA evident via MPO-DNA ELISA, and Quant-iT PicoGreen® dsDNA assay, respectively. Immunofluorescence showed an increased representation of citrullinated histone 3, MPO, and neutrophil elastase in the lung tissues of infected cats. Differential gene expression analysis following GO and KEGG pathway analysis supported significant upregulation in neutrophil activation-related genes and pathways in the infected cats. Importantly, correlation analysis of selected NET-specific markers with clinical scores, and histopathological scores demonstrated significant correlations indicating the association of neutrophilia and NETs with disease severity and pulmonary damage during SARS-CoV-2 infection in a feline model.

Conclusion

This study emphasizes the impact of neutrophil-related mechanisms in SARS-CoV-2 infection within a feline model, highlighting potential therapeutic strategies to mitigate excessive inflammatory responses and disease severity in COVID-19.

PML: A novel predictive and prognostic biomarker for neuroblastoma.

Sreenidhi Mohanvelu and Natarajan Aravindan

Departments of Physiological Sciences,
College of Veterinary Medicine, Oklahoma State University

Abstract

Promyelocytic Leukemia (PML) is a tumor suppressor and a critical regulator of DNA-damage, and cell death. PML is localized in nucleoplasm and cytoplasmic compartments as PML- nuclear bodies (PML-NB). Chromosomal translocation (15:17) of PML and retinoic acid [RA] receptor alpha (RARα) results in formation of an oncofusion protein and impairs PML-NB formation regulating tumor aggressiveness, metastatic features. Herein, we investigated the spatio-temporal expression of PML in neuroblastoma patients (n=140). Neuroblastoma is the deadliest cancer in infants accounting for 1/10th of all childhood cancers. PML enhances RA mediated signal transduction and induces neuroblastoma differentiation promoting RA treatment responsiveness. To study the spatio-temporal expression of PML at specific tumor sites, we custom archived the tumor samples in tissue microarrays and effectuated a chromogenic IHC. The analytical endeavor was accomplished by using the Multiplex IHC v 3.4.9 module integrated into Indica Lab's HALO platform. Our analysis revealed a positive correlation of PML expression to the patient survival (overall-, relapse-free-, progression-free survival) and recognizes its relevance in favorable prognosis. Furthermore, we observed a gradient decrease of PML at advanced disease stages iterating the crucial role of PML in disease aggravation. We also observed a significant loss of PML expression in patients presenting with metastatic disease or relapse disease emphasizing the prominence of PML-loss in disease dissemination and recurrence. Together, these outcomes recognize PML loss as a promising predictive and prognostic marker in the realm of neuroblastoma. Moving forward, it is crucial to identify the mechanism(s) that dictate PML loss in neuroblastoma, if we are to successfully develop maintenance therapeutic strategies for this deadly disease.

Combating Multidrug Resistant Pathogens with Phenolic Acids: A Study of Antibacterial Activity and Mechanism

Tanim Islam, Phurt Harnvoravongchai, Joy Scaria

Department of Veterinary Pathobiology,
College of Veterinary Medicine, Oklahoma State University

Background

Antibiotic resistance is a worldwide health crisis, rendering broad classes of antibiotics ineffective against emerging pathogens. Hence, it is an imperative to find alternative therapeutics. Phenolic acids (PAs) have been widely known to have numerous beneficial effects (antibacterial, antioxidant) when consumed in daily diet. Here we focus on the broad-spectrum antibacterial activity of PAs as a potential alternative to last resort antibiotics.

Methods

We tested antimicrobial activity of 3,4-dihydrobenzoic acid (DHBA), 2,4,6-trihydroxybenzoic acid (THBA) and 3,4-Dihydroxyphenylacetic acid (DOPAC) against panel of gram-negative and gram-positive pathogens. Checkerboard assay determined the efficacy of these compounds in combination with several classes of antibiotics. To reveal the mode of action, we performed membrane permeability assay and ROS assay. The MiniBioReactor model was run for 14 days to evaluate the impact of DHBA and colistin on gut microbial dysbiosis. The in vivo antimicrobial efficacy of DHBA was further validated using Caenorhabditis elegans as an animal model. Specific genes or pathways targeted by DHBA were elucidated using a high-throughput antimicrobial susceptibility test of single-gene deletion E. coli library.

Results

PAs displayed MICs of 3000-4000 µg/ml against all the pathogens tested, including the multidrug resistance (MDR) clinical isolates. Addition of PAs increased the susceptibility of Escherichia coli and Enterococcus faecium to colistin and vancomycin respectively. DHBA showed the strongest antimicrobial activity against the panel of pathogens and caused less community level disruption compared to colistin. PAs target the cell envelope in E. coli by permeabilizing the inner and the outer membrane, as well as reducing efflux activity. Furthermore, DHBA reduced the burden of E. coli in an in-vivo C. elegans model. We proposed genes and pathways associated with cell envelope proteins as potential targets.

Conclusion

PAs have broad spectrum antibacterial action in-vitro and in-vivo. These compounds target the cell envelope with less community level disruption. PAs enhance the efficacy of antibiotics in combination and thus lower the risk of emerging antibacterial resistance if used as a therapy against MDR infections.

Mucin-Adhering Bacteria Alter Gut Mucosal Microbiome Assembly via Priority Effects

Vishnu Thayil Valappil, Abhijit Maji, Sudeep Ghimire, Joy Scaria

Department of Veterinary Pathobiology,
College of Veterinary Medicine, Oklahoma State University

Background

Human gut microbiota is now well appreciated for its association with various diseases and physiological states. Key species presence in early life plays a significant role in determining the compositional signature of the microbiome, a phenomenon known as the priority effect. However, the principle that governs species selection based on the primary colonizer is not yet clear, and very little is known about these species in early-life gut microbiota that contribute to this assembly. In this study, we perform a high throughput screening of mucosal adhesion using a culture library collection to find out possible species involved in this assembly. Using strong-mucin adhering species, we further investigate the gut microbiome assembly using a continuous-flow bioreactor system in vitro.

Method

Biofilm formation by the human gut microbiome library was assessed using a crystal violet screening assay. Confocal microscopy and scanning electron microscopy were utilized to visualize the structure and viability of biofilms formed on mucin-coated surfaces. A packed column and drip flow bioreactor were utilized to mimic the mucosal gut environment for exploring the microbial recruitment. The microbial composition was determined through metagenomic analysis.

Results

Among 102 gut microbial species from a healthy human population, we could identify strong mucin adherers, including Bifidobacterium longum, Bacteroides caccae, B. finegoldi, Olsenella umbonate. Our bioreactor results demonstrated that early microbial colonization is depended on mucin, as a very minimum number of bacteria were able to adhere, and their adherence remained consistent even within a community. Notably, our metagenome analysis revealed that specific strong mucin utilizers bifidobacterium longum show no detection of pathogenic bacteria recruitment, underscoring the effectiveness of the mucosal barrier and the early colonizers in preventing pathogenic invasion. Additionally, by examining pediatric gut microbiome data and mucosal data across various health conditions, we sought to correlate early microbial configuration with potential health outcomes.

Conclusion

Our findings indicate that initial colonizers significantly shape the subsequent microbial community structure by influence the mucosal environment. This study advances our understanding of gut-microbiome assembly and its implications for health by demonstrating that the initial microbial colonizers, through priority effects, set the stage for subsequent community development

Assessing the Effectiveness of Automatic Speech Recognition Technology in Emergency Medicine Settings: A Comparative Study of Four AI-powered Engines

Xiao Luo, Le Zhou, Kathleen Adelgais, Zhan Zhang

Department of Management Science and Information Systems,
Oklahoma State University
Department of Biostatistics and Health Data Science,
Indiana University School of Medicine
School of Computer Science and Information Systems,
Pace University, New York, USA
School of Medicine, University of Colorado

Background

Cutting-edge automatic speech recognition (ASR) technology holds significant promise in transcribing and recognizing medical information during patient encounters, thereby enabling automatic and real-time clinical documentation, which could significantly alleviate care clinicians' burdens. Nevertheless, the performance of current-generation ASR technology in analyzing conversations in noisy and dynamic medical settings, such as Emergency Medical Services (EMS), lacks sufficient validation. This study explores the current technological limitations and future potential of deploying ASR technology for clinical documentation in fast-paced and noisy medical settings such as EMS.

Methods

In this study, we developed a computational framework and evaluated four ASR engines, including Google Speech-to-Text Clinical Conversation, OpenAI Speech-to-Text, Amazon Transcribe Medical, and Azure Speech-to-Text engine. The computational framework includes speech-to-text transcription, text analysis, and evaluation components. The empirical data used for evaluation were 40 EMS simulation recordings. The transcribed texts were analyzed for accuracy against 23 Electronic Health Records (EHR) categories of EMS. The common types of errors in transcription were also analyzed.

Results

Among all four ASR engines, Google Speech-to-Text Clinical Conversation performed the best. Among all EHR categories, better performance was observed in categories "mental state" (F1=1.0), "allergies" (F1=0.917), "past medical history" (F1=0.804), "electrolytes" (F1=1.0), and "blood glucose level" (F1=0.813). However, all four ASR engines demonstrated low performance in transcribing certain critical categories, such as "treatment" (F1=0.650) and "medication" (F1=0.577).

Conclusion

Current ASR solutions are insufficient for fully automating clinical documentation in EMS settings. Our findings underscore the need for further advancement of automated clinical documentation technology to enhance recognition accuracy in these time-sensitive and dynamic environments. With the development of generative AI technologies, future efforts can focus on automating error correction in medical content transcription and improving the extraction and documentation of clinical information in EMS settings.

Beyond the Spike: Unraveling the Symphony of Non-Spike Mutations in SARS-CoV-2 Omicron BA.1 Attenuation

Xiaoming Zhang, Xiang Chi, Xufang Deng

Departments of Physiological Sciences,
College of Veterinary Medicine, Oklahoma State University

Abstract

The Omicron BA.1 variant, characterized by over 50 amino acid mutations, represents an attenuated form of SARS-CoV-2. While it is established that the BA.1 spike gene, carrying over 30 mutations, plays a pivotal role in antibody escape and altered cell entry, the significance of the nearly 20 non-spike mutations in BA.1 attenuation remains unclear. Addressing this gap, we initially tested the null hypothesis that if the spike is solely responsible for BA.1's impaired replication, the non-spike mutations should not impact viral propagation. To examine this, we engineered a recombinant virus (icBA1-SWA1) carrying all non-spike mutations of BA.1 and assessed its replication compared to the isogenic wild-type WA1 virus (icWA1). Surprisingly, icBA1-SWA1 displayed significant reductions in viral titer and plaque size, indicating that these non-spike mutations indeed impair BA.1's replication in cell cultures. These mutations are located in non-structural proteins 3 (nsp3), nsp4, nsp5, nsp6, nsp12, nsp14, envelope (E), membrane (M), and N proteins when compared to the wild-type WA1 strain. To identify the major contributors, we generated a panel of recombinant viruses and individually or combinatorially tested these mutated genes for their effects on viral replication. The results revealed that the nsp6 mutations exhibited reduced replication and high genome instability, with compensatory mutations rapidly emerging. Notably, the nsp6 mutant virus displayed increased resistance to genome instability in the presence of nsp3, nsp4, and nsp5 mutations. Additionally, a recombinant virus carrying all non-spike mutations except nsp6 exhibited similar replication to icWA1. These findings suggest that BA.1 nsp6 is an important factor to cause significant replication defect, and mutations in nsp3, nsp4, and nsp5 stabilize the nsp6 mutations, indicating an epistatic relationship among these non-structural proteins. In summary, our study sheds light on viral non-structural proteins contributing to SARS-CoV-2 pathogenicity and enhances our understanding of SARS-CoV-2 adaptation and natural attenuation.

SPONSORS

VETERINARY MEDICINE

RESEARCH

CENTER FOR HEALTH SCIENCES

THE INNOVATION FOUNDATION
at Oklahoma State University

DEPARTMENT OF
MICROBIOLOGY AND MOLECULAR GENETICS
College of Arts and Sciences

DEPARTMENT OF
ANIMAL AND FOOD SCIENCES
Ferguson College of Agriculture

GRADUATE COLLEGE