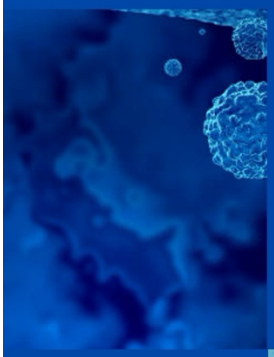
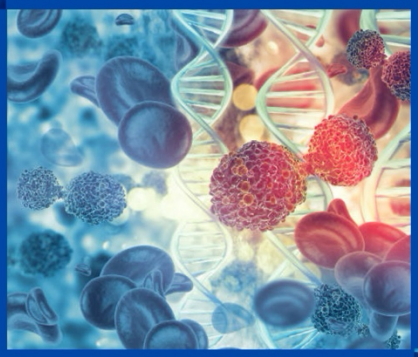
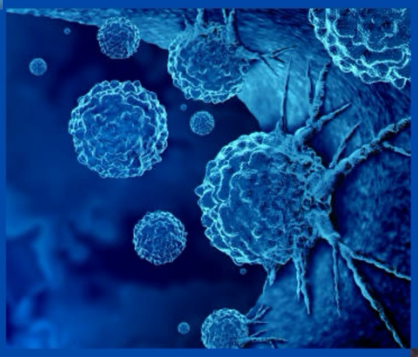
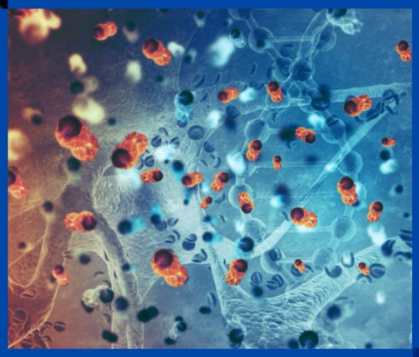


COBRE

CANCER & METABOLISM SYMPOSIUM







NOVEMBER 20, 2025
GATTON STUDENT CENTER
UNIVERSITY OF KENTUCKY

Markey Cancer Center
UNIVERSITY OF ARKANSAS
UNIVERSITY OF LOUISVILLE SCHOOL OF MEDICINE
HEALTH SCIENCES CENTER The UNIVERSITY of OKLAHOMA
Tulane University

ACKNOWLEDGEMENTS

College of Medicine, University of Kentucky

Markey Cancer Center, University of Kentucky

National Institutes of Health

School of Medicine, University of Louisville

The University of Oklahoma Health Sciences Center

Tulane University

University of Arkansas

COBRE

CANCER & METABOLISM SYMPOSIUM

PROGRAM CONTENTS

AGENDA

8:30 am Registration & Breakfast

9:00 am Welcome

Nathan Vanderford, PhD
Associate Professor of Toxicology and Cancer Biology
Director, Administrative Core, COBRE in Cancer and Metabolism
Director, Appalachian Career Training in Oncology Program
Assistant Director of Pathway Program and Student Success
University of Kentucky Markey Cancer Center

SESSION 1 COBRE AT THE UNIVERSITY OF KENTUCKY COLLEGE OF MEDICINE

Chair | Andrew Lane, PhD

9:05 am Decoding Core Fucosylation-Dependent Signaling in High-Risk Neuroblastoma

Eric Rellinger, MD
Assistant Professor, Department of Surgery
University of Kentucky Markey Cancer Center

9:40 am Patient-derived models for probing metabolic interactions in tumor microenvironment

Teresa Fan, PhD
Professor, Edith D. Gardner Chair in Cancer Research
Department of Toxicology and Cancer Biology
University of Kentucky Markey Cancer Center

SESSION 2 COBRE AT THE UNIVERSITY OF ARKANSAS

Chair | Caigang Zhu, PhD

10:15 am Quantitative optical biomarkers of aging through label-free multiphoton microscopy

Kyle Quinn, PhD
Professor of Biomedical Engineering, Former AIMRC Director
College of Engineering
University of Arkansas

10:50 am Break

11:00 am Investigating metabolic dysregulation roles in breast tumor innervation using tissue-engineered Models

Younghye Song, PhD
Associate Professor of Biomedical Engineering
College of Engineering
University of Arkansas

11:35 pm Optical imaging and spectroscopy approaches to evaluating long-term outcome in tumors

Narasimhan Rajaram, PhD
Professor of Biomedical Engineering, AIMRC Director
College of Engineering
University of Arkansas

LUNCH | POSTER SESSION 1&2

12:10 pm Poster Session 1 | even numbers | wrap up 12:45pm
12:30 pm Lunch (Pre-Registered Attendees) | wrap up 1:30pm
12:55 pm Poster Session 2 | odd numbers | wrap up 1:30pm

SESSION 3 COBRE AT TULANE UNIVERSITY

Featured Presenter: Chair | Jianhang Jia, PhD

1:30 pm The role of Phldb3 of the Liprin-a1-Rictor-mTOR C3-AKT pathway

Hua Lu, MD, PhD
Professor of Biochemistry and Molecular Biology
Chair, Department of Biochemistry and Molecular Biology
Reynolds and Ryan Families Chair in Translational Cancer
Tulane Cancer Center, Tulane University

2:05 pm Tumor-Host Interactions: A *Drosophila* Perspective

Wu-Min Deng, PhD
Professor of Biochemistry and Molecular Biology,
Gerald & Flora Jo Mansfield Piltz Endowed Professor in Cancer Research
Tulane Cancer Center, Tulane University

SESSION 4 COBRE AT THE UNIVERSITY OF LOUISVILLE

Chair | Richard Higashi, PhD

2:40 pm Harnessing the power of Trained immunity for cancer treatment

Jun Yan, MD, PhD
Professor of Medicine, Endowed Chair in Translational Research
Director, Center for Cancer Immunology and Immunotherapy
Brown Cancer Center, University of Louisville

3:15 pm Metabolic reprogramming of tumor-associated SiglecF+ neutrophils in lung cancer

Kavitha Yaddanapudi, PhD
Professor of Surgery, Department of Surgery
Vogt Endowed Chair in Immuno-Oncology
Brown Cancer Center, University of Louisville

3:50 pm Break

SESSION 5 COBRE AT THE UNIVERSITY OF OKLAHOMA HEALTH SCIENCES CENTER

Chair | Peter Zhou, MD, PhD

4:00 pm ExoLnc-driven Metabolic Reprogramming of Stromal Fibroblasts Fuels Ovarian Cancer

Revathy Nadhan, PhD
Research Scientist, Stephenson Cancer Center
University of Oklahoma Health Sciences Center

4:35 pm Advancing Translational Cancer Research: COBRE Phase III Core Resources at Stephenson Cancer Center

Muralidharan Jayaraman, PhD
Core Director, MTCRO COBRE, Stephenson Cancer Center
University of Oklahoma Health Sciences Center

Winner of Poster Award Announcement

5:10 pm Nathan Vanderford, PhD

5:30 pm Closing Remarks

Peter Zhou, MD, PhD
Professor, Madeline F. James & Edith D. Gardner Endowed Chair
Co-leader, Molecular and Cellular Oncology Program, NCI-designed Markey Cancer Center
University of Kentucky College of Medicine

PRESENTERS

Eric R. Rellinger, MD

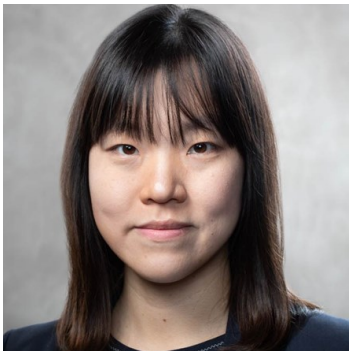
Dr. Rellinger is an Assistant Professor of Pediatric Surgery at the University of Kentucky, where he specializes in advanced minimally invasive, complex neonatal, and pediatric surgical oncology care. As a surgeon-scientist, he directs a basic science and translational research program focused on metabolic vulnerabilities in high-risk neuroblastomas, supported by funding from the COBRE Cancer Metabolism program, the V Foundation, and the Kentucky Pediatric Cancer Research Trust Fund. His work has earned national recognition through the American Pediatric Surgical Association's Grosfeld Research Scholarship and the American College of Surgeons' George H. A. Clowes, Jr., MD, FACS Research Career Development Award.

Teresa Fan, PhD

Dr. Fan is a professor of Toxicology and Cancer Biology at the University of Kentucky. She is a member of the Center for Environmental and Systems Biochemistry. Her research focuses on applying systems biochemical approaches including novel Stable Isotope-Resolved Metabolomic (SIRM), metabolomics-edited transcriptomic (META), and proteomic approaches (METPA) which we have developed 1) to investigate the anticancer mechanism of natural products and novel therapeutic agents; 2) to explore the functional role(s) of tumor microenvironment and extrinsic environmental factors in cancer development, progression, and therapy; 3) to uncover novel therapeutic targets and distinct mechanism-informed biochemical marker patterns for human diseases by interrogating the human metabolome; and 4) to decipher the gene networks that govern cancer metabolism. The central tool that she has used to achieve these goals is the integration of nuclear magnetic resonance (NMR) with mass spectrometry (MS) technologies, which enables a systematic interrogation of human metabolic networks and their perturbations by diseases.

Kyle P. Quinn, PhD

Dr. Quinn is a professor of biomedical engineering at Tufts University and director of the Tufts Advanced Microscopic Imaging Center (TAMIC). Dr. Quinn received his B.S. in Biomedical Engineering from the University of Wisconsin-Madison, and earned his Ph.D. in Bioengineering from the University of Pennsylvania. As a postdoctoral fellow at Tufts University, he was awarded both a Ruth L. Kirschstein National Research Service Award and Pathway to Independence Award from the NIH. In 2015, he joined the faculty of the Department of Biomedical Engineering at the University of Arkansas, where his research lab was continuously funded by NIH, and he received the NSF CAREER award. In 2021, he founded the NIH COBRE-funded Arkansas Integrative Metabolic Research Center, which integrates optical imaging, bioenergetics, and data science approaches to solve biomedical research problems involving cell and tissue metabolism. Dr. Quinn returned to Tufts in 2025, where he now directs TAMIC. His overall research interests are in developing and utilizing non-invasive quantitative optical methods to characterize the spatiotemporal patterns of disease progression and tissue repair. His research group is exploring how label-free multiphoton microscopy and artificial intelligence can be used to establish optical biomarkers for use in characterizing wound healing and aging. Link to slides: https://tufts.box.com/s/fw9edkkzegq46fqjysqtftyqbp9fl109

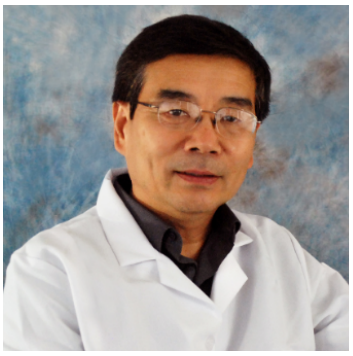


Younghye Song, PhD

Dr. Younghye Song is an Associate Professor and Graduate Coordinator of Biomedical Engineering at the University of Arkansas. Dr. Song's research interests lie in understanding the interactions between cells and the surrounding extracellular matrices (ECM) to identify novel mechanisms and therapeutic targets of diseases and injuries, with current focus on cancer-nerve crosstalk and traumatic injury to the nervous system. Her lab is supported by NIH, including the recent R37 MERIT award from NCI, as well as NSF, and Arkansas Biosciences Institute. She received the 2023 Dean's Excellence Award - Rising Star Research Award from the University of Arkansas College of Engineering. She is an Early Career Editorial Board Member of ACS Biomaterials Science & Engineering and a Review Editor of Frontiers in Medical Technology. Dr. Song is a member of the Biomedical Engineering Society, Society for Biomaterials, American Association for Cancer Research, Korean-American Scientists and Engineers Association, and American Society for Engineering Education. She received her B.S. in Chemical Engineering and Biomedical Engineering from Carnegie Mellon University, Ph.D. in Biomedical Engineering from Cornell University, and postdoctoral training in Biomedical Engineering at the University of Florida.

Narasimhan Rajaram, PhD

Dr. Rajaram is a Professor of Biomedical Engineering at the University of Arkansas, where he directs the Laboratory for Functional Optical Imaging and Spectroscopy. Dr. Rajaram recently took over as also the director of the NIH P20-funded Arkansas Integrative Metabolic Research Center. His's lab is focused on using non-ionizing light-based techniques to noninvasively investigate the tumor microenvironment to determine biomarkers of cancer progression, metastasis, and treatment resistance. He is a recipient of the National Science Foundation's CAREER Award and the Arkansas Biosciences Institute New Investigator of the Year. Dr. Rajaram's research has been funded by grants from the NIH and DoD. He received his Bachelor's degree in Electronics and Instrumentation Engineering from Anna University in India, PhD in Biomedical Engineering from the University of Texas at Austin and completed postdoctoral training at Duke University.



Hua Lu, MD, PhD

Professor Hua Lu received a Medical Bachelor (MB) degree (US MD equivalent) from Jiangxi Medical College in 1983, a Master of Science from Peking Union Medical College/Chinese Academy of Medical Science in 1986, and a PhD from Rutgers University / Robert Wood Johnson Medical School in 1993. After his postdoctoral research at Princeton University, he became a tenure-track Assistant Professor at Oregon Health & Science University in 1997 and was promoted to a tenured Associate Professor in 2003. He joined Indiana University as a Professor of Biochemistry and Molecular Biology/Daniel and Lori Efryomson Chair of Oncology in 2007 before joining Tulane University School of Medicine in 2012. He serves as an editorial board member for 4 scientific journals and is a standing / ad hoc reviewer for NIH, various national/international funding agents, and more than 90 scientific journals. He served an Associate Editor for the *Journal of Molecular Cell Biology* (Oxford press in Shanghai) and *Gene & Disease*, and a scientific advisory board member for the Diamond Blackfan Anemia (DBA) Foundation in New York. His lab discovered how cellular stresses activate p53 by blocking the MDM2/MDMX-p53 feedback loop. Recently, his lab identified anti-cancer small molecules that target p53, c-Myc, and PSMD3-TBK1-NF-kB pathways as potential anti-cancer agents. His lab has been funded by NIH/NCI grants for more than 28 years and resulted in more than 180 high-quality publications in peer-reviewed scientific journals. He was elected as a Fellow by the American Association of Advanced Sciences and an honored member of Sigma Society in 2018. He received several awards including the Award of Tulane Spirit as honored by Tulane University in 2022 and the Faculty Research Award for Basic Research by Tulane University School of Medicine 2025.

Wu-Min Deng, PhD

Dr. Deng's laboratory is interested in how cell–cell and cell–tissue microenvironment interactions are involved in normal development and how disruption of such interactions leads to tumorigenesis in the *Drosophila* model system. Dr. Deng's research focuses on fundamental questions in cancer biology and developmental biology. The research topics range from understanding how tissue microenvironment contributes to neoplastic tumor transformation and progression to studying how growth and tissue homeostasis are regulated during development and tumorigenesis. The research in his laboratory has led to the development of novel concepts such as tissue "tumor hotspots" and "compensatory cellular hypertrophy."

Jun Yan, MD, PhD

Dr. Yan is an endowed Chair Professor and Chief in the Division of Immunotherapy, Department of Surgery, and the Director of the Immuno-Oncology Program at the Brown Cancer Center (BCC), University of Louisville. He also leads the Center for Cancer Immunology and Immunotherapy (CCII) CoBRE. Dr. Yan has been working on tumor immunotherapy and tumor immunobiology for more than two decades and has been continuously funded by the NIH/NCI since 2004. His research program spans both basic and translational studies in cancer immunology and inflammation, with a specific focus on mechanisms of immune regulation. In recent years, he has expanded his work to include more in-depth mechanistic studies on the tumor microenvironment (TME), with particular attention to immune cell subsets that promote tumor progression and metastasis. A key emerging direction in his laboratory is the development of innovative therapeutic strategies, including the induction of trained immunity using natural compounds such as β-glucan. Dr. Yan has published more than 180 peer-reviewed papers, including article in leading journals such as *Nature Immunology*, *Cell Metabolism*, and *Immunity*, and has a Google Scholar H-index of 80. His research is currently supported by three NIH R01 grants and an award from the American Cancer Society.

Kavitha Yaddanapudi, PhD

Dr. Yaddanapudi is a Professor in the Division of Immunotherapy in the Department of Surgery at the University of Louisville and Henry Vogt Endowed Chair in Immuno-Oncology. Her research program focuses on different approaches that tumors use to suppress the immune system, and on the development of novel immune-based strategies for the treatment of cancer. A major focus of the lab is on understanding the role of tumor microenvironment in the regulation of immune responses in cancer with a specific focus on the role of cancer-driven pathological myelopoiesis.

Revathy Nadhan, PhD

Dr. Nadhan is a biomedical scientist whose research focuses on elucidating the molecular mechanisms underlying ovarian cancer progression, particularly through long non-coding RNAs and lysophosphatidic acid signaling pathways. Her work investigates the oncogenic roles of long non-coding RNAs, specifically in regulating mRNA splicing, stability and metabolism. She also explores the role of exosomal long non-coding RNAs in shaping tumor–microenvironment interactions. By integrating molecular biology with computational methods, Dr. Nadhan aims to identify novel therapeutic targets and prognostic biomarkers in gynecologic malignancies. She has authored and co-authored several peer-reviewed publications, highlighting the translational potential of her studies. With extensive experience in cancer signaling, bioinformatics, and translational research, she contributes to advancing precision oncology through multidisciplinary collaboration. Dr. Nadhan is also actively engaged in scientific communication and mentoring within the biomedical research community.

Muralidharan Jayaraman, PhD

Dr. Jayaraman directs the Cell and Tissue Analysis Core within the MTCRO-COBRE program at the NCI-designated Stephenson Cancer Center, where he leads a comprehensive suite of advanced services including cell line authentication, multiplex fluorescent immunohistochemistry, RNA in situ hybridization, biochemical assays, and tissue microarray construction and analysis. He also serves as Assistant Director for Basic and Translational Shared Resources and Core leader for the Cancer Functional Genomics Core under the P30 Cancer Center Support Grant. As a core director, he plays a pivotal role in enabling cutting-edge cancer research by collaborating on NCI grant applications, administering multiple research projects, and providing technical consultation on complex experimental design and execution. His commitment to advancing research capabilities extends to developing investigator training programs, implementing standardized protocols, and maintaining rigorous compliance with regulatory standards. Through presentations, workshops, and tours for both established and newly recruited investigators, he actively promotes core facility utilization and fosters collaborative relationships within the scientific community.

POSTER AWARDS

Staff / Postdoc

1st Place $200

2nd Place $100

3rd Place $50

Student

1st Place $200

2nd Place $100

3rd Place $50

ABSTRACTS

1 Liver-Specific Deletion of Carnitine Palmitoyltransferase 1a Promotes Tumorigenesis in a Mouse Model of Obesity-Driven Hepatocellular Carcinoma

Garrett B. Anspach[1-6], Robert Flight[6,7], Sehyung Park[1-6], Nikitha Dharanipragada[1-6], Hunter Moseley[6,7], Robert N. Helsley[1-6]

[1]Department of Internal Medicine–Division of Endocrinology, Diabetes, and Metabolism; [2]Department of Physiology; [3]Department of Pharmacology and Nutritional Sciences; [4]Saha Cardiovascular Research Center; [5]Barnstable Brown Diabetes and Obesity Center; [6]Markey Cancer Center; [7]Department of Molecular & Cellular Biochemistry; University of Kentucky, College of Medicine, Lexington, KY

Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) is the fastest-growing etiology of hepatocellular carcinoma (HCC). The primary goal of this project is to determine the contribution of carnitine palmitoyltransferase 1a (CPT1a)-mediated fatty acid oxidation (FAO) in MASLD-driven HCC.

Methods: Paired tumor (n=8) and adjacent non-tumor tissue (n=8) were collected from patients with suspected MASLD-driven HCC at the University of Kentucky Markey Cancer Center. Hematoxylin and eosin (H&E) staining was used for pathological determination of tumor and adjacent nontumor tissue. Lipids were extracted via methyl-tert-butyl ether and subjected to lipidomics by the West Coast Metabolomics Center. Bulk RNA-sequencing was employed to assess gene expression changes across paired samples. For murine studies, four-to-five-day old CPT1a$^{F/F}$ and liver-specific CPT1a KO (LKO) pups were treated with 7,12-dimethylbenz[a]anthracene and fed GAN diet (40% kcal fat; Research Diets) until 34 weeks of age. Mice were necropsied after a 24-hour fast. Livers were excised, and total tumor number was calculated.

Results: H&E staining showed significant lipid vacuole accumulation in human HCC tumors relative to nontumor tissue. Lipidomic analysis revealed significant increases in long-chain nonesterified monounsaturated fatty acids (MUFAs; C16:1, C18:1, C20:1) and MUFA-enriched phospholipids (PC30:1, PC32:1, PE32:1, and PC36:1) in HCC. On the contrary, both MUFA- (C14:1, C18:1) and PUFA-enriched acylcarnitines (C18:2, C18:3) were collectively reduced in human tumors. Consistent with this lipid profile, fatty acid oxidation genes (CPT1A, CPT2, ACADL, ACADM, ACADS, HADHA) were significantly reduced in tumor versus nontumor tissue. In mice, CPT1a deletion increased liver to body weight ratios by 50% (P=0.0003) and increased overall tumor number by ~3.4X (*3.7 vs. 12.4 average nodules per mouse;* P=0.0055). H&E analysis suggests that tumors in mice replicate the histopathology of human HCC.

Conclusions: These results suggest human HCC tumors exhibit a reduced capacity to undergo mitochondrial β-oxidation resulting in accumulation of free- and esterified-MUFAs with a concomitant reduction in MUFA-carnitines. Complimentary mouse studies show that CPT1a deletion in hepatocytes promotes HCC in male mice. Future studies are underway to identify mechanisms governing these differences. These findings identify mitochondrial FAO as a potential therapeutic target for MASLD-HCC prevention and treatment.

2 Lactate Promotes Epithelial–Mesenchymal Transition (EMT) of Prostate Cancer Via c-Myc Lactylation

Mithu Howlader, Amos Akinyemi, Md Rakibul Alam, Lixiang Gu, Xiongjian Rao, Zhiguo Li*

Department of Toxicology and Cancer Biology, College of Medicine, University of Kentucky, Lexington, KY

Prostate cancer (PCa) is a leading cause of cancer-related morbidity and mortality, characterized by complex metabolic reprogramming that supports tumour growth and progression. Lactate has emerged as a critical factor in PCa metabolism, produced via aerobic glycolysis and influenced by oncogene/tumour suppressor gene mutations, androgen signaling, and hypoxia. Lactate accumulation promoting the lactylation of proteins which is a novel post-translational modification (PTM) involving proteins that is induced by lactate. Excessive lactate production and rapid lactate transport in cancer cells are mainly driven by the upregulation of c-Myc which is a master regulator of multiple biological programs. Its persistent activation results in abnormal expression of glycolytic enzymes and lactate transporters, contributing to PCa progression. While c-Myc undergoes various PTM, the potential for c-Myc lactylation and its role in PCa development remain unexplored. Preliminary data indicate that sodium lactate induces lactylation in PCa cells and that c-Myc is subject to this modification in androgen-independent cell lines. Epithelial-mesenchymal transition (EMT), characterized by the loss of epithelial cells polarity and cell-cell adhesion, leading to the acquisition of mesenchymal-like phenotypes, is widely recognized as a key contributor to prostate cancer progression. Overexpression of c-Myc plays a central role in inducing EMT. Here, we investigated the role of lactate in promoting EMT in prostate cancer and the underlying regulatory mechanisms. High levels of lactate significantly promoted EMT progression in prostate cancer cells by lactylation of c-Myc. Thus, our findings will provide novel insights into the regulatory role of lactate in EMT in prostate cancer pathogenesis. Additionally, targeting of lactate-driven lactylation of c-Myc may boost the therapeutic strategy for prostate cancer.

3 PLK1-Mediated Phosphorylation of NANOG Drives Lineage Plasticity in Prostate Cancer in Response to AR-Targeted Therapy

Hamed Maasoumyhaghighi, Jinghui Liu, Mansoureh Nouri, Xiaoqi Liu*

Department of Toxicology and Cancer Biology, College of Medicine, University of Kentucky, Lexington, KY

Prostate cancer is a predominant form of cancer among American men, commonly treated with local therapy followed by androgen deprivation therapy (ADT). However, resistance to ADT often occurs, resulting in castration-resistant prostate cancer (CRPC). Enzalutamide, a second-generation androgen receptor antagonist, has been approved for metastatic CRPC and has shown improved survival rates. Nevertheless, Enzalutamide eventually loses its effectiveness, and resistance develops in the majority of patients. Currently, there are no effective treatments available for Enzalutamide-resistant CRPC. This emphasizes the urgent need for the development of new therapeutic approaches to address this challenge and provide better options for both current and future patients. Cancer stem cells (CSCs) exhibit similar characteristics to embryonic stem cells (ESCs) and express pluripotency-related transcription factors such as NANOG, OCT4, and SOX2. The expression levels of these transcription factors in cancer cells are closely linked to the development and progression of different types of cancer. NANOG, an ESC transcription factor, is responsible for maintaining the self-renewal and pluripotency of ESCs. In various cancers, NANOG is highly expressed and plays an important role in the process of tumorigenesis, including chemotherapy and radiation resistance. Studies have shown that prostate cancer (PCa) cells acquire stem-like properties through the expression of NANOG, particularly in stable and accumulated conditions. Phosphorylation of NANOG at certain sites is required for sustaining NANOG stability and thus promoting tumorigenic properties. Our central hypothesis is that the Plk1 phosphorylation of NANOG promotes lineage plasticity in CRPC. We will ask whether the expression of S to A mutation inhibits lineage plasticity.

4 Prevalence of Type 2 Diabetes Mellitus in Patients Diagnosed with Oropharyngeal Squamous Cell Carcinoma in Kentucky Based on HPV-Tumor Status

Monica McGrath[1], Krystle Kuhs[2], Alena Smith[3], Justin Levens[3], Mingguang Chang[3], Melina Windon[4]

[1]College of Medicine, [2]College of Public Health, [3]Markey Cancer Center, [4]Department of Otolaryngology – Head and Neck Surgery, University of Kentucky, Lexington, KY

Introduction: Oropharyngeal squamous cell carcinoma (OPSCC) can be classified by HPV tumor status (+/- HPV). Health outcomes including 5-year survival rates are vastly different between the two OPSCC patient cohorts (1). Reported comorbidities differ greatly with HPV(-) OPSCC patients suffering from a greater burden of comorbidities than their HPV(+) counterparts (2). Diabetes Mellitus has been shown to negatively affect progression-free survival in patients with OPSCC (3); however, evidence for effects of Type 2 Diabetes Mellitus on survival remains mixed (4). Interestingly, there is data to support the protective effect of taking metformin against metastasis and or recurrence in head and neck cancers (5-7). Kentucky (KY) offers a unique setting to further explore this relationship, as the burden of T2DM is estimated to be on the high side at around 12.9% of the adult population (8) and the rate of HPV-mediated oropharyngeal cancers is estimated at 6.2%, the highest in the US (9). **Objective:** This study aims to assess the prevalence of T2DM by HPV tumor status among OPSCC patients. **Methods:** OPSCC patients who were diagnosed or treated for a primary or recurrent oropharyngeal squamous cell carcinoma from 6/2021 to 10/2024 at Markey Cancer Center were included. Data were obtained from Kentucky Cancer Registry and a retrospective chart review. Patients whose HPV status or diabetes type were unknown were excluded. Prevalence of T2DM was compared by chi-squared test. **Results:** The cohort included 207 HPV (+) OPSCC patients, 50 (24.1%) of whom were found to have T2DM at diagnosis, and 53 HPV (-) OPSCC patients, 10 (18.9%) of whom had T2DM ($p = 0.40$). **Conclusion:** The KY OPSCC population demonstrates a uniquely high prevalence of T2DM regardless of HPV tumor status. This data provies justification for an expanded study examining metformin use among OPSCC treated with radiation therapy and its impact on recurrence. **Acknowledgements and Funding:** Thank you to the PSMRF Program through the University of Kentucky College of Medicine and the Markey Cancer Center CRI Team. This research was supported by the Cancer Research Informatics Shared Resource of the University of Kentucky Markey Cancer Center (P30 CA177558).The Professional Student Mentored Research Fellowship (PSMRF) Project is supported by the National Center for Advancing Translational Sciences (UL1TR001998), UK HealthCare and the University of Kentucky College of Medicine. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

References: (1) Goodman MT, et al. *Eur J Cancer*. 2015;51(18):2759-67. **(2)** Gronhoj C, et al. *Oral Oncol*. 2019;96:1-6. **(3)** Xu W, et al. *J Diabetes Investig*. 2024;15(8):1140-50. **(4)** Foreman A, et al. *Otolaryngol Head Neck Surg*. 2017;157(4):657-63. (**5)** Curry J, et al. *Laryngoscope*. 2017;127(8):1808-15. **(6)** Wu X, et al. *Cancer Res*. 2019;79(17):4360-70. **(7)** Hu X, et al. *Surg Oncol*. 2020;35:453-9. **(8)** American Diabetes Association. The Burden of Diabetes in Kentucky. 2023. **(9)** U.S. Dept of Health and Human Services, CDC. United States Cancer Statistics Data Brief: Cancers Associated with Human Papillomavirus By State, 2010-2014. CDC, March 2018.

5 Regulation of Lipogenesis Pathway through Phosphorylation of HOXB13 by Plk1

Mansoureh Nouri[1], Hamed Maasoumyhaghighi[1], Jinghui Liu[1], Xiaoqi Liu[1,2]

[1]Department of Toxicology and Cancer Biology, [2]Markey Cancer Center, College of Medicine, University of Kentucky, Lexington, KY

Prostate cancer (PCa) remains a leading cause of cancer-related mortality in men, particularly in its advanced stages. While androgen deprivation therapy (ADT) and next-generation androgen receptor (AR)-targeted therapies have improved outcomes, castration-resistant prostate cancer (CRPC) continues to pose significant therapeutic challenges due to tumor heterogeneity and resistance mechanisms, including AR splice variants like AR-V7. Emerging evidence suggests that lipid metabolism plays a crucial role in CRPC progression, with de novo lipogenesis (DNL) and lipid accumulation contributing to tumor aggressiveness and resistance to AR-targeted treatments. FASN and SREBPs are key mediators of this process, regulated by AR signaling. HOXB13, a homeobox transcription factor essential for prostate development, has been implicated in PCa progression through its interaction with AR and its role in reprogramming the AR cistrome. However, the molecular mechanisms underlying HOXB13 regulation remain largely unexplored. Our study aims to investigate the post-translational modification of HOXB13, specifically its phosphorylation by polo-like kinase 1 (Plk1), a mitotic kinase frequently overexpressed in PCa. We hypothesize that Plk1-mediated phosphorylation of HOXB13 alters its stability and enhances its interaction with HDAC3 through the MEIS domain, thereby influencing lipid metabolism and CRPC progression. To test this hypothesis, we will pursue three specific aims: (1) determine whether HOXB13 is a substrate of Plk1 and identify its phosphorylation site; (2) assess the functional significance of HOXB13 phosphorylation in regulating lipogenesis and CRPC progression through gene expression analysis, RNA sequencing, and lipid metabolism assays; and (3) evaluate the physiological relevance of HOXB13 phosphorylation using xenograft model and performing prostate reconstitution in SCID mice to investigate its impact on tumor formation and metastasis. By elucidating the regulatory role of Plk1 in HOXB13-mediated lipid metabolism and CRPC progression, this study will provide critical insights into the molecular mechanisms driving prostate cancer aggressiveness and may identify novel therapeutic targets for advanced disease.

6 Using a Syngeneic Model for Triple Negative Breast Cancer to Explore the Impact of Integrin α6β4 on Glucose Metabolism

Yiming Sheng[1], Parvanee A. Karimpour[2], Andrew Elliott[2], Jayla Fenderson[2], Min Chen[2,3], Kathleen L. O'Connor[2,4]

[1]Lewis Honors College, [2]Markey Cancer Center, [3]Department of Toxicology and Cancer Biology, [4]Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY

Triple negative breast cancer (TNBC) is the deadliest subtype of breast cancer. Integrin α6β4, a laminin receptor, is highly expressed in more than 80% of TNBC cases and contributes to the aggressive nature of TNBC. The reprograming of cellular metabolic processes such as glycolysis is a hallmark of cancer. Recent studies from our lab demonstrate that integrin α6β4 enhances Hif-1α nuclear accumulation, suggesting its role in promoting a pseudohypoxia signature. However, whether glucose metabolism is part of this signature and how integrin α6β4 impacts TNBC glucose metabolism remain unclear. To examine if integrin α6β4 expression impacts glycolysis, we first performed a glycolysis stress test to measure the extracellular acidification rate in BT549 cells that endogenously lack integrin α6β4 expression and stably transfected with empty vector (BT549-EV) or construct containing integrin β4 (BT549-β4). Our data show that BT549-β4 cells display significantly increased glycolysis and glycolytic capacity. To further investigate how integrin α6β4 impacts glucose metabolism in these cells, we performed stable isotope resolved metabolomics with $^{13}C_6$ glucose. We found that integrin α6β4 increased glucose uptake and shunted it into the pentose phosphate pathway compared to control, resulting in increased production of five-carbon ribose units. Interestingly, the increased ribose production in BT549-β4 cells was used to generate ATP, which enters into the production of NAD(H), both of which are essential coenzymes and substrates for TNBC metabolism. We then assessed the expression levels of key proteins in the glycolysis pathway by immunoblotting analysis and found that glucose transporters (Glut1 and Glut3) and several enzymes such as hexokinases and LDHA, are upregulated in BT549-β4 cells. Using the recently established mouse TNBC cell line EMT6 (EV vs β4), we further validated that integrin β4 promotes an invasive phenotype, as well as upregulates hexokinase I and II expression in EMT6-β4 cells. We further treated EMT6-EV and EMT6-β4 cells with various doses of 2-DG, a glucose analog, for 3 days followed by the MTT assay to assess how cell viability is affected when integrin β4-promoted glycolysis is inhibited. The results demonstrated that EMT6-β4 cells are more sensitive to 2-DG treatment compared to EMT6-EV cells. Similar results were obtained from BT549 cells. Data from culturing BT549 (EV vs. β4) and MDA-MB-231 (Control vs. β4 KO) cells in low and high glucose media, demonstrated that the impact of integrin β4-mediated cell proliferation depends on the availability and levels of glucose. In summary, our data highlight a novel function of integrin α6β4 in altering glycolysis. Our study used a recently generated syngeneic TNBC cell model to validate the impact of integrin α6β4 on glycolysis, which is complementary to our studies on human TNBC cell lines. Future studies will focus on investigating the mechanisms of how integrin α6β4 regulates the expression of key glycolysis pathway genes. Further, we will determine if the syngeneic EMT6 cell model exhibits similar glucose influx and metabolites compared to the human TNBC cell lines and if this model can be used for the *in vivo* testing of the response of these models to therapies targeting glucose metabolism.

7 The role of Eukaryotic elongation factor 2 kinase in sex disparities in melanoma immune response and tumor persistence

Andrew T. Shinkle, Felix Oyelami, Xingcong Ren, Jin-Ming Yang

Department of Toxicology and Cancer Biology, University of Kentucky, Lexington, KY

Melanoma exhibits stark sex disparities, with males accounting for well over half of global cases and having over 5,000 deaths projected in the United States in 2025. Despite these trends, the biological drivers of sex differences in melanoma progression and treatment response remain poorly understood. Here, we identify Eukaryotic elongation factor 2 kinase (eEF2K) as a key mediator of these disparities. eEF2K, a master regulator of protein translation and cancer cell survival, displays sex-differential expression, with males exhibiting a broader expression range and higher capacity on average compared to females, correlating with more aggressive and persistent tumor behavior and worse clinical outcomes. Our meta-analyses of national cancer databases reveal that while high eEF2K expression is linked to poorer survival in males, females show a relative survival advantage. We further uncover androgen receptor (AR)-mediated upregulation of eEF2K, implicating AR signaling as a driver of these sex differences. To test this, we will evaluate AR inhibitors like enzalutamide in combination with eEF2K targeting, aiming to enhance treatment efficacy, particularly in male patients who are most affected by disparities. Beyond survival, eEF2K promotes immune evasion by reducing melanoma antigenicity, a mechanism that may contribute to the female survival advantage. By dissecting eEF2K's role in tumor metabolism and stress responses, we will determine how male melanomas exploit these pathways to drive tumor growth and evade immune surveillance. This work will position eEF2K as both a prognostic marker and a therapeutic target in melanoma sex disparities, with implications for precision medicine strategies across multiple cancers. Ultimately, our findings will inform sex-stratified therapeutic approaches, improving clinical outcomes for melanoma patients.

8 Decoding Cellular Drivers of Glioblastoma Onset and Invasion Using Next-Gen Human Brain Model

Mitchell D. Thielen, Olga Liaudanskaya

Department of Biomedical Engineering, University of Cincinnati, College of Engineering and Applied Sciences

Glioblastoma multiforme (GBM) remains one of the most aggressive, invasive, and recurring cancers, with a five-year survival rate of around five percent. The aggressive nature of GBM stems from its remarkable ability to hijack the surrounding brain microenvironment, adopting the neuronal network, glial support system, and extracellular matrix to fuel invasion and therapeutic resistance. While GBM is a devastating disorder, we still struggle with treatment options, due to high chemo resistance of reoccurrence rates, making research in understanding what drives these devastating effects of crucial importance.

To investigate cell-specific regulation of GBM growth, migration and overall survival, we developed a 3D human *in vitro* GBM model, utilizing bioengineered silk scaffolds seeded with cocultures of GBM LN-229 cells with either mature cortical neurons, astrocytes, or human brain microvascular endothelial cells. Next, to understand the interaction (invasion and spread) of GBM with the host microenvironment, we injected GBM cells pre-labeled with fluorescent markers into established triculture scaffolds containing RFP-labeled neuronal mitochondria, allowing real-time tracking of cellular infiltration and potential mitochondria transfer events. This platform replicates the architectural and cellular complexity of the native brain microenvironment, enabling mechanistic interrogation of GBM behavior in a mature, vascularized neural context.

Preliminary findings demonstrated that astrocytes significantly enhance GBM development and proliferation compared to GBM monocultures, while endothelial cells contribute similarly once a mature vascular network forms by the 4-week mark, supporting previous findings that vascular niches sustain glioma stem-like cells [Hinrichsen et al.]. Interestingly, we observed that GBM cells tend to migrate toward and invade neuronal territories, forming the most tumorigenic domains around the 2-week mark. Our injection studies revealed that GBM cells actively engage with and potentially acquire neuronal mitochondria, supporting emerging reports that mitochondrial transfer from healthy brain cells drives metabolic reprogramming and enhances tumorigenicity [Watson et al.].

The observations underscore the need to investigate whether blocking neuronal mitochondrial transfer represents a viable therapeutic strategy to disrupt GBM's metabolic hijacking. Ongoing quantitative analyses, including proliferation assays, scaffold infiltration, and metabolic profiling via Seahorse assay, α-ketoglutarate/2-hydroxyglutarate ratio, and Western blot for oxidative phosphorylation markers, will elucidate cell-type-specific contributions to GBM aggressiveness.

9 SIRT7: A Metabolic Regulator Bridges Normal Hematopoiesis, Clonal Hematopoiesis, and Hematological Malignancies

Xiangzhen Wei, Mary Mohrin*

Department of Toxicology and Cancer Biology, University of Kentucky, Lexington, KY

Hematopoiesis is a strictly regulated process that lasts a lifetime to maintain blood cell homeostasis, while clonal hematopoiesis and subsequent transformation into leukemia represent stages of dysregulated proliferation and metabolic adaptation. Although Sirtuin 7 (SIRT7), a NAD^+-dependent deacetylase predominantly localized in the nucleolus, has been extensively studied in solid tumor progression, its role in hematologic malignancies remains largely unexplored. Based on previous work from our lab and recent immunofluorescence results, we found that SIRT7 can sense cellular stress and change its cellular localization. We hypothesize that in clonal hematopoiesis, SIRT7 may influence mutant clone expansion through metabolic, inflammatory, and genomic mechanisms. Downregulation of SIRT7 relieves its restraint on mitochondrial biogenesis, leading to enhanced energy metabolism and ROS accumulation that confer a growth advantage to mutant HSCs. Simultaneously, loss of SIRT7 augments NF-κB–mediated inflammatory signaling, promoting the adaptation of mutant clones to an inflammatory bone marrow environment. Moreover, impaired chromatin stability due to SIRT7 deficiency increases DNA damage and epigenetic plasticity, providing additional genetic and epigenetic drivers for clonal evolution. In the future, we will use the SIRT7 TG and SIRT7 KO mouse models in our lab to conduct additional in vivo research to validate its function in clonal hematopoiesis and hematological malignancies. This study highlights the importance of integrating metabolic regulation into understanding hematopoietic transformation and positions SIRT7 as a potential biomarker and intervention target across multiple stages of hematologic disease.

10 USP21 Promotes Castration-Resistant Prostate Cancer (CRPC) Progression by Deubiquitinating and Stabilizing AR and Sustaining AR Cytoplasmic–Nuclear Shuttling

Cheng Zhang, Dr. Ka-wing Fang (Research Mentor)

Department of Toxicology and Cancer Biology, University of Kentucky, Lexington, KY

The Ubiquitin-specific protease 21 (USP21) is a deubiquitinase with pro-carcinogenic effects in various cancers. While its role and molecular mechanism in CRPC remain elusive. To investigate the role of USP21 in promoting CRPC, we evaluated that USP21 shows the highest mRNA expression in CRPC patients, and high USP21 levels predicted a poor prognosis in patients with CRPC. Additionally, suppression of USP21 significantly inhibited CRPC cell proliferation. *In vivo*, USP21 depletion significantly suppressed tumor growth in xenograft models. Moreover, we identified the androgen receptor (AR) as a potential substrate of USP21 by tandem affinity purification. Mechanistically, i) USP21 directly interacted and stabilized AR by deubiquitinating its K48-linked polyubiquitination, which is a main driver for CRPC. Silencing of USP21 enhances polyubiquitination of AR, promotes its proteasomal degradation, and ultimately attenuates PSA, while these effects could be largely restored by reintroduction of AR. ii) USP21 regulates transcriptional activity of AR according to our RNA-seq studies, we revealed that siUSP21 decreases both androgen response and AR-induced pathways, and suppresses USP21 decrease mRNA level of AR target genes in C4-2B cells. iii) Interestingly, we revealed that USP21 facilitates AR nuclear transportation through recruiting and stabilizing AR primarily in the microtubule cytoskeleton. iiii) Furthermore, we found disulfiram (DSF), an inhibitor against USP21 deubiquitylation activity, significantly displayed an anti-tumor effect on USP21-AR driving CPRC progression. We also identify synergistic effects between enzalutamide (ENZ) and DSF in CRPC cells crossing a wide range of concentrations. In conclusion, our project gives insight into the mechanism underlying how USP21 regulates AR to promote the progression of CRPC, therefore providing a novel approach for CRPC treatment.

11 Targeting PGK1 to Control Metabolic Plasticity in Prostate Cancer Progression

Min Zhang, Ricky Wang, Xiaoqi Liu

Toxicology and Cancer Biology, College of Medicine, University of Kentucky, Lexington, KY

Prostate cancer (PCa) undergoes dynamic metabolic reprogramming during progression, shifting from citrate oxidation in early-stage tumors to glycolysis in advanced, treatment-resistant states. Phosphoglycerate kinase 1 (PGK1), a glycolytic enzyme, has emerged as a key metabolic regulator with additional roles in oncogenic signaling and therapeutic resistance. However, its precise role in driving metabolic adaptation and resistance in PCa remains unclear. This study aims to elucidate PGK1's role in driving metabolic adaptation in PCa and assess its potential as a therapeutic target to overcome treatment resistance. Our preliminary data demonstrate that PGK1 inhibitors significantly reduce cell viability in 22Rv1 and N2P1 PCa cell lines, as shown by MTT assays. Additionally, colony formation assays reveal impaired long-term proliferative capacity upon PGK1 inhibition, while transwell assays indicate a marked reduction in cell migration and invasion. These findings suggest that PGK1 is critical for maintaining the aggressive phenotype of late-stage PCa. To further elucidate the molecular mechanisms underlying PGK1-mediated metabolic adaptation, we will investigate its role in regulating key metabolic and survival pathways. Future studies will explore PGK1 inhibition in vivo to assess its therapeutic efficacy in overcoming treatment resistance. By targeting PGK1-driven metabolic reprogramming, this study aims to provide novel insights into the metabolic vulnerabilities of advanced PCa and establish PGK1 as a promising therapeutic target.

12 Pdcd4/mTORC2 Axis Regulates Tumorigenesis through PFKFB3 in NSCLC

Elham Zokaei[1], Qing Wang[1], Yanquan Zhang[1], Liang Zeng[1], Yumeng Xin[1], Hsin-Sheng Yang [1,2]

[1]Toxicology and Cancer Biology, [2]Markey Cancer Center, University of Kentucky, Lexington, KY

Programmed cell death 4 (PDCD4) is a tumor suppressor whose expression is frequently downregulated in various cancers, including lung cancer. PDCD4 has been shown to suppress tumor cell proliferation, migration, invasion, and metastasis in cultured cells and in mouse models. It has also been suggested to play a role in regulating glucose metabolism; however, the detailed mechanisms remain unclear. Using reverse phase protein array, we found that knockdown of PDCD4 upregulated 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3), a critical enzyme in regulation of glycolysis. PFKFB3 converts fructose-6-phosphate to fructose-2,6- bisphosphate to activate the rate-limiting enzyme, 6-phosphofructo-1-kinase, in glycolysis. PFKFB3 expression is upregulated in lung cancer and correlates with tumor stage progression. Inhibition or down-regulation of PFKFB3 in lung cancer cells induces cell apoptosis and inhibits tumor growth in nude mice. Given that PDCD4 inhibits mTORC2 activity, we next examined how mTORC2 affects PFKFB3 expression. We found that mTORC2 interacted with PFKFB3 in NSCLC cells. Using In vitro kinase assays demonstrated that can phosphorylate PFKFB3. Further analysis pinpointed a Ser residue as the target site, leading to the generation of phosphorylation deficient (S to A) and phospho-mimetic (S to D) mutants. Stability assays revealed that phosphorylation at this specific Ser is essential for maintaining PFKFB3 protein stability; as the phosphorylation-deficient mutant underwent rapidly degradation, whereas the phospho-mimetic mutant showed increased its half-life. Functional assays showed that WT PFKFB3 promoted high proliferation rates, whereas the phosphorylation-deficient mutant impaired colony formation and proliferation. Taken together, these findings uncover a key mechanism of metabolic reprogramming in NSCLC, in which mTORC2 drives glycolysis by directly phosphorylating PFKFB3, highlighting the therapeutic potential of targeting the PDCD4/mTORC2/PFKFB3 axis in NSCLC treatment.

13 Targeting KLRG1 Restores Anti-PD-1 Efficacy in Obesity-Associated Renal Cancer

Francesca Dempsey[1], Lyse Norian, PhD[2,3]

[1]Graduate Biomedical Sciences, Cancer Biology Theme, University of Alabama at Birmingham; [2]College of Medicine, [3]Microbiology, Immunology, and Molecular Genetics, University of Kentucky, Lexington, KY

Understanding the effects of obesity on cancer progression and treatment outcomes is a critically important area of research. Obesity is known to increase the risk for many cancers, including renal cell carcinoma (RCC). Our lab has shown that obesity leads to worse outcomes in advanced renal cancer patients treated with anti-PD-1 immunotherapy as compared to lean patients, but the cause for this difference is unknown. There is mounting evidence from our group and others that suggests immune senescence, a dysfunctional state in which immune cells lose proliferative capacity and exhibit reduced activation and effector function, could be a contributing factor to these disparate outcomes. We hypothesize that obesity is promoting immune senescence, thereby decreasing responsiveness to immunotherapy. In support of this idea, Norian lab NanoString profiling data from tumors of RCC patients revealed senescence as one of the most upregulated pathways in patients with obesity. To investigate this further, we compared intratumoral immune senescence in diet-induced obese (DIO) versus lean mice using flow cytometry and multiplex immunofluorescence on human RCC samples from patients -/+ obesity. We found that RCC patients with obesity had more senescent T cells in tumors than lean patients. In tumors from DIO mice, we observed increased expression of senescence markers particularly in CD4+ T cells. Importantly, blockade of KLRG1, an inhibitory receptor associated with T cell senescence and reduced effector function, in combination with anti-PD-1 restored responsiveness and improved outcomes in DIO mice to levels comparable to those observed in lean mice treated with anti-PD-1 alone. These findings suggest that T cell senescence contributes to impaired immunotherapy responses in obesity and highlight senescence blockade as a potential therapeutic strategy to improve responses in cancer patients with obesity.

14 Utilizing a Combination of Epigenetic and Lipid Metabolism Inhibitors as a Potential Therapeutic Strategy for BRAFi-Resistant Colorectal Cancer

E. Elias-Ferreira[1]*, J.W. Tessmann1*, M.E. Geisen1, Q. Wei1, S. Rock3, Y.Y. Zaytseva[1,2]

*[1]Department of Toxicology and Cancer Biology, [2]Markey Cancer Center, University of Kentucky, [3]Caris Life Sciences, Irving, TX; *Authors contributed equally to this work*

Despite advances in the development of novel therapies, drug resistance remains a major challenge in colorectal cancer (CRC), the second leading cause of cancer-related death in the U.S. BRAF-mutant CRC, mainly driven by the BRAFV600E mutation, is associated with reduced response to chemotherapy and poor prognosis. BRAF inhibitors (BRAFi) are FDA-approved and effective for these patients; although resistance typically develops within 4–6 months. Our recent study has shown that BRAFi resistance is associated with upregulation of fatty acid synthase (FASN), a crucial enzyme in lipid metabolism and a therapeutic target in CRC. Screening the APExBIO DiscoveryProbe FDA-approved drug library identified histone deacetylase inhibitors (HDACi) as highly efficacious compounds in BRAFi-resistant cells. HDACs are frequently dysregulated and drive epigenetic reprogramming in cancer. However, HDACi show limited efficacy as monotherapy or in combination with chemotherapy in solid tumors. New combinational approaches are needed to fully achieve their therapeutic potential. Therefore, the goal of this study is to evaluate the efficacy of combining HDACi with TVB2640 (FASN inhibitor) in BRAFi-resistant cells and investigate the underlying mechanisms behind the synergy between BRAFi and FASN-targeted therapy.

We utilized organoids and cell lines resistant to PLX8394 (a second-generation BRAFi) and encorafenib/cetuximab (an FDA-approved treatment for $BRAF^{V600E}$ CRC). Cell viability was assessed using CellTitle-Glo assay, and synergy scores were calculated with SynergyFinder using the Bliss model. Western blotting was conducted to evaluate changes in acetylation and protein expression.

We found that combination of romidepsin (HDACi) with TVB2640 significantly reduces cell viability in BRAFi-resistant CRC cell lines and human organoids as compared to monotherapy. Combination of romidepsin and TVB2640 shows a particular high synergy in inhibiting cell viability, in BRAFi-resistant CRC cell lines. Consistently, the combinational treatment significantly increases caspase-3/7 activity and cell death markers, such as cleaved caspase-7. The analysis of protein expression demonstrates that resistant cells show higher expression of HDACs and FASN as compared with parental cell lines. Clinical data from Caris Life Science patient cohort further support these findings, showing that upregulation of FASN is associated with upregulation of HDACs expression in $BRAF^{V600E}$ CRC patients. Both HDACs and FASN can potentially alter an Acetyl-CoA pool and acetylation of proteins. Indeed, we found that combination of romidepsin and TVB2640 alters histone acetylation levels on histone 3 lysine residues, H3K27 and H3K9, suggesting that epigenetic modulation is a contributing factor to the combinational effect of HDAC and FASN inhibition.

In summary, our study demonstrates that combination of FASN and HDAC inhibitors is an effective therapeutical strategy for BRAFi-resistant CRC. Further studies are needed to elucidate the mechanisms behind the combinational effect of HDACi and FASN-targeted therapy.

15 Information-Content-Informed Kendall-Tau Correlation Methodology: Interpreting Missing Values as Useful Information

Robert M. Flight[1,2], Praneeth S. Bhatt[3], Hunter N.B. Moseley[1,2,4,5]

[1]Markey Cancer Center, [2]Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY; [3]University of Washington Graduate School, Seattle, WA; [4]Institute for Biomedical Informatics, [5]Department of Toxicology and Cancer Biology, University of Kentucky, Lexington, KY

Introduction: Almost all correlation measures currently available are unable to directly handle missing values. Either missing values are ignored, or they are imputed and used in the calculation of the correlation coefficient. In either case, the correlation values are impacted based on a perspective that the missing data represents no useful information. Missing values occur in real metabolomics data sets for a variety of reasons, but frequently due to specific analytes falling below the limit-of-detection of the analytical instrumentation (left-censored values). These missing data are not missing at random, but represent potentially useful information by virtue of their "missingness" at one end of the data distribution.

Methods: To use this left-censorship missingness in correlation, we propose the information-content-informed Kendall-tau (ICI-Kt) methodology. We show how left-censored missing values can be included within the definition of the Kendall-tau correlation coefficient, and how that inclusion leads to an interpretation of information being added to the correlation. We also implement calculations for additional measures of theoretical maxima and pairwise completeness that provide additional interpretive perspectives to the methodology.

To test and validate the ICI-Kt methodology, we used a variety of simulated datasets with different types, amounts, and causes of missingness, as well as over 100 metabolomics experimental datasets from Metabolomics Workbench (MW); comparing ICI-Kt to Kendall-tau and Pearson correlations in each case.

Results: Tests on both simulated and real metabolomics datasets demonstrate that ICI-Kt performs better than Kendall-tau, Spearman, or Pearson correlation methods when left-censored values are present, especially for outlier detection.

We provide parallel processing implementations of these algorithms in both R and Python packages that enable fast calculations when applied to large datasets.

16 Machine Learning Predicted Pathway Annotations Greatly Improve Pathway Enrichment Analyses of Metabolomics Datasets

Erik D. Huckvale[1], P. Travis Thompson[1], Robert M. Flight[1], Hunter N.B. Moseley[1–4*]

[1]Markey Cancer Center, [2]Superfund Research Center, [3]Department of Molecular and Cellular Biochemistry, [4]Institute for Biomedical Informatics, University of Kentucky, Lexington, KY

Introduction: In metabolomics data analysis, annotation enrichment analysis (AEA) is a method for detecting over-representation (enrichment) of annotations for metabolite features that have appreciable covariance across an analytical dataset. Enriched annotations, especially enriched pathway annotations, provide biological and biomedical interpretation of the experimental groups represented in the dataset. AEA applied only to pathway annotations is often called pathway enrichment analysis (PEA). Several knowledgebases have pathway annotations associated with metabolites, including the Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome, and MetaCyc. However, these three knowledgebases combined have pathway annotations for less than 19,000 metabolites, which is a small fraction of detectable metabolites. For most metabolomics datasets, only 30% to 40% of the detected metabolites have pathway annotations, greatly limiting PEA in detecting enriched pathways.

Methods: In this work, we have developed a convolutional network-like, multitask classification model using a dataset constructed from KEGG, Reactome, and MetaCyc that can predict metabolite-pathway annotations based on metabolite chemical structure.

Results: Our modeling approach scored a Matthews correlation coefficient (MCC) of 0.9036 ± 0.0033(sd) across 100 train-test splits. Using our model, we generated high-quality metabolite-pathway annotations for PEA applied to 990 metabolomics datasets from the Metabolomics Workbench repository. Finally, we demonstrate an over 10-fold increase in the median number of detected enriched pathways across these metabolomics datasets over using only knowledgebase-derived annotations.

Conclusions: Based on our results, we can confirm that large improvements can be made to PEA for metabolomics experimental datasets using high-quality predicted metabolite-pathway annotations. The over 10-fold improvement to PEA demonstrates both the accuracy of the predictions of our machine learning model and the potential to greatly improve the biological and biomedical interpretation of metabolomics experiments at a perturbed pathway-level.

17 Seeing is Believing: The Evolving Role of Muscle Ultrasound in Comprehensive Nutritional Care of Pediatric Oncology Patients – A Case Series

Corey Hawes, DCN, RD, CSO, CNSC, FASPEN; Vlad Călin Rădulescu, MD; Senna Munnikhuysen, MD

University of Kentucky, Kentucky Children's Hospital, Lexington, KY

Nutritional assessments in pediatric oncology often fail to detect critical changes in body composition. This case series highlights the novel use of point-of-care muscle ultrasound (mUS) by a dietitian to objectively guide interventions. In three patients, serial mUS measurements revealed muscle mass changes that were discordant with body weight trends. These objective findings directly informed nutritional therapies and documented their efficacy. mUS is a promising tool for assessing muscle status, overcoming the limitations of conventional methods. Larger prospective studies are warranted to validate these results and establish standardized protocols.

18 Reprogramming of Nucleic Acid and Protein Methylation by EZH2

Mohamed M.Y. Kaddah[1,2,3], Jahid Islam[1], Penghui Lin[1], Teresa Cassel[1], Richard Cunningham[4], Tom Brown[4], Richard M. Higashi[1,2], Andrew N. Lane[1,2], Teresa W-M. Fan[1,2]

[1]Center for Environmental and Systems Biochemistry, [2]Department of Toxicology and Cancer Biology, College of Medicine, University of Kentucky, Lexington, KY; [3]Pharmaceutical and Fermentation Industries Development Center, City of Scientific Research and Technological Applications, Alexandria, Egypt; [4]Magdalen Centre, The Oxford Science Park, Littlemore, Oxford, United Kingdom

EZH2, a histone H3K27 trimethyltransferase, is a key epigenetic regulator frequently dysregulated in cancer. Its impact on nucleotide biosynthesis and nucleic acid modifications remains elusive. We developed ion chromatography–ultra-high-resolution Fourier transform mass spectrometry (IC-UHR-FTMS) with a 9.3 femtomole low limit of quantification to determine changes in methylation of DNA, RNA, and mRNA in A549 cells following EZH2 knockdown (KD). Using dual tracers, L-methionine-(methyl-^{13}C) and L-glutamine-($^{15}N_2$), we quantified positional ^{13}C/^{15}N isotopomers of methylated nucleotides and their precursors. EZH2 KD reduced ^{15}N incorporation into deoxynucleotides, indicating impaired *de novo* synthesis from glutamine. It also attenuated ^{15}N and/or ^{13}C labeling of nucleotides and methylated nucleotides in total RNA and mRNA at various atomic positions, reflecting global losses in biosynthesis and S-adenosylmethionine (SAM)-dependent methylation. Notably, AMP methylation at N6 and 2′-O positions was most responsive to EZH2 KD, which implicates reduced capped-RNA translation. Some of the EZH2 KD-induced changes in RNA methylation corresponded with the altered expression of their writer or eraser enzymes. This study demonstrates multiplex tracers-coupled IC-UHR-MS as a powerful tool for comprehensive tracing of methylation dynamics in mammalian cells and reveals EZH2's role in metabolic-epitranscriptomic regulation by modulating SAM availability via glutamine-fueled *de novo* purine biosynthesis and RNA methylation.

19 Application of LAICPMS on Tumor Slices

Penghui Lin, Richard Higashi, Andrew N. Lane, Teresa W-M. Fan

CESB and Markey Cancer Center, University of Kentucky, Lexington, KY

Breast cancer is the 2nd most common type of cancer and affects 1/8 of the women in their lifetime [1]. It is also the 2nd leading cause of death among women. To better understand, treat, and prevent breast cancer, new techniques are needed at the microscopic level to assess disease and drug resistance biomarkers. LAICPMS (Laser Ablation Inductively Coupled Plasma Mass Spectrometry) is a powerful tool for simultaneous quantitative imaging of endo- and exogenous metals, metal containing drugs as well as metal-tagged antibodies to biomarkers in tissue sections. It has been applied to a range of biological samples and pathologies [2]. With the newly improved rapid response laser ablation sample cells and analyte transport technologies, the sub ppm range sensitivities at a spatial resolution ≤ 10 μm, or near single cell can be achieved [3-5]. With the multiplex ACCLAIMS (ACcumulated elements and Cyto-proteomics/metabolomics by Laser Ablation Imaging Mass Spectrometry) we are developing, contamination metals and the specific designed lanthanide-conjugated antibodies to biomarkers of these tissue can be detected simultaneously for an unprecedented understanding of the mechanism of how these metals impact tumorigenesis, progression, and drug resistance. Compared with other imaging techniques, ACCLAIMS has the advantages of low background, multi-channel simultaneous acquisition, with no signal overlap between channels. Here we demonstrate the approach with images of a pair of cancerous breast tissue vs its benign counterpart showing the distribution of two contaminant metals together with six different cell/functional markers at 10 μm resolution. These images provide valuable spatially resolved information for further study of the interaction between contaminant metals and cancer cell markers in the tumor microenvironment of these patients.

1. Siegel RL, et al. Cancer statistics, 2025. *CA: A Cancer Journal for Clinicians*, 2025. 75:10-45.
2. Theiner S, et al. Mass spectrometry techniques for imaging and detection of metallodrugs. *Curr Opin Chem Biol*, 2021. 61:123-134.
3. Van Malderen SJM, et al. Sub-micrometer nanosecond LA-ICP-MS imaging at pixel acquisition rates above 250 Hz via a low-dispersion setup. *Anal Chem*, 2020. 92(8):5756-64.
4. Van Malderen SJM, et al. Development of a fast laser ablation-inductively coupled plasma-mass spectrometry cell for sub-μm scanning of layered materials. J Anal Atomic Spectrometry, 2015;30(1): 119-125.
5. Van Acker T, et al. High-resolution laser ablation-inductively coupled plasma-mass spectrometry imaging of cisplatin-induced nephrotoxic side effects. Anal Chimi Acta, 2016;945: 23-30.

20 ExoLnc-Signaling: UCA1-Driven Metabolic Reprogramming in the Tumor Microenvironment of Ovarian Cancer

Revathy Nadhan, Sneha Basu, Ji Hee Ha, Muralidharan Jayaraman, Danny N. Dhanasekaran

Stephenson Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK

Tumor progression is orchestrated by intricate communication between cancer cells and the tumor microenvironment (TME) through autocrine, paracrine, and exocrine signaling networks. Among these, ExoLnc signaling, mediated by exosomal long non-coding RNAs (lncRNAs), has emerged as a pivotal mechanism that remodels the TME and drives oncogenic transformation. Here, we identify UCA1 (Urothelial Cancer Associated 1) as a key ExoLnc signaling component in high-grade serous ovarian carcinoma (HGSOC). Comprehensive profiling of exosomes derived from HGSOC cell lines, patient-derived ovarian cancer cells, and ascitic fluids revealed that UCA1 is selectively packaged and delivered via exosomes to the fibroblasts in the TME. PKH67-labeled exosome uptake assays demonstrated efficient transfer of UCA1 into stromal fibroblasts, defining the functional ExoLnc communication between tumor and stromal compartments. Analyses using the Agilent Seahorse glycolytic stress assay indicated that UCA1-enriched exosomes markedly enhanced glycolytic activity in fibroblasts, whereas depletion of UCA1 abrogated this metabolic switch. Correspondingly, key glycolytic enzymes were upregulated following exosomal UCA1 uptake, confirming metabolic reprogramming in fibroblasts toward a pro-tumorigenic metabolic phenotype. Collectively, these findings uncover a previously unrecognized ExoLnc signaling axis in which exosomal UCA1 reprograms glucose metabolism to promote ovarian cancer progression. Targeting ExoLnc signaling, particularly through UCA1 inhibition, may offer a novel precision therapeutic strategy to disrupt the oncogenic crosstalk between cancer cells and the TME in ovarian cancer.

Acknowledgement: This research was supported by the Department of Defense Ovarian Cancer Research Program Award W81XWH-18-1-0066, W81XWH-22-1-0415, the National Institute of General Medical Sciences grant P20 GM103639, P30GM154635, the National Cancer Institute of the National Institutes of Health grant P30 CA225520.

21 PLK1-Mediated PDHA1 Phosphorylation Drives Metabolic Reprogramming and Reveals a Therapeutic Strategy for Lung Cancer

Jia Peng[1], Qiongsi Zhang[1], Xiongjian Rao[1], Derek B. Allison[2,3], Yifan Kong[1], Ruixin Wang[1], Jinghui Liu[1], Yanquan Zhang[1], Wendy Katz[4], Zhiguo Li[1,2], Xiaoqi Liu[1,2]

[1]Department of Toxicology and Cancer Biology, [2]Markey Cancer Center, [3]Department of Pathology and Laboratory Medicine, [4]Department of Pharmacology and Nutritional Sciences, University of Kentucky, Lexington, KY

PLK1 has been implicated in driving the metabolic shift from oxidative phosphorylation (OXPHOS) to glycolysis, yet the underlying molecular mechanism remains elusive. Pyruvate dehydrogenase (PDH) catalyzes the conversion of pyruvate into acetyl-CoA, the key substrate for the tricarboxylic acid (TCA) cycle. We identified PLK1-dependent phosphorylation of the PDH E1α subunit (PDHA1) at threonine 57 (PDHA1-T57) as a critical modification that promotes PDHA1 degradation through mitophagy activation. Using stable-isotope–resolved metabolomics (SIRM), we also demonstrate that PLK1-mediated phosphorylation of PDHA1-T57 induces a metabolic reprogramming from OXPHOS toward glycolysis.

Cells expressing a phospho-mimetic PDHA1-T57D variant show increased dependence on the aspartate–malate shuttle rather than glucose-derived pyruvate to sustain TCA cycle activity. This metabolic adaptation was recapitulated in mouse embryonic fibroblasts and in transgenic mice conditionally expressing PDHA1-T57D, confirming the physiological relevance of PLK1-driven reprogramming *in vivo*. Because pyruvate dehydrogenase kinase (PDK)–mediated PDH inhibition can be reversed by dichloroacetic acid (DCA), we evaluated DCA in combination with the PLK1 inhibitor Onvansertib. The dual treatment synergistically suppressed lung tumor growth by enhancing mitochondrial reactive oxygen species, inhibiting glycolysis, and inducing apoptosis.

Collectively, these findings identify PLK1-mediated PDHA1 phosphorylation as a mechanistic driver of metabolic reprogramming during lung carcinogenesis and suggest that combined PLK1 and PDK inhibition may represent a promising therapeutic strategy warranting clinical evaluation.

22 Pharmacologic and Genetic Alteration of Neuroblastoma Fucosylation Impacts Antigen Display and Macrophage Recruitment

Michelle G. Pitts, Johan Pijenborg, Emiel Rossing, Thomas Boltje, Beibei Zhu, Eric J. Rellinger

University of Kentucky, Lexington, KY

Neuroblastoma (NB) is a devastating solid tumor that most commonly forms in the adrenal gland and along the sympathetic nervous axis in very young children and infants. Significant recent advances, including monoclonal antibody therapy, have improved the odds, yet long-term event-free survival remains dismal for children whose tumor is driven by amplification of the *MYCN* oncogene. *MYCN* amplification results in profound metabolic alterations that are only beginning to be understood, including increased usage of the hexosamine biosynthetic pathway, not traditionally understood to be part of core cancer metabolism. This complex metabolic shunt yields enhanced availability of substrates for aberrant glycosylation, or modification of proteins with carbohydrate motifs, a phenomenon that may in effect shield NB cells from immune recognition.

To study how aberrant glycosylation, particularly core fucosylation, impacts immune cell recognition of NB, we have employed both genetic and pharmacological methods to lessen or prevent the addition of fucose to the innermost asparagine residue on N-linked glycans. These methods have resulted in changes in surface antigens displayed in vitro on *MYCN*-amp NB cells, including immune checkpoint molecules and markers of stemness. Using an immune competent model of mouse NB with Fucotrim I, a proprietary fucosylation inhibitor, has resulted in changes in macrophage recruitment to the tumor along with significant reductions in tumor growth, suggesting that macrophage recruitment and phenotype in this immune-cold tumor may be critical for changing its trajectory.

Future directions for this work include in-depth analysis of phagocytosis and antibody dependent cellular cytotoxicity, migration assays, and enumeration and characterization of extracellular vesicles with both genetic and pharmacological alteration of fucosylation status. The results of this work, particularly in immune competent models and in combination with monoclonal antibody therapy, will inform future treatment strategies centered on directing the immune system to better control this insidious tumor.

23 PLK1 Phosphorylation of FSP1 and ACSL4 Confers Ferroptosis Resistance in Therapy-Refractory Cancer

Xiongjian Rao[1,2], Jia Peng[1,2], Oluwafunminiyi E. Obaleye[1,2], Felix Oyelami[1,2], Derek B. Allison[2], Ruixing Wang[1,2], Jinhui Liu[1,2], Zhiguo Li[1,2], Xiaoqi Liu[1,2]*

[1]Department of Toxicology and Cancer Biology, [2]Markey Cancer Center, University of Kentucky, Lexington, KY

Cancers acquire insensitivity after therapy and advanced cancers usually resist treatment, while the mechanisms involved in insensitivity and resistance remain elusive. Here, we found that Polo-like kinase 1(PLK1) is highly elevated in therapy resistant cancers and advanced cancers. Specifically, PLK1 contributes to the treatment resistance in cancers by regulating ferroptosis, an iron-dependent programmed cell death which is morphologically, biochemically and genetically distinct from other forms of cell death. Ferroptosis specific protein 1(FSP1), a parallel regulator of glutathione peroxidase 4 (GPX4), protecting the cells from ferroptotic cell death, is phosphorylated by PLK1 at Thr 291 and its phosphorylation promotes FSP1 stabilization as well as protein expression. ACSL4, a negative regulator of ferroptosis causing lipid ROS, is phosphorylated by PLK1 at Ser569 and its phosphorylation leads to the degradation of ACSL4. We also found that the combination of PLK1 inhibitor Onvansertib and ferroptosis inducers is a promising therapeutic strategy for therapy resistant cancers. Our findings provide novel mechanisms of therapy resistance and potential therapeutic approaches for advanced cancers.

24 E2F1 Regulates FLVCR1 Expression and Links Lipid Metabolism to Castration-Resistant Prostate Cancer Progression

Chengtao Sun, Cheng Zhang, Ka-Wing Fong

Department of Toxicology and Cancer Biology, Markey Cancer Center, University of Kentucky, Lexington, KY

Phosphatidylcholine and phosphatidylethanolamine, the major phospholipids in mammalian membranes, are synthesized via the Kennedy pathway, which depends on the uptake of choline and ethanolamine. FLVCR1, a recently identified transporter of these metabolites, plays a crucial role in phospholipid biosynthesis and membrane homeostasis. However, its transcriptional regulation and role in prostate cancer remain unclear. Here, we report that FLVCR1 is an E2F1 target in castration-resistant prostate cancer (CRPC) cell models (C4-2B, 22Rv1, and DU145). Using E2F1 CUT&RUN-seq, we observed strong E2F1 enrichment at the FLVCR1 gene locus compared with IgG controls. E2F1 knockdown by two independent shRNAs significantly reduced FLVCR1 mRNA and protein levels. Functionally, FLVCR1 silencing inhibited cell proliferation, colony formation, migration, and invasion, as demonstrated by colony formation, wound-healing, and transwell assays. These findings suggest that E2F1-driven FLVCR1 expression contributes to membrane dynamics and cell motility in prostate cancer cells. Together, our results identify an E2F1–FLVCR1 regulatory axis linking transcriptional control with lipid metabolism, providing insights into metabolic regulation underlying CRPC progression.

25 Fatty Acid Synthase Enhances Extracellular Vesicle Secretion in Colorectal Cancer Cells and Promotes Activation of Hepatic Fibroblasts

Josiane Weber Tessmann[1], Daheng He[2], Chi Wang[2], Yekaterina Zaytseva[1,3]

[1]Department of Toxicology and Cancer Biology, [2]Markey Cancer Center, 3Biostatistics and Bioinformatics Shared Resource, University of Kentucky, Lexington, KY

More than 50% of colorectal cancer (CRC) patients develop metastases over the course of their disease, with the liver being the most common site of metastasis. Fatty acid synthase (FASN), a key enzyme of de novo lipid synthesis, is overexpressed and strongly associated with metastasis and poor prognosis in CRC. FASN catalyzes the biosynthesis of palmitate, an essential component of structural lipids. Exosomes are composed of a lipid bilayer membrane and carry a wide range of bioactive molecules, including lipids, proteins, and nucleic acids. Tumor-derived exosomes play a critical role in establishing a pre-metastatic niche (PMN) to support metastasis. Hepatic stellate cells (HSCs) can be activated by tumor signals and transformed to cancer-associated fibroblast, thus, contributing to PMN formation. However, the mechanisms by which exosomes promote HSCs activation and their subsequent contribution to PMN formation during CRC liver metastasis are not fully understood. Therefore, this study aims to investigate the contribution of FASN in exosomes formation, release, and HSCs activation in CRC. To delineate the role of FASN in exosomes formation, FASN-knockdown (FASN KD), FASN-overexpression (FASN OE), and pharmacological inhibition of FASN in CRC cells were utilized. Cells were cultured in media containing exosome-free FBS for 48h. Exosomes were isolated from cell culture media using ultracentrifugation. The concentration and size of exosomes were measured using nanoparticle tracking analysis. Exosomes markers were assessed using western blot. Proteomics analysis was conducted to assess the impact of FASN on exosome cargo. To investigate the functional impact of CRC-derived exosomes, HSCs LX-2 was used. The purity of isolated exosomes was confirmed by assessing the expression of CD9, Alix, TSG101, and syntenin. Secretion of exosomes by FASN-KD cells ($Exo^{FASN\text{-}KD}$) and by cells treated with FASN inhibitors was significantly reduced as compared to control cells. Moreover, FASN expression was decreased in $Exo^{FASN\text{-}KD}$. In contrast, secretion of exosomes by FASN-OE cells ($Exo^{FASN\text{-}OE}$) was significantly increased as compared to control cells. Consistently, we observed an increase in expression of FASN in $Exo^{FASN\text{-}OE}$. Proteomic analysis revealed that FASN modulates exosome cargo, with changes in inflammatory response, metabolism, exocytosis, and adhesion pathways. To test if FASN expression in CRC cells affects the ability of secreted exosomes to activate HSCs, LX-2 cells were incubated with exosomes from control and FASN-KD CRC cells. We found that the exposure to exosomes from control CRC cells leads to robust activation of LX-2 cells as determined by FAP and α-SMA expression; however, the level of LX-2 activation was significantly reduced when cells were exposed to $Exo^{FASN\text{-}KD}$. In summary, our data suggests that FASN promotes exosomes secretion in CRC. Importantly, the association between the level of FASN expression in CRC cells and activation of LX-2 cells by secreted exosomes suggests a potential role for FASN in PMN formation. Further studies are needed to elucidate the functional importance of FASN in PMN formation via exosomes secretion in CRC.

26 Uncovering a Metabolic Gatekeeper: The Role of NAD+ Metabolism and SIRT7 in Age-Related Retinal Degeneration and Glaucoma

Chih-Ling Wang[1], Xiangzhen Wei[1], Chezney Boothe[2], Mary Mohrin[1]

[1]Department of Toxicology and Cancer Biology, [2]Department of Biology, University of Kentucky, Lexington, KY (ACTION program)

Glaucoma is a complex, multifactorial eye disease characterized by the progressive loss of retinal ganglion cells (RGC), occurring in one of the body's most metabolically active tissues- the retina. Understanding how these ultra metabolically active cells deal with damage and stress under homeostatic conditions might uncover features that can be targeted in metabolically overactive cancer cells from any tissue type. Aging is a major risk factor for glaucoma as well as for most types of cancer. In glaucoma, increasing evidence links age-related stress, DNA damage and *metabolic imbalance* to RGC degeneration. Declining NAD^+ levels during aging impair DNA repair and mitochondrial function, promoting cellular senescence and neurodegeneration. Conversely, senescent cells can further accelerate NAD^+ depletion. Despite many groups showing that increased NAD^+ may limit glaucoma progression, the molecular mechanism is still unknown. A number of protein families like PARPs, Sirtuins, Cyclic ADP-Ribose (cADPr) Synthases like CD38 and CD157, and SARM1 rely on NAD^+ as a co-factor and consume cellular NAD^+ reserves. Here we will focus on sirtuins, whose activity is directly regulated by NAD^+ levels. Sirtuin expression decreases with age and Sirtuin 7 (SIRT7) stands out as uniquely positioned to potential influence glaucomatous pathogenesis through its role in nucleolar biology, DNA damage responses and epigenetic regulation. SIRT7 acts as a versatile regulator in tumorigenesis, displaying both pro-tumorigenic and tumor-suppressive functions in a context-dependent manner. Studying SIRT7's function in the retina might provide insights into strategies for targeting it in cancer. In retina, SIRT7 is highly expressed in RGCs compared to other cells type. Our previous studies have shown the protective role of SIRT7 in both neural and hematopoietic stem cell (HSC) aging. Loss of SIRT7 in HSCs leads to smaller nucleoli and less heterochromatin. Moreover, our preliminary immunofluorescence data from human donor retinas revealed fewer SIRT7 signals in RGC from glaucoma samples. Interestingly, aged control samples exhibited increased pan-nuclear gH2AX signals, indicative of excessive DNA damage signaling- potential early events preceding RGC loss. Since aging-related metabolic changes, such as NAD^+ decline, can drive neurodegeneration, understanding how these pathways impact RGC survival is critical. Notably, SIRT7 activity depends on NAD+ levels, allowing it to function as a metabolic sensor that influences cellular metabolism and stress response. We hypothesize that SIRT7 acts as a metabolic-neuroprotective gatekeeper, preserving nucleolar and genomic stability to protect RGCs from age-related neurodegeneration. To test this hypothesis, we will use our SIRT7 novel overexpression and knockout mouse models, combined with in vitro and ex vivo retinal assays, to pursue two aims: (Aim 1) Define the role of SIRT7 in regulating nucleolar stress responses and DNA damage repair in glaucoma. (Aim 2) Investigate how aging and NAD^+ decline impairs SIRT7 function and exacerbates glaucoma progression. Together, this work will provide novel mechanistic insights and uncover a potential therapeutic target for age-related retinal degeneration and glaucoma. **Keywords:** SIRT7, retinal ganglion cells, glaucoma, aging, NAD^+, DNA damage, nucleolar stress

27 Core Fucosylation is a Novel Regulator of Neuroblastoma Transcriptional Cell-State

Beibei Zhu[1], Michelle Pitts[1], Michael Buoncristiani[1], Lindsay Ahmad[1], Chi Wang[2], B. Mark Evers[1,3], Eric Rellinger[1,3]

[1]Department of Surgery, [2]Department of Biostatistics, [3]Markey Cancer Center, University of Kentucky, Lexington, KY

Neuroblastoma (NB) is a devastating childhood cancer that accounts for 15% of all childhood cancer deaths despite aggressive treatments. NB cancer cells adopt two major transcriptional cell-states. The adrenergic (ADRN) state is a more differentiated, neuronal state of the cancer cells that is typically more sensitive to therapy, while the mesenchymal (MES) state is less differentiated and more resistant to treatment. Altered glycosylation is a common feature of adult cancer progression. Our previous studies revealed that increased core fucosylated glycan abundance is present within neuroblast-rich regions of human MYCN-amplified NB tumors. Core fucosylation is a specific type of N-linked glycosylation in which a fucose residue is attached via an α1,6 linkage to the innermost N-acetylglucosamine of glycoproteins. This modification is catalyzed by the enzyme fucosyltransferase 8 (FUT8) using GDP-fucose as the donor substrate, which can be generated through the de novo fucose synthesis pathway involving enzymes such as GDP-mannose 4,6 dehydratase (GMDS). Given their key roles in the GDP-fucose synthesis and transfer pathways, respectively, GMDS and FUT8 represent potential actionable targets for therapeutic blockade of core fucosylation in NB. Herein, we performed CRISPR knockout of both GMDS and FUT8 in BE(2)-C cells to delineate their role in NB tumor initiation and progression. CRISPR KO of both GMDS and FUT8 impedes NB tumor formation within immunodeficient models of NB. Intriguingly, genetic knockdown of both GMDS and FUT8 also drives NB cells towards an adrenergic (ADRN) state featuring increased expression of ASCL1, PHOX2B, PHOX2A, GD2 with concomitant suppression of a key mesenchymal markers (SOX9, CD44). Notably, fucosylation-deficient neuroblastoma cells exhibit altered SMAD activation. We are actively investigating differential BMP/TGF-β signaling as a potential mechanism by which this post-translational modification shapes therapeutically relevant transcriptional states. These findings may reveal new opportunities to modulate signaling plasticity and enhance therapeutic sensitivity in NB.

28 Advancing Translational Cancer Research: COBRE Phase III Core Resources at Stephenson Cancer Center

Muralidharan Jayaraman

Director, NIH MTCRO-COBRE Cell and Tissue Analysis Core, Stephenson Cancer Center, The University of Oklahoma Health, Oklahoma City, OK

The battle against cancer demands sophisticated tools, to unravel its multifaceted biological mechanisms and to develop effective therapeutic strategies. The COBRE grant awarded to Stephenson Cancer Center in The University of Oklahoma Health addresses this challenge through two synergistic core facilities that provide comprehensive cancer research capabilities. The Cell and Molecular Imaging (CMI) Core, led by Dr. Ji Hee Ha, offers cutting-edge imaging technologies to visualize cellular and molecular processes in real-time. Through three specific aims, the CMI Core provides molecular imaging consultation, labeled cells for tracking gene expression and disease progression, and high-throughput live cell imaging capabilities. Complementing these efforts, the Cell and Tissue Analysis (CTA) Core, directed by Drs. Muralidharan Jayaraman and Kar-Ming Fung, focuses on histopathological investigation of the tumor microenvironment. The CTA Core delivers consultation services, comprehensive tissue analysis including tissue microarray construction, circulating tumor cell isolation, metabolic flux analysis, immunohistochemical staining, and in situ hybridization, alongside critical human cell authentication services. During Phases 1 and 2 of the COBRE initiative, both cores supported 10 research project leaders and 4 pilot project leaders, contributing to numerous publications and successful grant applications. In Phase 3 of the COBRE, these cores will further the specific aims, by integrating advanced imaging modalities with comprehensive tissue analysis capabilities. This infrastructure positions Oklahoma as a transformative hub for cancer research, fostering innovation and discovery in the battle against cancer.