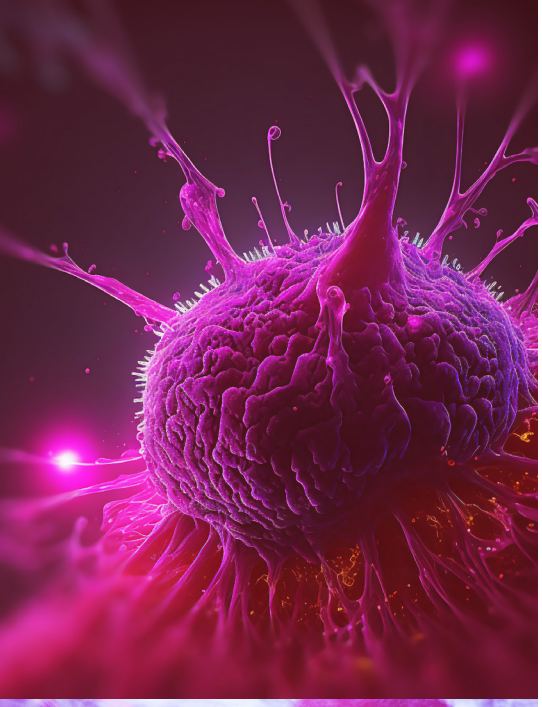
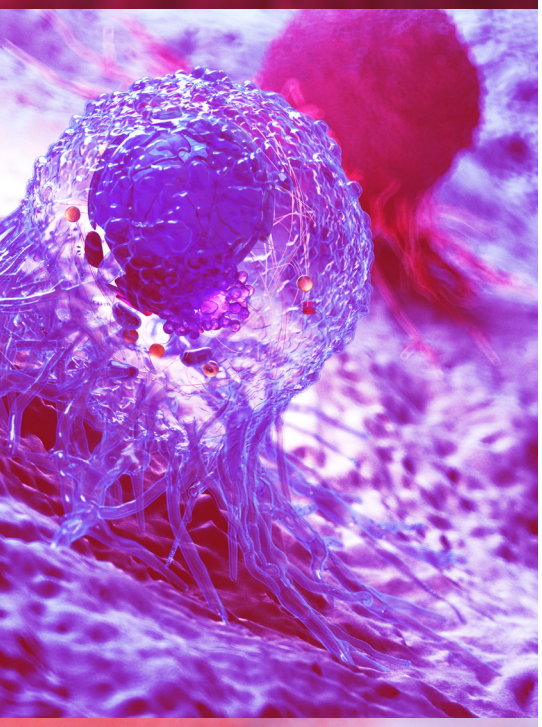
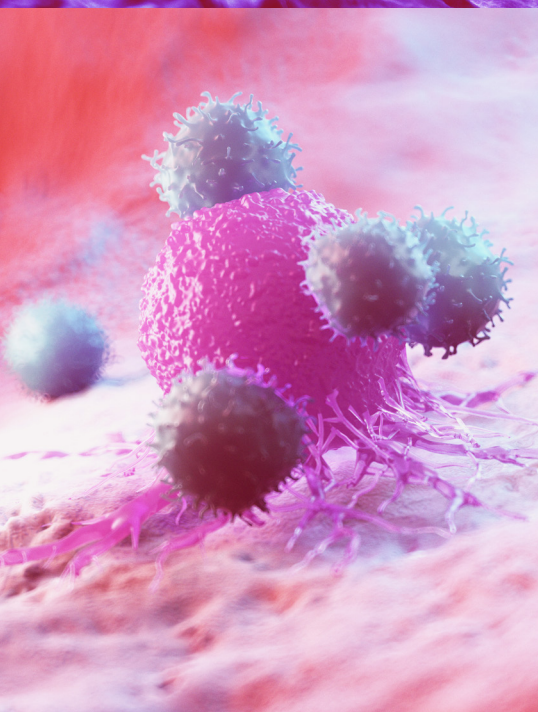






College of Medicine

CANCER METABOLISM SYMPOSIUM

July 18 | 8:30 a.m.–5:30 p.m.
Gatton Student Center
University of Kentucky

UAMS
University of Arkansas for Medical Sciences

HEALTH SCIENCES CENTER
The UNIVERSITY of OKLAHOMA

UNIVERSITY OF LOUISVILLE
SCHOOL OF MEDICINE

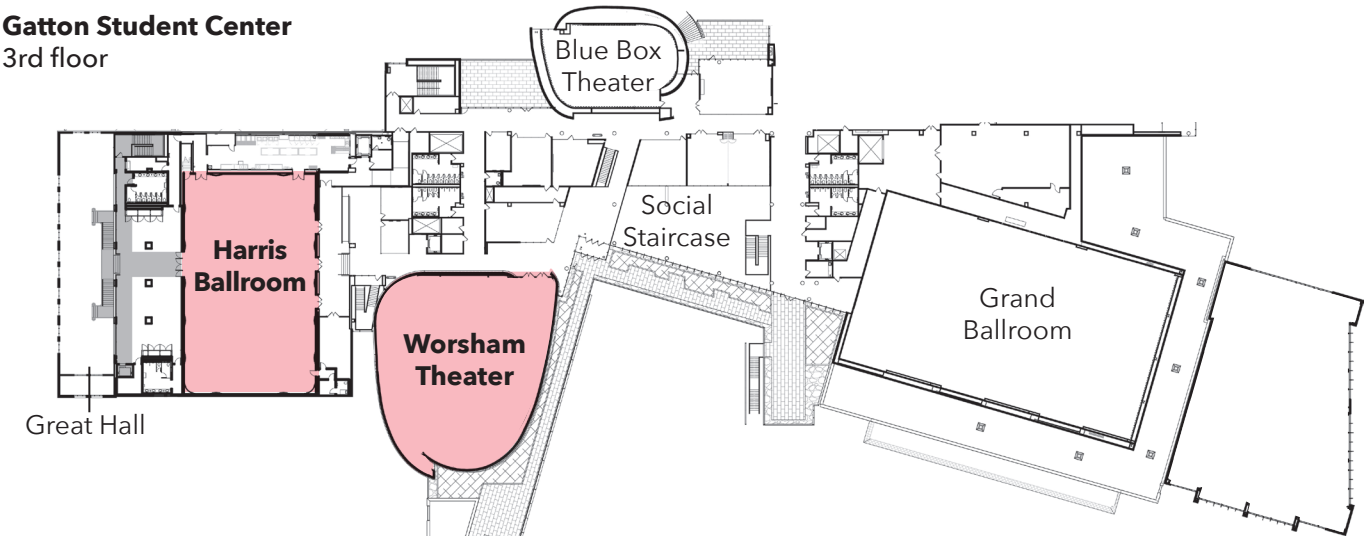
HealthCare
MARKEY CANCER CENTER
An NCI-Designated Cancer Center

College of Medicine

CANCER METABOLISM SYMPOSIUM

Program Contents

Gatton Student Center
3rd floor



Agenda

8:30 a.m. **Registration**
Harris Ballroom Poster Assembly, Continental Breakfast

9:00 a.m. **Introduction**
Worsham Theater
Mark Evers, MD
Director, University of Kentucky Markey Cancer Center
McDowell Foundation Endowed Chair
Associate Vice President for Oncology Research and Strategic Development
Physician-in Chief, Oncology Service Line
UK Health Care

9:05 a.m. **Session 1: COBRE at the University of Kentucky College of Medicine**
Worsham Theater Chair: Andrew Lane, PhD
Interrogating cancer metabolism in-patient and patient-derived models
Teresa Fan, PhD
Edith D. Gardner Chair in Cancer Research
Department of Toxicology and Cancer Biology
University of Kentucky Markey Cancer Center

9:50 a.m. ***Lipid synthesis as a potential therapeutic target in colorectal cancer***
Yekaterina Zaytseva, PhD
Associate Professor
Department of Toxicology and Cancer Biology
University of Kentucky Markey Cancer Center

10:35 a.m. **Break**
Harris Ballroom Poster Session, Refreshments

11:00 a.m. **Session 2: COBRE at the University of Arkansas**
Worsham Theater Chair: Jianhang Jia, PhD
Label-Free Optical Imaging of Skin Wound Metabolism
Kyle Quinn, PhD
Associate Professor of Biomedical Engineering
Director, Arkansas Integrative Metabolic Research Center
University of Arkansas

11:45 a.m. ***Optical spectroscopy and imaging approaches to evaluating long-term outcome in tumors***

Narasimhan Rajaram, PhD
Associate Professor of Biomedical Engineering
Imaging & Spectroscopy Core Director, Arkansas Integrative Metabolic Research Center
University of Arkansas

12:30 p.m. **Lunch**
Harris Ballroom

1:30 p.m. **Session 3: COBRE at the University of Louisville School of Medicine**
Worsham Theater Chair: Rich Higashi, PhD
Metabolic Reprogramming in Pro-metastatic Macrophages to Control Tumor Metastasis

Jun Yan, MD, PhD
Professor of Surgery, Microbiology and Immunology, and Pharmacology and Toxicology
Endowed Chair in Translational Research, Brown Cancer Center
Director, Division of Immunotherapy, UofL School of Medicine
Program Leader, Immuno-Oncology Program, Brown Cancer Center
University of Louisville School of Medicine

2:15 p.m. ***Targeting Adenosine Metabolic Pathway to Reverse Immunotherapeutic Resistance in Cancer***

Kavitha Yaddanapudi, PhD
Associate Professor, Department of Medicine
Associate Scientist, James Graham Brown Cancer Center
Associate Faculty Member, Department of Microbiology & Immunology
University of Louisville School of Medicine

3:00 p.m. **Tea Break**
Harris Ballroom Poster Session

3:30 p.m. **Session 4: COBRE at the University of Oklahoma Health Sciences Center**
Worsham Theater Chair: Peter Zhou, MD, PhD
Ethnicity-Specific Differences in Ovarian Cancer Metabolic Signatures: Implications for Precision Cancer

Danny N. Dhanasekaran, PhD
Professor and Samuel Roberts Noble Foundation Endowed Chair in Cancer Research
Director, SCC-COBRE & Center for Basic Cancer Research
Deputy Director for Basic Research
Peggy and Charles Stephenson Cancer Center
University of Oklahoma Health Sciences Center

4:15 p.m. ***DCLK1 drives chemoresistance and alters metabolic pathways in ovarian cancer***

Bethany Hannafon, PhD
Assistant Professor
Department of Cell Biology
Peggy and Charles Stephenson Cancer Center
University of Oklahoma Health Sciences Center

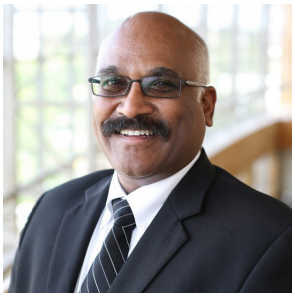
5:05 p.m. **Winner of Poster Award Announcement**
Worsham Theater

Peter Zhou, MD, PhD
Co-Director, COBRE in Cancer and Metabolism
University of Kentucky College of Medicine

5:15 p.m. **Concluding Remarks**
Worsham Theater

Daret St. Clair, PhD
Co-Director, COBRE in Cancer and Metabolism
University of Kentucky College of Medicine

Presenters



Danny Dhanasekaran, PhD

Dr. Danny Dhanasekaran obtained his Ph.D. in Biochemistry from the Indian Institute of Science. Following his postdoctoral studies at the University of Wisconsin–Madison and the National Jewish Center for Cancer Research and Respiratory Medicine, he became a tenured Professor at the Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine. He later joined the University of Oklahoma Health Science Center as a Professor of Cell Biology and assumed the Samuel Noble Foundation Endowed Chair in Cancer Research at the Stephenson Cancer Center. He also serves as the Director of MTCRO-COBRE and Associate Director for Basic Research at the Stephenson Cancer Center.

Dr. Dhanasekaran has received prestigious accolades including the Acres of Diamond Award and the Million Dollar Research Award from Temple University School of Medicine. He has held significant positions such as the WCU Professorship at Seoul National University in South Korea and Visiting Professorships at the University of Tokyo Medical and Dental University in Japan and the Università del Piemonte Orientale in Italy. In addition to his academic roles, Dr. Dhanasekaran served as a Charter Member of the NIH/CSR Study Section on Tumor Cell Biology and continues to contribute as a member on several grant review panels for esteemed organizations including the NIH, DOD, and AHA. He is actively involved as an editor and serves on the editorial board for several international journals.

Dr. Dhanasekaran's research focuses on analyzing transcriptomic and metabolomic variations in ovarian cancer patients with a specific interest in understanding their implications in precision cancer medicine. His work also explores the role of non-coding RNAs in ovarian cancer pathogenesis and their potential as diagnostic and therapeutic targets. By integrating omics data analysis with other molecular profiling techniques, Dr. Dhanasekaran aims to advance our understanding of ovarian cancer and develop personalized treatment approaches to improve patient outcomes.

Teresa Fan, PhD

Dr. Fan obtained her BSc in Public Health from National Taiwan University in 1977, MSc in Food Science from University of Hawaii in 1979, and PhD in Biochemistry from University of California, Davis in 1983. She established and directed the Center for Regulatory and Environmental Analytical Metabolomics (CREAM) in 2007 after joining the Department of Chemistry at University of Louisville. She then started the Center for Environmental and Systems Biochemistry (CESB) in 2014 after relocating to University of Kentucky. She pioneered the Stable Isotope-Resolved Metabolomics (SIRM) approach, which integrates state-of-the-art NMR spectroscopy with mass spectrometry (ultra high-resolution MS, in particular) to rigorously map metabolic networks via stable isotope tracer atom incorporation into numerous metabolites. It is applicable to any biological system including human subjects *in vivo* and cultured human tissues *ex vivo* to gain system-level knowledge on reprogrammed metabolism in response to disease development and stressors. This approach is well-accepted by the cancer/metabolic disease research community for the discovery of novel metabolic targets. She has recently expanded the SIRM approach from single to multiple tracers to widen the network coverage while reducing sample requirement and variable batch-to-batch artifacts. Dr. Fan's long-term goal is to translate the metabolic knowledge into effective chemoprevention and patient care.

Bethany Hannafon, PhD

Dr. Hannafon is an Assistant Professor in the Division of Gynecologic Oncology at Stephenson Cancer Center and the Department of Obstetrics and Gynecology at the University of Oklahoma Health Sciences Center. She is also an Adjunct Assistant Professor of Cell Biology at the University of Oklahoma Health Sciences Center. Dr. Hannafon's laboratory is actively exploring the cellular and molecular underpinnings of cancers that primarily affect women. Our lab seeks to understand the biology and improve the detection, prevention, and treatment of ovarian and breast cancers. Specifically, we aim to understand the molecular and cellular signaling events that drive cancer progression and therapeutic resistance. We are interested in understanding the mechanisms of chemoresistance, which results in tumor recurrence and poor outcomes for women with cancer. We are actively developing new therapeutic combinations aimed at overcoming this clinical challenge.

Kate Zaytseva, PhD

Dr. Zaytseva completed a bachelor's degree at Rostov-on-Don State University in Russia with a major in biology and a minor in chemistry. She started her career in cancer research during her graduate years at the University of Kentucky, Department of Biomedical Pharmacology. In 2010, she joined Dr. Mark Evers' group as a post-doctoral scholar at the Markey Cancer Center. Dr. Zaytseva was awarded an NCI K22 career development grant to investigate the contribution of lipid metabolism to colorectal cancer progression and metastasis as an independent investigator. Dr. Zaytseva is an Associate Professor in the Department of Toxicology and Cancer Biology at the University of Kentucky, and is funded by an NCI R01 grant to study lipid metabolism in colorectal cancer and an NIEHS UK-SRC grant to study the contribution of PFAS, the "forever chemicals", to gastrointestinal pathology including colorectal carcinogenesis.

Narasimhan Rajaram, PhD

Dr. Rajaram is an Associate Professor of Biomedical Engineering at the University of Arkansas and Director of the Metabolic Imaging and Spectroscopy Core of the Arkansas Integrative Metabolic Research Center. Dr. Rajaram's Laboratory for Functional Optical Imaging and Spectroscopy is focused on using non-ionizing light-based techniques to non-invasively investigate the tumor microenvironment to determine biomarkers of cancer progression, metastasis, and treatment resistance. He is a recipient of the National Science Foundation's Early Career Award (CAREER) and the Arkansas Biosciences Institute New Investigator of the Year. Dr. Rajaram's lab has received funding from the National Cancer Institute and the Department of Defense. Narasimhan Rajaram received his PhD in Biomedical Engineering from the University of Texas at Austin and completed postdoctoral training at Duke University.

Kyle P. Quinn, PhD

Dr. Quinn is a tenured Associate Professor of Biomedical Engineering at the University of Arkansas and Director of the NIH COBRE-funded Arkansas Integrative Metabolic Research Center (AIMRC). Dr. Quinn received his B.S. degree 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 a Ruth L. Kirschstein National Research Service Award and Pathway to Independence Award from the NIH. Since joining the Department of Biomedical Engineering at the University of Arkansas, his lab has been continuously funded by NIH and received an NSF CAREER award. In 2021, he established the AIMRC, which integrates optical imaging, bioenergetics, and data science approaches to solve biomedical research problems involving cell and tissue metabolism. 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 processes.

Kavitha Yaddanapudi, PhD

Dr. Yaddanapudi completed her post-doctoral work in immunology at Columbia University in New York and joined the University of Louisville's Brown Cancer Center. She is an Associate Professor of Surgery and Henry Vogt Endowed Chair in Immuno-Oncology in the Division of Immunotherapy at the University of Louisville. The goal of her research program is to develop novel immune-based strategies for the treatment of cancer by identifying and targeting mechanisms that tumors use to suppress the immune system. A major focus of the lab is on understanding the role of the tumor microenvironment in the regulation of immune responses with a specific focus on the role of cancer-driven pathological myelopoiesis. One group of cells is the myeloid-derived suppressor cells (MDSCs) that accumulate in tumor-bearing animals and in late-stage cancer patients. Her current research efforts are focused on unraveling what impact MDSCs might have on cancer immunotherapy since these cells negatively regulate anti-tumor activity, and on developing therapeutic strategies to overcome immune suppression.

Jun Yan, MD, PhD

Dr. Yan is a Professor and Chief of the Division of Immunotherapy in the Department of Surgery, and the Director of the Immuno-Oncology Program at 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 over 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 immune regulation. Recently, he has expanded his research to include more in-depth mechanistic studies on the tumor microenvironment (TME), with a focus on immune cell subsets that promote tumor progression and metastasis, aiming to develop effective interventions such as induction of trained immunity by natural compound ß-glucan for cancer therapeutics. He has published over 170 peer-reviewed papers and is currently supported by three R01s and an American Cancer Society Mission Boost Award.

His publications include: (1) Liu M, et al. Transcription factor c-Maf is a checkpoint that programs macrophages in lung cancer, *JCI 2020*;130:2081; (2) Morrissey SM, et al. Tumor-derived exosomes drive immunosuppressive macrophages in a pre-metastatic niche through glycolytic dominate metabolic reprogramming, *Cell Metabol* 2021;33:2040; (3) Geller AE, et al. The induction of peripheral trained immunity in the pancreas incites antitumor activity to control pancreatic cancer progression, *Nature Commun* 2022;13:759; (4) Chen X, et al. Differential metabolic requirement governed by transcription factor c-Maf dictates innate γδT17 effector functionality in mice and humans, *Sci Advances* 2022;8:eabm9120; and (5) Ding C, et al. Inducing trained immunity in pro-metastatic macrophages to control tumor metastasis, *Nature Immunol* 2023;24:239.

Alphabetical List of Abstracts

Poster Awards

Staff Employee/Post-doc		Student	
1st place	*$200*	*1st place*	*$200*
2nd place	*$100*	*2nd place*	*$100*
3rd place	*$50*	*3rd place*	*$50*

Abstracts

Abstract 1

Spatial MALDI-MSI Reveals Differential Glycogen Accumulation in Pediatric Neuroblastic Tumors

Lindsay Bryant[1], Michael Buoncristiani[1], Michelle Pitts[1,2], Beibei Zhu[1,2], Oscar Lopez-Nunez[3], Juan Gurria[4], Derek Allison[5], Nathan Shelman[5], Cameron Shedlock[2], Roberto Ribas[2], Shannon Keohane[2], Ramon Sun[2], Matt Gentry[2], B. Mark Evers[6], Eric J. Rellinger[1,6]

[1]University of Kentucky, Department of Pediatric Surgery
[2]University of Florida, Department of Biochemistry
[3]Cincinnati Children's Hospital and Medical Center, Department of Pathology
[4]Cincinnati Children's Hospital and Medical Center, Department of Pediatric Surgery
[5]University of Kentucky, Department of Pathology
[6]University of Kentucky, Markey Cancer Center

Introduction: Neuroblastoma (NB) is the most common extracranial solid tumor in children. MYCN-amplification metabolically transforms cancer cells and occurs in nearly half of high-risk NBs. Children with high-risk disease have poor survival despite aggressive multimodal treatments, thus highlighting the need to better understand mechanisms of NB progression and therapeutic resistance. Glycogen accumulation has an increasingly recognized role in cancer progression, invasion, and chemoresistance. Little is known about the role of glycogen in neuroblastoma progression. Herein, we used spatial metabolomics to quantify glycogen content across the spectrum of neuroblastic tumors.

Methods: We performed matrix-assisted laser desorption ionization mass spectroscopy imaging (MALDI-MSI) on 42 formalin-fixed, paraffin-embedded human samples including 5 adrenal glands, 5 ganglioneuromas, 5 ganglioneuroblastomas, 9 stage I NBs, 9 stage IV MYCN non-amplified NBs, and 9 stage IV MYCN-amplified NBs using isoamylase. Isoamylase was used for glycogen liberation and a mass spectrometer equipped with a Nd:YAG UV laser was used to detect glycogen release on X and Y coordinates. Regions of interest were defined by H&E overlaid on the MALDI-spectra. T-test was completed for comparisons between two groups. For comparisons between ≥2 groups, ANOVA was completed.

Results: All neuroblastic tumors featured lower levels of all glycogen polymers compared to normal adrenals. Glycogen chain lengths of 5-17 glucose units were identified with the 7-glucose unit polymer being most abundant. Neuroblastic cancers had 3 to 10-fold decreased of glycogen relative to normal adrenal glands. Stage IV neuroblastomas had the highest levels among the neuroblastic tumor (ganglioneuroblastomas, Stage 1, and Stage 4; $p \leq 0.0001$). Of the stage IV neuroblastomas, we observed a two-fold increase in glycogen abundance within MYCN-amplified neuroblastomas ($p \leq 0.0001$).

Conclusion: This in situ analysis of human NBs reveals glycogen accumulation is greatest within MYCN-amplified NBs. These findings invite further preclinical analysis into the role of glycogen accumulation in MYCN-amplified NB progression and therapeutic resistance.

Abstract 2

Porcupine Inhibition via LGK974 Enhances Drug-Resistant Prostate Cancer to Enzalutamide Therapy

Katelyn M. Jones, Xiaoqi Liu

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

Androgen receptor (AR) signaling continues to participate as a vital component of castration-resistant prostate cancer (CRPC). Subsequently, this has led to the development of Androgen Signaling Inhibitors (ASI), specifically Enzalutamide (ENZ), which is a direct inhibitor of AR, to clinically manage CRPC. Inevitably, ENZ treatment only provides improvement for approximately two months before advancing to an incurable form, ENZ-resistant CRPC. With prostate cancer (PCa) ranking as the second leading cause of cancer-related deaths in U.S. males, there is an urgency and necessity for the discovery and development of novel therapeutic approaches for CRPC. Wnt signaling has been extensively documented in its involvement in PCa and the tumor microenvironment (TME), however the mechanism of how the Wnt signaling cascades contribute to ENZ resistance is still ambiguous. Recently we have published that the activation of the canonical Wnt pathway contributes to the progression of ENZ resistance in CRPC and using a combination of β-catenin inhibitor with ENZ resulted in the synergistic inhibition of patient derived xenograft (PDX) tumor growth. Regarding the non-canonical Wnt pathway, we confirmed its contribution to invasion and migration which leads to metastasis in ENZ-resistant CRPC, and when the downstream effector ROCK1/2 is depleted and cells are treated with ENZ, there is a significant hindering of cell migration and invasion. Also, utilizing a combination therapy of ROCK1/2 inhibitor with ENZ synergistically inhibited the growth of PDX tumors. Hence the reasoning that by simultaneously inhibiting both the canonical and non-canonical Wnt signaling cascade will result in the inhibition of cell proliferation, migration, and invasion.

The objective of the proposed research is to determine how Porcupine (PORCN) is associated with CRPC progression to ENZ-resistance and develop a novel approach sensitizing ENZ-resistant CRPC to ENZ therapy, providing terminal patients with clinical options. The central hypothesis of this proposal is PORCN and Wnt signaling engage in a paramount role contributing to AR activation, promoting CRPC progression and development of ENZ resistance. My hypothesis will be validated by pursuing three Specific Aims: (1) Define the role of PORCN in Enzalutamide-resistant prostate cancer; (2) Define TME involvement in mediating ENZ resistance and promoting metastasis; (3) Inhibition of PORCN sensitizes ENZ-resistant PCa to ENZ treatment. The rationale is that by defining PORCN in autocrine and paracrine AR regulation, AR signaling potentially could be influenced by pharmacological inhibition of Wnt secretion, resulting in a novel approach to CRPC therapy. The contribution is significant because it will (i) define PORCN and Wnt signaling involvement in the progression of CRPC to ENZ-resistant CRPC; (ii) evaluate the TME contribution to advancing ENZ resistance; and (iii) introduce an effective therapeutic approach to ENZ-resistant CRPC, providing patients with alternative options. With cutting-edge techniques and exceptional mentorship that will enhance my knowledge in Wnt signaling, CRPC, and the development of mouse models, the research will be finished in a timely manner.

Abstract 3

Leveraging Artificial Intelligence in the Characterization of Non-Small Cell Lung Cancer

Erika M. Skaggs[1], Christine F. Brainson[2]

[1]University of Kentucky, Arts and Sciences, Chemistry
[2]University of Kentucky, College of Medicine, Toxicology and Cancer Biology

Adenocarcinoma (ADC) and squamous cell carcinoma (SCC) are the two most common presentations of lung cancer, accounting for a combined 65% of annual diagnoses. While both forms are classified as non-small cell (NSCLC), they are histologically distinct, and each is marked by a wide array of genetic, epigenetic, and morphologic variables which underpin their lethality. Unfortunately, this often makes them a moving target for oncologists, as the cells readily adapt to various treatment regimens. For example, adenocarcinomas can evolve more squamous phenotypes as a resistance mechanism to therapy. This "resistance through transition" is of great clinical relevance since SCC is significantly more aggressive and generally has fewer treatment options than ADC. However, the mechanisms which allow tumors to make the switch are still unclear, though one possible explanation is epigenetic reprogramming.

The focus of this experiment was to examine the role of a major epigenetic marker, the trimethylation of lysine 27 on histone 3 tails (H3K27me3), in NSCLC. Specifically, we sought to understand H3K27me3's ability to repress immune signaling, and how cystathionine β-synthase (CBS), a determinant of methyl availability and regulator of oxidative stress, might influence the cells capacity to methylate at the target loci. We hypothesized that tumors that are experiencing a higher oxidative stress burden will siphon away methyl groups via CBS and the glutathione pathway, ultimately leading to less trimethylation of H3K27 within the chromatin. To study this, we employed a novel investigatory technique which combined traditional immunohistochemistry (IHC) with an image-based artificial intelligence (AI) software. Using a tissue microarray containing samples from 216 NSCLC patients, we trained the AI to isolate tumor cells among the exceedingly diverse tumor micro-environment and analyze their IHC staining independent of the surrounding stroma. Six stains were evaluated: H3K27me3; EZH2, the methyltransferase responsible for H3K27me3; B2M, an immune marker representing MHC I; HLA-DR,DQ,DP, an immune marker representing MHC II, PD-L1, programmed death ligand; and CBS, a stress response molecule that alters methionine metabolism. Consistent with the theory of ADC to SCC transitions being driven by oxidative stress, results indicate that tumors which are mid-transition (as signified by adeno-squamous histology) display the highest CBS levels, followed by ADC, which have not yet begun to switch, and squamous tumors, which have already resolved their stress challenge. This is in contrast, however, with the correlation analysis of H3K27me3 and CBS. Current theory places CBS upstream of H3K27me3 in terms of methyl group availability, which would predict a negative association between the two. Yet the results here show a positive correlation, meaning that H3K27me3 may actually be the stronger driver, or that the ability of EZH2 to methylate at H3K27 may depend on the methyl donor concentration in specific cellular compartments. More investigation is needed to confirm these findings, but if successful, this project could eventually give clinicians much needed insight into how, when and why their patients undergo disease progression.

Abstract 4

Targeting Lipid Metabolism to Improve Efficacy of BRAF-Targeted Therapy in Colorectal Cancer

Mariah E. Geisen[1], Daheng He[2], Chi Wang[3], Jill M. Kolesar[4], Yekaterina Zaytseva[1]

[1]University of Kentucky, College of Medicine, Toxicology and Cancer Biology
[2]University of Kentucky, College of Medicine, Markey Cancer Center
[3]University of Kentucky, College of Medicine, Internal Medicine
[4]University of Kentucky, College of Pharmacy, Pharmacy Practice and Science

Introduction: Aberrant lipid metabolism is a hallmark of cancer associated with poor prognosis in colorectal cancer (CRC). Fatty acid synthase (FASN), a key enzyme of lipid synthesis, is overexpressed and a potential therapeutic target in CRC. Overexpression of CD36, a fatty acid translocase, is one of the mechanisms of compensation to FASN-targeted therapy and plays a pro-tumorigenic role in CRC. BRAFV600E is the mutation occurring in about 10-15% of CRC cases. BRAF-targeted therapy is effective, but quickly developed resistance is an issue. Our preliminary data show that development of resistance to BRAF-targeted therapy is associated with an increase of expression of FASN, CD36, accumulation of triglycerides (TGs) and mitochondrial respiration. Therefore, our central hypothesis is that inhibition of lipid metabolism will sensitize CRC cells to BRAF inhibitors and overcome acquired resistance.

Methods: We established HT29 cells and primary PT130 and PT2449pt cells resistant to PLX8394, a novel BRAF inhibitor. IC50 curves, PrestoBlue viability, CytoSelect™ 24-Well Cell Invasion, and Triglyceride Assays, Seahorse XF analysis, western blot, and confocal microscopy were used to evaluate differences between parental and resistant cells. RNA-seq and lipid analysis are used to evaluate changes in gene expression and lipids levels. A combination of PLX8394 and TVB3664 (FASN inhibitor) was tested on cell viability in parental and resistant cells.

Results: PLX8394 resistant cells have a higher IC50, increased cellular proliferation and invasion than parental cells. An increase in invasion of resistant cells is associated with a decrease in e-cadherin. RNA-sequencing show a significant increase in FASN expression. Western blot analysis of resistant cells confirm upregulation of FASN, CD36 and other lipogenic markers. Consistently, resistant cells have an increase in levels of triglycerides as compared to parental cells. Seahorse XF Cell Mito Stress Test shows that resistant cells forgo the Warburg effect and instead rely more heavily on oxidative phosphorylation. To further support our hypothesis, FASN shRNA knockdown of FASN in parental HT29 cells were more susceptible to PLX8394 treatment compared to control. We also observed the synergetic effect of PLX8394 and TVB3664 treatment on cell viability in parental cells, but not in resistant cells.

Conclusion: Our study demonstrates that resistance to BRAF inhibitors is associated with a significant increase in proliferation, metastasis, and upregulation of lipid metabolism. We show that a combination of FASN and BRAF inhibitors has a combinational effect on inhibiting cell viability in parental but not resistant cells, suggesting that the addition of FASN inhibitor to the standard regimen for BRAFV600E mutation positive patients can improve the efficacy of these therapies. Additional screening of lipid metabolism-targeted therapies in combination with standard BRAF regimens are needed to develop novel and more efficacious strategies for CRC patients with BRAF mutations.

Abstract 5

Investigating the Roles of LKB1 in Lung Tumorigenesis

Kassie J. Naughton, Xiulong Song, Christine F. Brainson

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

Two major subtypes of non-small cell lung cancer are adenocarcinoma (ADC) and squamous cell carcinoma (SCC), which are vastly different histologically, and require divergent treatment strategies. In ADCs, tumors harboring mutations in both KRAS and LKB1 (aka STK11) have lower survival rates than the KRAS-only tumors. These tumors are not only aggressive, but also respond poorly to immunotherapy, reducing the number of available effective therapies. However, this data is limited to only ADCs, and it is unclear if tumors of this genotype with SCC characteristics are also resistant to immunotherapy. We developed a mouse model of Kras/Lkb1 capable of producing aggressive ADCs, and a proportion of tumors can transition to a SCC state driven by a reduction in Polycomb Repressive Complex 2 (PRC2) activity. PRC2 requires S-adenosyl methionine to methylate histones, indicating an important role for methionine metabolism in this lineage switch. Therefore, we predict that methionine restriction will enhance treatment efficacy in KRAS/LKB1 mutant NSCLCs via alterations to PRC2 activity. From our Kras/Lkb1 mouse model, we have developed two tumoroid models: one ADC (3690) and one SCC (3650). Characterization of these tumoroids have shown higher expression of SCC markers in the 3650s (Sox2 and Krt5) and higher expression of ADC markers in the 3690s (Ccsp and Spc). In addition, when treated with a combination of EZH2 (of PRC2) inhibitor (EPZ-6438) and IFN-gamma, we saw increases in PD-L1, MHCI, and MHCII in the 3650s but not in the 3690s. This indicates an important role for histology of a tumor in immunotherapy response, driven by PRC2 methylation status. Furthermore, we are investigating the role of methionine metabolism in these Kras/Lkb1 mice via methionine diet restriction. We found that dietary methionine restriction, started a week after tumor initiation, significantly reduces tumor number and size. In addition, dietary methionine restriction in concert with carboplatin treatment decreases tumor burden in our Kras/Lkb1 mice. The tumor immune microenvironment (TIME) in these mice on restricted methionine diets is also being investigated. We have found that in mice on a restricted methionine diet developed tumors with more macrophages and fewer neutrophils. This change in the TIME of the mice on the restricted methionine diet could indicate these mice may respond differently to immunotherapy than those on a regular methionine diet. However, more data is needed to determine if these TIME cells are tumor-promoting or -eliminating and how it could impact immunotherapy treatment efficacy. These investigations will provide a greater understanding of how methionine restriction regulates PRC2 activity, as well as aid in determining which NSCLC histology may respond more positively to specific treatments. Work funded by American Cancer Society 133123-RSG-19-081-01-TBG, R01 CA237643 and American Institute for Cancer Research, and P30 CA177558 for MCC Shared Resources.

Abstract 6

ICAM-1-suPAR-CD11b Axis is a Novel Therapeutic Target for Metastatic Triple-Negative Breast Cancer

Dong Li[1], Hami Hemati[1], Younhee Park[1], Rokana Taftaf[2], Youbin Zhang[2], Jinpeng Liu[3], Massimo Cristofanilli[2], Xia Liu[1]

[1]University of Kentucky, College of Medicine, Department of Toxicology and Cancer Biology
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Accumulating evidence demonstrates that circulating tumor cell (CTC) clusters have higher metastatic ability than single CTCs and negatively correlate with cancer patient outcomes. Along with homotypic CTC clusters, heterotypic CTC clusters (such as neutrophil–CTC clusters), which have been identified in both cancer mouse models and cancer patients, lead to more efficient metastasis formation and worse patient outcomes. However, the mechanism by which neutrophils bind to CTCs remains elusive. In this study, we found that intercellular adhesion molecule-1 (ICAM-1) on triple-negative breast cancer (TNBC) cells and CD11b on neutrophils mediate tumor cell–neutrophil binding. Consequently, CD11b deficiency inhibited tumor cell–neutrophil binding and TNBC metastasis. Furthermore, CD11b mediated hydrogen peroxide (H_2O_2) production from neutrophils. Moreover, we found that ICAM-1 in TNBC cells promotes tumor cells to secrete suPAR, which functions as a chemoattractant for neutrophils. Knockdown of uPAR in ICAM-1+ TNBC cells reduced lung-infiltrating neutrophils and lung metastasis. Bioinformatics analysis confirmed that uPAR is highly expressed in TNBCs, which positively correlates with higher neutrophil infiltration and negatively correlates with breast cancer patient survival. Collectively, our findings provide new insight into how neutrophils bind to CTC to facilitate metastasis and discover a novel potential therapeutic strategy by blocking the ICAM-1-suPAR-CD11b axis to inhibit TNBC metastasis.

Abstract 7

Different Metabolic Changes Captured by Optical Image Techniques at Radioresistant and Radiosensitive Head and Neck Squamous Cell Carcinomas under Radiation Stress

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Radiotherapy (RT) has emerged as one of the most popular treatments for head and neck squamous cell carcinoma (HNSCC). However, over half of RT-treated patients with advanced local HNSCC tumors will not respond to RT, which leads to an increased patient death rate. Hypoxia is a common condition observed in solid tumors and Hypoxia-inducible factor 1 (HIF1) has been shown to be associated with RT resistance. Because of the high recurrence and death rates for radioresistant HNSCC patients, it becomes significant to understand the mechanisms involved in tumor cell resistance acquisition to develop improved radiation for HNSCC patients. Using optical imaging techniques, we identified metabolic changes in the radioresistance development, using radioresistant (rSCC-61) and radio-sensitive (SCC-61) HNSCC cells under radiation stresses. Specifically, we used glucose analog (2-NBDG) and tetramethyl rhodamine ethyl ester (TMRE) to image glucose uptake and mitochondrial membrane potential, respectively, to report the metabolic changes between rSCC-61 and SCC-61 cells under radiation stress with or without Hypoxia-Inducible Factor 1-alpha (HIF-1α) inhibition. We observed that the two HNSCC cell lines have different responses under RT stress. After 4Gy irradiation, both cell lines showed increased 2-NBDG uptake. Interestingly, in rSCC61 the TMRE staining was decreased after RT in the opposite way to SCC61. This suggests the radioresistant cell line has a decreased mitochondrial membrane potential activity and consequently decreased Krebs cycle after irradiation, even with the increased glucose uptake. At the same conditions, we observed that HIF1a protein expression increased significantly on rSCC-61 compared to the baseline condition, a pattern not observed at SCC61. Once incubated with YC-1, a HIF1a inhibitor, the different responses under RT stress were not observed, being similar to baseline conditions. Those results suggested that the higher HIF1a recruitment is responsible, at least in part, for the metabolic changes after radiation and possibly the radioresistance acquisition, in our model. The present study presents that the optical imaging technique can be a useful tool to observe metabolic changes in HNSCC cells under radiation stress, thereby acting as an efficient and non-destructive strategy to study the role of metabolism reprogramming in RT resistance development.

Abstract 8

Plk1 Phosphorylation of PHGDH to Regulate Serine Metabolism

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Polo-like kinase 1 (Plk1) has been reported to be highly expressed in most tumors, especially in advanced tumors, but how Plk1 elevation benefits tumor growth remains elusive. Metabolic reprogramming is one of the hallmarks of cancer, but how the tumors fully take advantage of deregulated metabolism is an enigma. Here, we found that Plk1 could divert serine metabolism from de novo synthesis to exogenous uptake by regulating PHGDH (phosphoglycerate dehydrogenase), the first rate-limiting enzyme of de novo serine biosynthesis. We show that PHGDH is phosphorylated by Plk1 at a cluster site (S512, S513, S517) and that Plk1-associated phosphorylation of PHGDH results in its protein degradation, thus reduced de novo serine biosynthesis. As a compensatory response, cells with an elevated level of Plk1 significantly increase the uptake of serine to produce more sphingosines, which are pivotal metabolites for the growth of cancer cells. Our finding may provide guidance on how to target de novo biosynthesis of serine, serine uptake or sphingosine metabolism to treat advanced prostate cancer.

Abstract 9

Targeting RSK-TRIM28-LDHA Axis in Neuroendocrine Prostate Cancer

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New generation androgen deprivation therapy (ADT) that target AR activity such as abiraterone and enzalutamide have been introduced in the clinics for treating metastatic castration-resistance prostate cancer (CRPC). Despite significant and often durable response, patients ultimately develop resistance to these AR-directed strategies. Around 15-20% of treatment resistance case is mediated by neuroendocrine prostate cancer (NEPC) which becomes complete AR independence. It has been suggested that NEPC develops as a result of lineage plasticity driven by loss of tumor suppressors RB1 and TP53, gain of lineage determinant transcription factors combined with significant epigenetic changes. To date, the only treatment regimen for confirmed or suspected NEPC is platinum-based chemotherapy similar to those employed for the treatment of other neuroendocrine small-cell carcinomas. Unfortunately, this regimen does not lead to long-term remission and carries significant toxicity. There is unmet need to develop for individualized targeted therapy for effective NEPC treatment. TRIM28 (tripartite motif containing 28) is an epigenetic regulator which plays a pivotal role in controlling gene expression. We previously reported that TRIM28 is aberrantly upregulated in CRPC and promotes disease progression. However, the role of TRIM28 in NEPC has yet to be explored. Our exciting preliminary study has uncovered that TRIM28 expression is upregulated in NEPC, and TRIM28 is phosphorylated by RSK1 at S473. Using epigenomic and metabolomic approaches, we have demonstrated that pS473-TRIM28 promotes the transcriptional activation and thereby function of metabolic enzyme, lactate dehydrogenase-A (LDHA). Our central hypothesis is RSK1 phosphorylates TRIM28 at S473 and pS473-TRIM28 level is elevated in NEPC, which promotes transcriptional activation and thus the function of LDHA, eventually contributing to NEPC growth and metastasis. Pharmacological inhibition of RSK and LDHA will impose a much more pronounced therapeutic effect on NEPC progression.

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