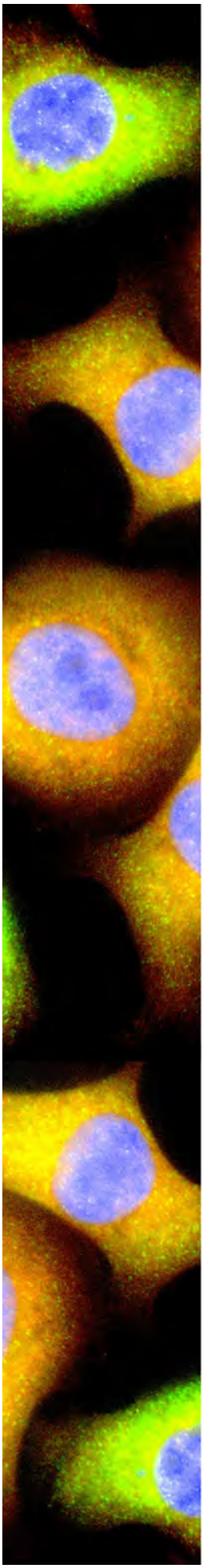

HealthCare
MARKEY CANCER CENTER
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MARKEY CANCER CENTER
RESEARCH DAY

PRESENTED BY THE MARKEY CANCER FOUNDATION

Discover the Latest Advances in Cancer Research

May 10, 2022 | 8:30 am – 5:00 pm
UK Gatton Student Center

HealthCare
MARKEY CANCER CENTER
An NCI-Designated Cancer Center

May 10, 2022

Dear Colleagues and Friends,

It's great to be back to our in-person event – after a two-year hiatus because of the COVID-19 pandemic. While grateful that we could conduct the 2021 event online, nothing compares to being able to see and hear each other in the same room. This year, the "room" has changed: The UK Gatton Student Center offers an expanded space for our event. The Grand Ballroom gives us plenty of room to present posters; the Worsham Theatre allows for a more immersive experience with our oral presentations; and the Harris Ballroom gives us a larger, brighter space for our Exhibit Hall and food service.

One thing stays the same this year: Our twelfth-annual Markey Cancer Center Research Day, remains *the* one-day event showcasing the work of researchers from myriad disciplines at the University of Kentucky. This year, you can see 97 reasons why "Markey Makes a Difference" as you peruse posters representing all aspects of cancer research, prevention and control, treatment and clinical care.

The Faculty Oral Presentations will feature two of Markey's leading scholars, and the morning Oral Presentations offer one graduate student, one research associate and one resident physician the opportunity to share their work.

Two keynote speakers highlight the day: Ann Richmond, PhD, Ingram Professor of Cancer Biology, Vanderbilt University School of Medicine, will present the Susan B. Lester Memorial Lecture; and Vanessa Sheppard, PhD, Professor and Chair, Health Behavior and Policy & Associate Director, COE and Health Disparities, Massey Cancer Center/Virginia Commonwealth University, will present the second-annual Gilbert H. Friedell, MD, Memorial Lecture.

Please join me in thanking our exhibitors, advertisers and the UK Markey Cancer Foundation for their support, not only of this event but for our educational mission across the University of Kentucky campus.

Enjoy the day, and please let me know your comments regarding this year's event as well as suggestions for future Markey Cancer Center events.

Sincerely

B. Mark Evers

B. Mark Evers, MD

MARKEY CANCER CENTER
RESEARCH DAY
PRESENTED BY THE MARKEY CANCER FOUNDATION

Program Contents

HealthCare
MARKEY CANCER CENTER
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About the Cover

Immunocytochemical analysis of cellular localization of Par-4 and vimentin. PC-3 prostate cancer cells were fixed and co-stained with antibodies against Par-4 (red fluorescence) and vimentin (green fluorescence). The presence of yellow color indicates the colocalization of the two proteins. DAPI staining was used to reveal cell nuclei (cyan fluorescence). The image was captured by Ravshan Burikhanov using the Nikon confocal microscope provided by the Markey Cancer Center.

Agenda
Markey Cancer Center Research Day 2022

Morning

7:45	Check-In/Onsite Registration (second floor)
	Continental Breakfast sponsored by **Specialty Underwriters** (Harris Ballroom, third floor)
8:30	Welcome Session (Worsham Theatre, second floor)
8:45 – 9:00	**Sumati R. Hasani**, MS, Graduate Student, Oral Presentation Awardee "Activation of Drp1 promotes fatty acids-induced metabolic reprogramming to potentiate Wnt signaling in colon cancer"
9:00 – 9:15	**Jennifer T. Castle**, MD, Surgery Resident, Oral Presentation Awardee "Inhibition of De Novo and Salvage Pathways for dNTP Synthesis Enhances Sensitivity to Ionizing Radiation in Pancreatic Neuroendocrine Tumor Cells"
9:15 – 9:30	**Yanquan Zhang**, PhD, Scientist, Oral Presentation Awardee "Taxol-elevated PLK1 Overcomes BETi-Resistant in Prostate Cancer via Triggering Phosphorylation-dependent Degradation of BRD4"
9:30 – 10:00	**Ren Xu**, PhD, Faculty, Oral Presentation "ECM Network in Breast Cancer Progression"
10:00 – 10:30	**Pamela C. Hull**, PhD, Faculty Oral Presentation "Improving HPV Vaccination for Cancer Prevention"
10:30 – 11:45	Poster Presentation #1 (Grand Ballroom)
11:30 – 1:00*	Lunch for pre-registered guests sponsored by **Jazz Pharmaceuticals** (Harris Ballroom, third floor)
*11:30 – 1:00	Exhibit Hall Hours (Harris Ballroom)

Afternoon

12:15 – 1:30	Poster Presentation #2 (Grand Ballroom)
1:30 – 2:30	Gilbert H. Friedell, MD, Memorial Lecture (Worsham) ***Vanessa B. Sheppard****, PhD, Professor and Chair, Health Behavior and Policy Associate Director, COE and Health Disparities, Massey Cancer Center/Virginia Commonwealth University*
2:30 – 2:40	Break
2:40 – 2:45	Markey Women Strong Research Award Presentation ***Katie Alford****, Founding Member, Markey Women Strong*
2:45 – 3:00	State of the Cancer Center Address ***B. Mark Evers****, MD, Director, Markey Cancer Center* *Introduction by Farra Alford, Chair, UK Markey Cancer Foundation*
3:00 – 4:00	Susan B. Lester Memorial Lecture ***Ann Richmond,*** *PhD, Ingram Professor of Cancer Biology Vanderbilt University School of Medicine*
4:05 – 4:15	Awards Presentation
4:15 – 5:30	Reception sponsored by **Abbvie** (Harris Ballroom)

Morning Oral Presenters

Sumati R. Hasani, MS
PhD Candidate
Molecular and Cellular Biochemistry, College of Medicine

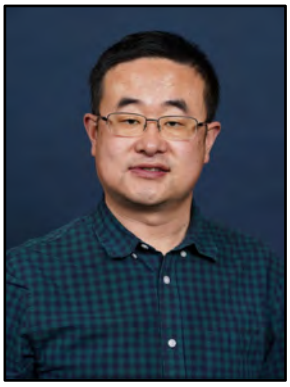
"Activation of Drp1 promotes fatty acids-induced metabolic reprogramming to potentiate Wnt signaling in colon cancer"

Sumati Hasani is a 4th Year PhD candidate in Dr. Tianyan Gao's laboratory at the Department of Molecular and Cellular Biochemistry in the College of Medicine. Her research interrogates the role of mitochondrial dynamics in colorectal cancer (CRC) signaling and metabolism. More specifically, she is interested in delineating the mechanisms by which CRC cells capitalize on fatty acid uptake to stimulate mitochondrial fission, leading to the activation of fatty acid metabolism and downstream oncogenic pathways. By exploring the crosstalk between cancer signaling and metabolism, Sumati hopes to obtain a mechanistic insight into how increased fatty acid metabolism functions as a tumor promoting factor in patients with poor prognosis. Sumati completed her BS degree at the University of California, Davis and has received her Master of Science degree from Georgetown University in Washington DC.

Jennifer T. Castle, MD
Resident Physician, General Surgery, College of Medicine
T32 Postdoctoral Fellow, Markey Cancer Center, College of Medicine
PhD Candidate, Clinical and Translational Sciences, UKCCTS

"Inhibition of De Novo and Salvage Pathways for dNTP Synthesis Enhances Sensitivity to Ionizing Radiation in Pancreatic Neuroendocrine Tumor Cells"

Dr. Jennifer T. Castle earned her medical degree at the University of Kentucky where she discovered a love for operating and has since continued her education at the university as a general surgery resident. She currently is a post-doctoral fellow in Dr. B. Mark Evers's lab and a PhD candidate in the Clinical and Translational Sciences program. Her research is focused on sensitizing neuroendocrine tumors to radiation therapy and delineating the mechanisms of resistance to these regimens. In particular, she is interested in discovering synergistic drug combinations that enhance efficacy and allow for lower concentrations to be used with radiation therapy. Her goal is to reduce the clinical toxicities of these drugs in patients by capitalizing on their synergy, and, thus, decrease the concentrations needed to treat these tumors.



Yanquan Zhang, PhD
Scientist I
Toxicology and Cancer Biology

"Taxol-elevated PLK1 Overcomes BETi-Resistant in Prostate Cancer via Triggering Phosphorylation-dependent Degradation of BRD4"

Dr. Yanquan Zhang is a Scientist in Dr. Xiaoqi Liu's laboratory in the Department of Toxicology and Cancer Biology, College of Medicine. He obtained his PhD in Biology Science at Tsinghua University, Beijing, China; and he has received his postdoctoral training at the Chinese University of Hong Kong and the University of Kentucky. His research focuses on understanding the kinase- and phosphorylation-dependent regulation of prostate cancer development and treatment response. In particular, he is interested in determining the role of PLK1, an oncogenic kinase in prostate cancer, by identifying novel substrates. Dr. Zhang's studies have discovered that BRD4 is directly phosphorylated by PLK1 and PLK1-dependent phosphorylation triggers BRD4 degradation. Importantly, treatment with Taxol, an FDZ-approved chemotherapy drug, overcomes resistance to BRD4 inhibitor both *in vitro* and *in vivo* as a consequence of increased PLK1 expression. Identification of PLK1 as a novel regulator of BRD4 provides a rationale for combining Taxol and BRD4 inhibitor in treating prostate cancer.

Faculty Oral Presenters

Ren Xu, PhD
Professor, Pharmacology and Nutritional Sciences

"ECM Network in Breast Cancer Progression"

Dr. Ren Xu is a professor in the UK School of Medicine's Department of Pharmacology and Nutritional Sciences. His research focuses on the function and regulation of tumor microenvironment, especially extracellular matrix (ECM), in breast cancer development and progression. He received extensive training in mammary gland biology and breast cancer research at the Lawrence Berkeley National Laboratory prior to the faculty appointment at UK Markey Cancer Center. Dr. Xu demonstrated for the first time that ECM remodeling is regulated at the transcription network levels during cancer progression. He identified several hubs, such as Hsp47 and collagen hydroxylation enzymes, from the network as potential therapeutic targets for breast cancer utilizing *ex vivo* and *in vivo* mammary tumor models (*Cancer Res*, 2012; *Breast Cancer Res*, 2018; *Nature communications*, 2018). His laboratory recently demonstrated novel function of Hsp47 in regulating CTC-platelet interaction and cancer metastasis by facilitating collagen secretion in cancer cells (*Cancer Res*, 2015; *PNAS*, 2020). These results indicate the crucial role of cancer cell-derived collagen in CTC survival and colonization. More recent data from his team revealed a novel link between adipocyte-derived Hsp47 and HFD-induced obesity and mammary tumor progression. His current research is funded by multiple NIH R01s. Dr. Xu has published more than 50 peer-reviewed articles and filed five patent applications. He has served on more than 30 review panels and is currently a regular member of the NIH TME study section.

Pamela C. Hull, PhD
Associate Director, Population Science and Community Impact
Associate Professor, Behavioral Science
William Stamps Farish Endowed Chair in Cancer Research

"Improving HPV Vaccination for Cancer Prevention"

Dr. Pamela Hull is a medical sociologist with expertise in the development, dissemination and implementation of behavioral and health service interventions to promote cancer prevention behaviors. Her research focuses on the implementation of evidence-based practices for cancer prevention and control, including HPV vaccination and obesity prevention, using implementation science and technology-based applications. She has over 17 years of experience in conducting community-engaged research with a focus on reducing health disparities among Hispanic, African American and low-income populations, in collaboration with community partners. She leads a National Cancer Institute (NCI)-funded R01 implementation science study focused on increasing HPV vaccination among adolescents in community-based pediatric primary care practices. She also leads the USDA-funded Children Eating Well (CHEW) grant, which focuses on a mobile phone application designed for early childhood obesity prevention among low-income families with preschool-aged children. In previous positions, Hull served as associate director of community outreach and engagement for the Vanderbilt-Ingram Cancer Center and held a faculty position in the Vanderbilt University Medical Center. She also served as the associate director of the Center for Prevention Research at Tennessee State University in Nashville. Dr. Hull earned her undergraduate degree in sociology from Duke University and completed both a master's degree and doctorate in sociology from Vanderbilt University.

Susan B. Lester Memorial Lecture

About Susan B. Lester

The family and friends of Susan B. Lester endowed a lectureship in her honor, and it is this generous donation that makes Markey Cancer Research Day possible. Mrs. Lester left an indelible mark on her world, serving as a clinical dietician for Eastern State Hospital and for nursing home patients in Eastern and Central Kentucky. This symposium benchmarks recent advances in cancer research and thus honors Mrs. Lester by underscoring both her generous life and the brave battle she fought against this disease.

Ann Richmond, PhD

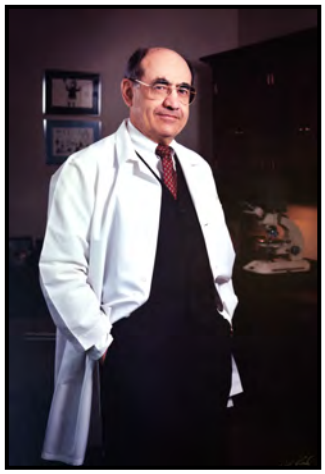
Ingram Professor of Cancer Biology
Vanderbilt University School of Medicine

Dr. Richmond received her Bachelor of Science degree from Northeast Louisiana University, her Master of Natural Sciences degree from Louisiana State University and her PhD in Developmental Biology at Emory University in 1979. She conducted postdoctoral research in Tumor Biology at Emory and then joined the faculty there, rising to the rank of associate professor of medicine before moving to Vanderbilt in 1989 as tenured associate professor of cell biology and medicine and as a research career scientist at the U.S. Department of Veterans Affairs Nashville campus. She was promoted to full professor in 1995, and she was appointed professor and vice chair of the Department of Cancer Biology in 2000.

Dr. Richmond is internationally known for her research on chemokines, small "chemotactic" proteins that attract inflammatory cells. She was the first to demonstrate that a chemokine can regulate tumor growth. Her early research involved purification and sequencing of one of the first known chemokines, CXCL1, and her lab played a major role in characterization of the role of its receptor, CXCR2, in leukocyte trafficking, inflammation, angiogenesis, wound healing and tumor progression. She and her colleagues helped elucidate the role that inhibitor of kappa-beta kinaseβ (IKKβ), an activator of the transcription factor NF-κB, plays in chemokine expression and melanoma cell survival, suggesting that IKKβ may be a potential target for melanoma therapy. They also have shown that targeting the NF-κB/IL-6/STAT3 pathway is a rational strategy for treating angiosarcoma.

Dr. Richmond's body of work — more than 200 publications cited by other scientists more than 10,000 times – has shed light on how the inflammatory process, combined with other genetic and environmental factors, contributes to tumor progression and metastasis. A goal of her research is the advancement of "personalized cancer therapy" — determining which genes are mutated or amplified in individual tumors and delivering drugs that specifically inhibit the activity of those genes. Antagonizing chemokine receptors may provide new therapeutic options. Toward that end, she and her colleagues are working to learn more about the effects of therapy on the tumor microenvironment, including the development of drug resistance.

Gilbert H. Friedell, MD, Lecture



About Gilbert H. Friedell, MD

In 1983, Dr. Friedell became the first director of the UK Markey Cancer Center, beginning a legacy of cancer care that continues to grow and make a difference in the lives of Kentuckians every day. At Markey, he co-founded the Kentucky Cancer Registry – now one of the premiere SEER databases in the country – and served as the principal investigator of the National Cancer Institute's Mid-South Cancer Information Service, a cancer education program that provides easy-to-understand information for cancer patients, survivors, health care providers and more.

Dr. Friedell was a passionate advocate for programs that provided education and increased access to healthcare for the medically underserved, particularly in Appalachian Kentucky. He famously believed that "If the problems are in the community, the solutions are in the community." With this in mind, he helped launch Kentucky Homeplace, an initiative that has linked tens of thousands of rural Kentuckians with medical, social and environmental services since it began in 1994.

Though he retired from UK in 2000, Dr. Friedell's influence is still felt strongly in the overarching mission of our cancer center: to conquer cancer in the Commonwealth. We at Markey are proud to uphold the vision and values of Dr. Friedell, building upon his contributions to public health as we continue to care for Kentuckians with cancer.

Vanessa B. Sheppard, PhD
Professor and Chair, Health Behavior and Policy
Associate Director, COE and Health Disparities
Massey Cancer Center/Virginia Commonwealth University

Dr. Vanessa B. Sheppard is professor and the Theresa B. Thomas Memorial Foundation Chair in Cancer Prevention and Control at Virginia Commonwealth University (VCU). At VCU, Dr. Sheppard serves as chair of the Department of Health Behavior and Policy in the School of Medicine and as the inaugural associate center director for Community Outreach Engagement and Health Disparities at the VCU Massey Cancer Center. She also has a fully funded lab that is focused on improving breast cancer outcomes for women from racial/ethnic minority backgrounds. Her long-term career goal is to reduce inequities in cancer outcomes through scholarship, teaching and service. She has harnessed her expertise in health services research, clinical trials and behavioral interventions to address gaps in care for African-American, African-immigrant and Latina populations. Dr. Sheppard has led studies focused on factors that have potential to improve cancer outcomes such as treatment adherence, patient-provider relationships, obesity and physical activity. Her team was the first to develop and test decision support and lifestyle interventions for African-American and Latino women. Prior to joining VCU, Dr. Sheppard was a tenured associate professor at Georgetown University in the Department of Oncology and was the assistant director of health disparities research at the Lombardi Comprehensive Cancer Center.

Exhibit Hall – Harris Ballroom

Visit our exhibitors in the Harris Ballroom (UKGSC Room 330) between 11:30 am and 1 pm.

Booth	Company
1	Abbvie
2	Jazz Pharmaceuticals
3	Specialty Underwriters
4	Nikon Instruments, Inc.
5	Eppendorf
6	Caris
7	Amgen
8	New England Biolabs
9	Merck

Booth	Company
10	Novogene
11	Roche
12	Regeneron
13	Seattle Genetics
14	Agilent – Cell Analysis
15	Agilent – Analytical Sciences
16	VWR
17	UK Federal Credit Union

For Exhibitor and Advertiser contact information, see next page.

Please extend your appreciation to our event sponsors:

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Reception and Food Breaks by:

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Exhibitor and Advertiser Contact Information

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15	Agilent - Analytical Sciences	www.agilent.com	Mike Purcell	513-702-4007	mikeanalytical@gmail.com
14	Agilent - Cell Analysis	www.agilent.com	Steve Kraynik	513-687-4022	steve.kraynik@agilent.com
Ad	American Welding & Gas	www.awggases.com	Angie Radebaugh	859-737-8142	angie.radebaugh@awggases.com
7	Amgen	www.amgen.com	Alison Price	859-229-0408	aprice01@amgen.com
7	Amgen	www.amgen.com	Jonathan Jordan	805-447-1000	jojordan@amgen.com
6	Caris	www.CarisLifeSciences.com	Diane Warner	502-645-8117	dwarner@carisls.com
6	Caris	www.CarisLifeSciences.com	Andrew Thieman	513-568-4011	athieman@carisls.com
5	Eppendorf	www.eppendorf.com	Jennifer Birchmore	615-712-1271	birchmore.j@eppendorf.com
2	Jazz Pharmaceuticals	www.jazzpharma.com	Chris Glasgow	614-600-9472	chris.glasgow@jazzpharma.com
9	Merck	www.merck.com	David Yount	859-338-6281	david.yount@merck.com
8	New England Biolabs	www.neb.com	Heidi Iuvino	716-777-0579	iuvino@neb.com
4	Nikon Instruments	www.nikoninstruments.com	Kelley Bevis	613-547-4331	kelley.bevis@nikon.com
4	Nikon Instruments	www.nikoninstruments.com	Fay Munro	513-473-7738	fay.munro2@nikon.com
10	Novogene	www.novogene.com	Kari Thomas	916-252-0068	kari.thomas@novogene.com
Ad	Precision Air Technology	www.precisionairtechnology.com	Customer Service	919-812-0340	info@precisionairtechnology.com
15	Quanta Bio	www.quantabio.com	Stephen Doore	410-340-0507	stephen.doore@quantabio.com
12	Regeneron	www.regeneron.com	Jacky Space	859-333-6004	jacqueline.space@regeneron.com
11	Roche	www.usdiagnostics.roche.com	Tony Morris	520-906.2982	tony.morris@roche.com
13	Seattle Genetics	www.seagen.com	Tony Berry	859-808-0115	tberry@seagen.com
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16	VWR	www.vwr.com	Riley Sigler	859-321-7764	riley.sigler@avantorsciences.com
16	VWR - Life Sciences	www.vwr.com	Kaylagh Hollen	562-825-8822	kaylagh.hollen@avantorsciences.com

Prize Giveaway Instructions

Visit the Exhibit Hall in the UKGSC Harris Ballroom for a chance to win one of the great prizes listed below – including the grand prize, a set of **Apple AirPods**, provided by **Specialty Underwriters**!

Instructions:

1. Go to Booth "G" to receive up to four tickets per person.
2. Print your name clearly on the ticket(s).
3. Visit the vendor booths and receive one stamp per visit per ticket.
4. Fill up all five boxes with stamps from five different vendors on a ticket and get a chance to win any of our fantastic prizes.

Drawings from all entrants will be announced during the Wine and Cheese Reception in the Harris Ballroom immediately following the Awards Presentation.

One prize per person. Must be present to win.

Grand Prize:

- **(1) Apple AirPods** provided by Specialty Underwriters

Exhibitor Prizes:

- **(1) Nikon Backpack & Yeti mug** provided by Nikon Instruments Inc.
- **(1) Gift Basket** provided by New England Biolab
- **(1) Swag Bag & $100 gift card** provided by VWR/Quanta
- Check at Booth "G" for any last-minute additions!

Sample Prize Ticket:

Markey Cancer Center Research Day Prize Ticket
Get stamps from FIVE different vendors for a chance to win!

Exhibitor Stamp Goes Here	Exhibitor Stamp Goes Here	Exhibitor Stamp Goes Here	Exhibitor Stamp Goes Here	Exhibitor Stamp Goes Here

PRINT name: _____

Return stamped ticket to Booth "G" at the Exhibit Hall Entrance.

Alphabetical List of Abstracts

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Abstract 1

Population-Based/Behavioral

Assessing Cancer Literacy and Risk Behaviors among Appalachian Kentuckians through an Oral History Approach

Courtney C. Martin[1], Nathan L. Vanderford[2]

[1]UK College of Arts & Sciences, Biology; [2]UK College of Medicine, Toxicology & Cancer Biology

Kentucky ranks first in the nation in cancer incidence and mortality with the greatest of these disparities being found in the Appalachian region of the state. Using an oral history approach, we aimed to better understand cancer in the region through individual perspectives. The purpose of this study was to assess cancer literacy and health behaviors and examine how these factors may be contributing to the cancer epidemic. Using convenience sampling, we recruited individuals from within the community that identified as currently residing in or having strong ties to the Appalachian region. Participants exhibited varying experiences with cancer including personal diagnosis, personal experience outside of their own such as that of a family member and/or working in a cancer-related field.

Interview responses were analyzed using qualitative content analysis and categorized into themes, subthemes, and subtopics, respectively. Themes that emerged include cancer literacy; experiences with cancer; impressions of the healthcare system; cancer risk behaviors & influences thereof; factors that influence healthcare seeking behaviors; potential solutions; and Appalachian characteristics.

Our findings demonstrate the need for educational interventions, healthcare outreach, increased access, the development of infrastructure (recreational), social support systems, and overall addressing the problem from within the region itself. These changes can take on various forms, such as integrating cancer education into the curriculum of schools, creating policies focused on expanded access to healthcare facilities and/or recreational activities for members of the community and using Appalachian voices as part of the solution. Overall, these changes, if acted upon, have the potential to reduce the cancer burden in this area.

Abstract 2

Basic Science

Exonuclease 1 Recruitment in Human DNA Mismatch Repair

Breanna G. Knicely, Eva Goellner

UK College of Medicine, Toxicology & Cancer Biology

DNA repair is the process by which cells identify and correct mutations present within the genome. These correction mechanisms maintain genomic stability and prevent the development of cancer. The DNA mismatch repair (MMR) pathway identifies and corrects small insertions, deletions, and misincorporations that arise within the genome as a result of errors during DNA replication. The process by which MMR corrects these mispairs includes recognition of the mispair by the MutS complex, recruitment of the MutL complex to the mutation site, excision of the mispair, and gap filling by DNA polymerase. MMR is also critical for inducing apoptosis after chemically induced mispairs, such as those from alkylating agents. Exonuclease 1 is the protein responsible for the excision step of MMR. However, MMR can occur in the absence of Exo1. Thus, MMR can be separated into two subpathways: Exo1-dependent MMR and Exo1-independent MMR. In Exo1-dependent MMR Exo-1 binds to both the MutS complex and MutL complex. The MutL interaction is facilitated by an MLH1 interaction peptide (MIP) box. We recently identified a Msh2 interaction peptide (SHIP) box in yeast Exo1. This project aims to understand how MIP and putative human SHIP box motifs influence human Exo1 recruitment to MMR processes. We have created mutations within the predicted binding domains of human Exo1. We observe changes in localization of Exo1-mutant proteins within the cell, suggesting that changes in the overall MMR process may be present. We also observe changes in MMR-mediated apoptotic response when a subset of Exo1-mutations are expressed in the presence of endogenous wildtype Exo1, indicating a potential for a dominant negative interaction. Our ongoing studies are expected to shed more light upon how the changes in these interactions may affect the human MMR process and overall genomic stability.

Abstract 3

Basic Science

Enhanced Anticancer Activity of Artesunate in Ovarian Cancer through Combination with Navitoclax

Rebecca Ahn[1], Joseph Robert McCorkle[2], Kristen Hill[2], Jill Kolesar[3]

[1]UK College of Arts & Sciences, Biology; [2]UK College of Medicine, Markey Cancer Center; [3]UK College of Pharmacy, Markey Cancer Center

Introduction: Ovarian cancer is the deadliest gynecologic malignancy, however, there has been limited development of new treatment modalities. The five-year overall survival rate remains below 50% and improved therapeutic strategies are needed. Studies have shown the antimalarial drug artesunate has antineoplastic activity against various malignancies, including ovarian cancer. Artesunate's primary mechanism of action is the generation of toxic levels of reactive oxygen species (ROS), triggering apoptosis. We have investigated the ability of the BCL-2 family inhibitor, navitoclax, to enhance artesunate efficacy in ovarian cancer cells, in vitro.

Methods: Cell viability was analyzed following treatment with artesunate and navitoclax, alone and in combination, in four human ovarian cancer cell lines: CAOV3, OVCAR3, OV-90 and UWB1.289. Cell viability was measured after 72 hour treatment using CellTiterGlo 2.0 cell viability assays (Promega). Dose response curves were fitted using 4-parameter log-logistic non-linear models to determine IC50 for each drug. Drug synergy was assessed using the Loewe additivity model.

Results: Mean artesunate IC50 for OVCAR3, UWB1.289, CAOV3, and OV-90 resulted as 5.95μM, 17.9μM, 26.7μM and 61.0μM respectively. The mean navitoclax IC50 were 3.33μM, 3.53μM, 7.93μM, and 13.3μM for CAOV3, UWB1.289, OV-90, and OVCAR3. Artesunate IC50 was significantly higher in OV-90 cells compared to the other three cell lines (one-way ANOVA $p=2x10^{-4}$; Tukey's Multiple Comparison $p<0.01$). Navitoclax IC50 was significantly higher in OVCAR3 cells (one-way ANOVA $p=8x10^{-4}$; Tukey's $p<0.05$). Combination treatment was synergistic in CAOV3 (score=8.97; $p=3.5x10^{-20}$), OVCAR3 (score=5.38; $p=8.5x10^{-4}$), UWB1.289 (score=4.04; $p=2.8x10^{-8}$) and OV-90 cells (score=6.6; $p=1.4x10^{-2}$).

Conclusion: Navitoclax enhances the anticancer efficacy of artesunate in ovarian cancer cells. Drug synergy was observed in both artesunate-sensitive and resistant cells. This drug combination could help fulfill the urgent need for new therapeutic options in ovarian cancer. Further investigation into the molecular mechanism of cytotoxic synergy for artesunate combined with navitoclax is ongoing.

Abstract 4

Basic Science

Assessing Glycogen Metabolism as a Therapeutic Target in Ewing's Sarcoma

Kayli E. Bolton[1], Lyndsay E. A. Young[1], Or Kakhlon[2], Ramon C. Sun[3], Matthew S. Gentry[1]

[1]UK College of Medicine, Molecular & Cellular Biochemistry; [2]Hadassah-Hebrew University Medical Center, Neurology; [3]UK College of Medicine, Neuroscience

Ewing's Sarcoma (ES) is the second most common pediatric bone cancer and affects the bone and surrounding soft tissues of adolescents typically between 10 and 15 years old. Roughly half of ES patients develop a metastatic disease due to the cancer's aggressiveness, and the long-term survival rate of those with metastatic disease is unfavorable at less than 20%. The current treatment options for ES include chemotherapy in addition to surgery and/or irradiation. This standard of care provides some improvement in disease; however, significant progress in effectively treating ES will likely be contingent on the development of novel approaches.

Glycogen is an important energy storage molecule and acts as an energy currency for the body. Glycogen accumulations have been identified in cancers of the breast, kidney, lung, uterus, head and neck, ovary, bladder, colorectal, and pancreatic tumors, yet the significance of these deposits remains largely unknown. One characteristic of ES is the occurrence of large accumulations that have recently been identified as glycogen. This project's aim was to explore the ability of pharmacological interventions to target ES-glycogen. The goal of this project was to identify how preventing the synthesis of glycogen by small molecule inhibition will affect ES cancer progression. We utilized two compounds identified by a collaborator called compounds A and E that target glycogen production and tested them as potential therapeutics in ES. We assessed glycogen levels and cell viability on ES tumor cells treated with compounds A and E and each compound's effects on tumor growth and metabolism in an animal model. Our data demonstrated that ES-glycogen is a novel therapeutic target and established the therapeutic potential of compounds A and E in a preclinical setting. Our next steps for this project include utilizing immunofluorescence to visualize the nuclei and glycogen in ES cells and coupling compounds A and E with chemotherapies for assays in vitro using ES cells and in vivo with a xenograft mouse model. Future studies will determine the minimum treatment regimen, assess re-accumulation of ES-glycogen after treatment, and define an optimal therapeutic window.

Abstract 5

Translational

Extracellular Vesicles as a Potential Mediator of Adaptive Redox State in Radiation Resistant Cancer

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Other than skin cancer, prostate cancer is the most diagnosed cancer in men. Radiation is a viable treatment option but due to tumor heterogeneity, some cases will become recurrent. Our goal is to elucidate the underlying mechanism(s) of the radiation resistant process. We used PC3, a grade IV adenocarcinoma from a bone metastasis and prostate cancer cells, clone 695, that survived in nude mice after exposure to radiation (5 x 2Gy). In performing a colony survival assay, we found that clone 695 is significantly more resistant to radiation at a 2Gy dosage than the parental cell line. Utilizing the Seahorse XF96 instrument, we found that clone 695 demonstrates a higher basal oxygen consumption rate (OCR) and higher ATP-linked OCR. Interestingly, Clone 695 showed an increase in proton leak in conjunction with a significantly lower respiratory capacity than PC3. Based on the fluorescent probe TMRE, clone 695 also had an increased mitochondrial membrane potential. Confocal microscope images of Mitotracker green coupled with IMARIS rendering software demonstrate the increase of mitochondrial mass of clone 695 at the single cell level. Moreover, Clone 695 not only showed an increase in H2O2 released into the media, but in mitochondria as well when compared to PC3. This data suggests redox state alteration in radioresistant cancer cells. In exploring what contributes to the characteristics of radioresistant cells, found that extracellular vesicles (EVs), double membrane bound particles, are being released upon radiation treatment. Although similar in size, compared to non-irradiated cells, EVs released upon radiation treatment is about 1.2x higher in PC3 cells exposed to 6Gy radiation. More importantly, we found that these EVs carried antioxidant proteins such as catalase, MnSOD, and chaperon proteins such as HSP70. Given this data, we plan to test if these EVs act as a mediator that contributes to redox imbalances in radiation resistant cancer cells.

Abstract 6

Basic Science

Mass Spectrometry Imaging of Ewing Sarcoma Patient Samples Identifies a Novel Therapeutic Target

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Ewing sarcoma (ES) is a rare pediatric cancer of the bone and soft tissues affecting adolescents and young adults, with peak incidence from ages 10 to 15 years. A clinical feature of ES is the accumulation of Periodic acid-Schiff positive (PAS+) aggregates. PAS+ staining in ES histopathology analysis has been speculated to be glycogen accumulation, however, this has not been confirmed. To interrogate these aggregates, we developed a novel workflow for the high-sensitive detection of in situ glycogen via enzymatic release of glycogen substrates coupled to matrix assisted laser desorption/ionization imaging mass spectrometry (MALDI-MSI) from formalin-fixed patient tissues. We utilized this method to study the nature of PAS+ staining in a cohort of ES patient samples surgically removed from tibia, rib, chest, shoulder, abdomen and testicle tissues. Using this innovative technique, we discovered profound intra-tumoral glycogen accumulation compared to adjacent stroma, normal, muscle and/or necrotic tissue. Further, intra-tumoral glycogen also has an increased phosphate content and distinct chain length profile compared to normal glycogen found in adjacent and patient-matched tissue. Notably, genetic manipulation and pharmacological inhibition of glycogen synthesis, or removal of glycogen phosphates, stunted ES tumor growth in xenografted mouse models. These findings suggest an important role of ES glycogen aggregates in tumor growth. Together, application of this novel MALDI-MSI technique, and the insights provided by formalin-fixed ES patient samples, reveals a distinct role of ES glycogen aggregates and uncovers a new therapeutic opportunity for this rare pediatric cancer.

Abstract 7

Basic Science

JNK1 Mediated PUMA Expression Contributes to Platycodin D Induced Apoptosis in Lung Cancer

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Platycodin D, a triterpenoid monomer, has been shown to possess anti-tumor effect on various types of cancer. However, the mechanism by which platycodin D suppresses tumorigenesis remains elusive. In this study, we aimed to investigate the effect of platycodin D on the growth of non-small lung cancer cells (NSCLC) and the underlying mechanism. The NSCLC cells treated with platycodin D significantly reduced the cell viability, decreased the number of colonies, impaired the mitochondrial function, and induced apoptosis. To understand the mechanism, the protein levels of the Bcl-2 related proteins were examined and the expression of p53 upregulated modulator of apoptosis (PUMA) was upregulated by platycodin D treatment. Knockdown of PUMA resulted in attenuation of platycodin D-induced apoptosis, indicating that up-regulation of PUMA contributed to platycodin D-induced apoptosis. The induction of PUMA expression by platycodin D treatment was through activation of AP-1 since mutation of AP-1 binding site in PUMA promoter abolished the PUMA promoter activity. The chromatin immunoprecipitation also demonstrated that AP-1 bound to PUMA promoter when the cells were treated with platycodin D. Moreover, knockdown of JNK1, but not JNK2, significantly decreased the platycodin D-induced expression of PUMA and cleaved caspase 3, indicating the essential role of JNK1 in platycodin D-induced apoptosis. Furthermore, platycodin D efficiently suppressed the tumor growth in the H1299 xenograft. Taken together, activation of JNK1/AP-1 axis by platycodin D is essential for apoptosis induction in suppression of NSCLC growth, providing a new mechanism of how platylcodin D suppresses NSCLC.

Abstract 8

Basic Science

Activation of Drp1 Promotes Fatty Acids-Induced Metabolic Reprograming to Potentiate Wnt Signaling in Colon Cancer

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Cancer cells are known for their ability to adapt variable metabolic programs depending on the availability of specific nutrients. Our previous studies have shown that uptake of fatty acids alters cellular metabolic pathways in colon cancer cells to favor fatty acid oxidation. Here, we show that fatty acids activate Drp1 to promote metabolic plasticity in cancer cells. Uptake of fatty acids (FAs) induces mitochondrial fragmentation by promoting ERK-dependent phosphorylation of Drp1 at the S616 site. This increased phosphorylation of Drp1 enhances its dimerization and interaction with Mitochondrial Fission Factor (MFF) at the mitochondria. Consequently, knockdown of Drp1 or MFF attenuates fatty acid-induced mitochondrial fission. In addition, uptake of fatty acids triggers mitophagy via a Drp1- and p62-dependent mechanism to protect mitochondrial integrity. Moreover, results from metabolic profiling analysis reveal that silencing Drp1 disrupts cellular metabolism and blocks fatty acid-induced metabolic reprograming by inhibiting fatty acid utilization. Functionally, knockdown of Drp1 decreases Wnt/b-catenin signaling by preventing fatty acid oxidation-dependent acetylation of b-catenin. As a result, Drp1 depletion inhibits the formation of tumor organoids in vitro and xenograft tumor growth in vivo. Taken together, our study identifies Drp1 as a key mediator that connects mitochondrial dynamics with fatty acid metabolism and cancer cell signaling.

Abstract 9

Basic Science

Targeting Plk1 Re-Establishes the Response to Immune Checkpoint Therapy in Pancreatic Cancer

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Background and aims: Polo-like Kinase 1 (Plk1) is the only gene that can distinguish gemcitabine-sensitive versus -resistant pancreatic ductal adenocarcinoma (PDAC). Targeting Plk1 as a plausible approach to treat PDAC has been tried in clinic. However, the mechanism of the failure of Plk1 inhibitor in treatment of PDAC is still poorly understood. In addition, little is understood, however, regarding the role of Plk1 in PDAC progression.

Methods: To recapitulate the role of Plk1 in the disease progression, we crossed *Plk1*^{LSL} with *Mist1*$^{CreER/+}$ or *Mist1*$^{CreER/+}$/*Kras*$^{G12D/+}$ mice and examined the roles of Plk1 in acute pancreatitis (AP), and PanIN development. Through bioinformatics analysis we identified the regulation networks by which Plk1 is involved in the disease progression. We also explored whether and how Plk1 regulates programmed death-ligand-1 (PD-L1) and the potential efficacy of combinatory treatment of inhibition of Plk1 plus α-PD-L1 *in vitro* and *in vivo*.

Results: We found that Plk1 was associated with poor outcomes in human patients and Plk1 overexpression significantly inhibited caerulein-induced acute pancreatitis and delayed development of acinar-to-ductal metaplasia (ADM) and PanIN by inactivating NFκB pathway. Unexpectedly, PD-L1 was upregulated upon inhibition/depletion of Plk1 via activation of NFκB pathway. Mechanistically, Plk1 phosphorylation of RB at S758 inactivated NFκB pathway by inferring its translocation to nucleus. Of interest, the distinct role of Plk1 in the precursor of PDAC and the negative regulation of PD-L1 by Plk1 in PDAC were both mediated by dysregulation of NFκB pathway.

Conclusions: Plk1 inhibition-induced unexpected elevation of PD-L1 re-sensitized PDAC to immune checkpoint blockade therapy through reactivating antitumor response.

Abstract 10

Basic Science

A Kinome-Wide CRISPR Screen Identifies CK1α as a Novel Target to Overcome Enzalutamide Resistance of Prostate Cancer

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Enzalutamide (ENZA), a second-generation androgen receptor antagonist, has significantly increased progression-free and overall survival of patients with metastatic prostate cancer (PCa). However, resistance remains a prominent obstacle in treatment, illustrating the urgent need to develop new approaches to increase ENZA efficacy. Utilizing a kinome-wide CRISPR-Cas9 knockout screen, we identified casein kinase 1 alpha (CK1α) as a novel therapeutic target to overcome ENZA resistance. Depletion or pharmacologic inhibition of CK1α significantly enhanced EZNA efficacy in ENZA-resistant cell lines and patient-derived xenografts. Mechanistically, CK1α phosphorylates and modulates the protein abundance of ataxia-telangiectasia mutated (ATM), a primary initiator of DNA double-strand break (DSB)-response signaling, which is compromised in ENZA-resistant cells and patients. Inhibition of CK1α stabilizes ATM, resulting in the restoration of DSB-response signaling, and thus increases ENZA-induced cell death and growth arrest in an ATM-dependent manner. Our study details an innovative therapeutic approach for ENZA-resistant PCa and characterizes a novel perspective for the function of CK1α in the regulation of DNA damage response signaling.

Abstract 11

Basic Science

Understanding the Role of Peroxiredoxin IV in Progression of Colorectal Cancer

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Peroxiredoxin IV (Prx4) is a multifunction enzyme with a primary antioxidant role of reducing free radicals such as hydrogen peroxide and peroxynitrite. Literature review suggests that Prx4 upregulation in colorectal cancer is associated with shorter survival of patients. However, the function and mechanisms of Prx4 in cancer development are not well understood. The goal of this study is to validate the oncogenic role of Prx4 and to identify potential mechanisms of invasion and metastasis. Western Blot was performed to compare the expression of Prx4 protein in seven colorectal cancer cell lines. shRNA mediated knockdown of Prx4 was performed in cancer cell lines HCT116 and RKO. Stable vector control and Prx4 knockdown cells were used to perform migration assay. Transwell Matrigel invasion assay was also performed using the same cell lines with 10% FBS containing-medium as chemoattractant. Vector control and Prx4 knockdown HCT116 cells were orthotopically implanted into the cecum wall of immunodeficient mice and the mice were humanely euthanized four weeks later. RNA-Sequencing was then performed to compare the transcriptome of control and Prx4 knockdown HCT116 cells. From these experiments, we found that there is high Prx4 expression in colorectal cancer cell lines. Stable knockdown of Prx4 in HCT116 and RKO cell lines resulted in a significant decrease in migration and invasion rates of cancer cell lines in vitro. Orthotopic implantation of HCT116 vector control and Prx4 knockdown cells into cecum wall resulted in a lower metastasis rate to the liver and the lungs. We are currently conducting mechanistic studies to determine how the loss of Prx4 leads to a decrease in malignancy of colorectal cancer cell lines. Thus, Prx4 promotes colorectal cancer cell invasion and metastasis. Our study suggests Prx4 is a critical oncogenic protein that can be used as a potential therapeutic target for patients.

Abstract 12

Basic Science

Generating Tools for Dissecting Mismatch Repair Sub Pathways

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DNA Mismatch Repair (MMR) is a regulatory pathway for identifying and repairing mispaired bases during DNA replication and the pathway is critical for maintaining genome stability. The machinery of MMR recognizes the mispaired bases with the MutS complex and generates a nick or cut in the sequence with the MutL complex. The exonuclease 1 protein removes a section of the daughter strand containing the mispair. DNA polymerase fills in the gap with the correct sequence. If left unrepaired these mispair disrupt the genetic stability of the cell, causing the cell to become mutated. An accumulation of the mutations is known to be a hallmark of cancer. Mutations of the MMR pathway are common in gastrointestinal, colorectal, and endometrial cancers and can also be found in an inherited cancer predisposition disorder called Lynch Syndrome. Further mechanistic understanding of the pathway can help with the diagnosis and treatment plans of tumors. MSH2 is one of the two heterodimers in the MutS complex of MMR. It binds with the MSH6 or MSH3. The MutS complex recognizes mispairs and is important in recruiting downstream proteins. Green Fluorescent Protein (GFP) expresses a green fluorescence when exposed to blue-ultraviolet light. It is used as a molecular tool for tracking and visualizing proteins. During my project, I have subcloned the MSH2 gene into a c-terminal tagged GFP vector so the expression of the MSH2 could be observed under the microscope. The eGOellner lab has an existing Exonuclease 1 (Exo!) vector with a red fluorescent tag. The SHIP1 and SHIP 2 motifs are binding sites for Exo 1 to bing to MSH2. The Exo 1 MIP motif is the binding site for the MLH1 protein of the MutL complex. During my lab project, I used site-directed mutagenesis to disrupt the SHIP1 and MIP binding sites. These mutant plasmids were then used as the building blocks for the lab to generate double and triple mutations disruption Exo1 recruitment. Together these molecular tools will allow us to observe changes in expression and localization within cells and to further study MSH2 recruitment of Exo1. These experiments will allow us to dissect the MMR pathways and further study the effects of mutations in the pathways.

Abstract 13

Basic Science

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

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Background: Colorectal cancer (CRC) is the second leading cause of cancer death. Aberrant activation of lipid metabolism is a hallmark of cancer. Fatty acid synthase (FASN), a key enzyme in lipid metabolism, is overexpressed and an anticancer target in CRC. BRAFV600E is the most common mutation occurring about 10-15% of CRC cases. Single-agent BRAF inhibition has proven ineffective due to acquired resistance. There is an urgent need for new therapeutic approaches to decrease MAPK reactivation and postpone/overcome resistance to BRAF inhibition. Literature suggests that resistance to BRAF-targeted therapy may be associated with an increase in de novo lipid synthesis. The approaches combining BRAF, and lipid targeted therapy have not been tested. Therefore, our central hypothesis of this study is that inhibition of fatty acid metabolism will sensitize CRC cells to BRAF inhibitors and overcome acquired resistance.

Methods. Both established (HT29) and primary (PT130 and PT2449pt) CRC cell lines were used. Cell lines resistant to PLX8394 (BRAF inhibitor) were created to be compared to the parental cells. Cells were treated with either PLX8394, TVB3664 (FASN inhibitor), Encorafenib (FDA-approved BRAF inhibitor), Cetuximab (FDA-approved EGFR inhibitor), or a combination of the drugs. IC50 curves, Cell Viability, Matrigel Invasion assays, and western blot analysis were used to evaluate cell responses to treatments. Morphological changes between parental versus resistant cells were evaluated by confocal microscopy. Xenograft models were used to determine the responses of PT130 resistant cells versus parental cells to treatment in vivo.

Results. Established PT130 PLX8394 resistant cells have a higher IC50 than parental PT130 cells when treated with this inhibitor, and show acquired resistance to encorafenib in CRC. Western blot analysis demonstrates that FASN, pACC, pACLY, CPT1, and CD36 proteins associated with lipid metabolism, are significantly overexpressed in resistant cells as compared to parental cells. We also show that the PT130 resistant cells grow significantly faster than parental cells as demonstrated by a growth curve response using PrestoBlue analysis. In vivo, we show that PT130 PLX resistant cells grow significantly faster than parental cells, along with upregulation of multiple markers associated with lipid metabolism. Also, morphological changes were observed by confocal microscopy PT130 resistant cells. Analysis of markers associated with migration and invasion demonstrates that PT130 resistant cells had a loss of e-cadherin, a key component of the adherens junctions that are integral in cell adhesion and maintaining epithelial phenotype of cells. Using cell viability assay in PT130 cells, we show that combined inhibition of BRAF signaling and FASN using PLX8394 and TVB3664 has more prominent effect inhibiting cell proliferation as compared to each drug alone.

Conclusion. Our study demonstrates that resistance to BRAF inhibitors is associated with a drastic increase in proliferation and upregulation of lipid metabolism in vitro and in vivo. We show that combination of FASN and BRAF inhibitors has a combinational effect on inhibiting cell viability, suggesting that targeting lipid metabolism in BRAF mutant CRC can improve efficacy of BRAF inhibitors and overcome development of resistance. We plan to further test the inhibition of BRAF/EGFR signaling in combination with lipid metabolism-targeted therapies to develop novel and more efficacious strategies for CRC patients with BRAF mutations.

Abstract 14

Basic Science

Repurposing FDA-Approved PI3K/Akt Inhibitors to Improve Brain Uptake of Anticancer Drugs in Glioblastoma Resection Models

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Background: Glioblastoma multiforme (GBM) has the lowest median survival (8 months) amongst primary malignant brain tumors. Despite resection and radiotherapy, invasive tumor cells remain along the resection cavity and spread into the contralateral hemisphere, highlighting the role of systemic chemotherapy in GBM standard of care. However, brain penetration of anticancer drugs is limited by drug efflux transporters at the blood-brain barrier (BBB).

Objectives: Our laboratory has shown that GBM upregulates BBB drug efflux transporters via a mechanism that involves PI3K/Akt. Our goal is to increase anticancer drug brain concentrations by downregulating BBB drug efflux transporters using repurposed FDA-approved PI3K/Akt inhibitors, which holds the potential for translation into the neuro-oncology clinic.

Methods: GL261 Red-FLuc cells (2.5K cells/µl; 2µl/2min) were injected into 8-week-old female J:NU mice. Tumor burden, volume, and invasiveness were assessed with IVIS Æ Spectrum imaging, MRI, and histopathology, respectively. On day 14 post-injection, mice received 5-aminolevulinic acid (200 mg/kg i.p.), and tumors were resected with a 2mm punch biopsy tool and surgical fluorescence microscope (ex/em: 405/635nm). Drug efflux transporter expression and activity in isolated brain capillaries were determined by Western blotting and substrate fluorescence assays, respectively. In addition, cytotoxicity was assessed after 48-hour drug incubation using CyQuant MTT Assays.

Results & Anticipated Results: For GL261 Red-FLuc cells, IC50 values from cell viability assays with temozolomide, lapatinib, alpelisib, and miltefosine were, 32, 20, and 190 µM, respectively. Median survival of GBM mice was 26.5d and resecting the tumor significantly increased survival to 34d ($p=0.0116$). Drug efflux transporter expression and activity levels in contralateral brain capillaries were significantly upregulated compared to sham controls. Based on preliminary data, we expect the FDA-approved PI3K/Akt inhibitors, alpelisib and miltefosine, to significantly reduce drug efflux transporter levels, increase brain uptake of anticancer drugs, and prolong GBM mouse survival.

Discussion & Significance of Impact: We have previously shown that PI3K/Akt inhibition reduces P-gp/BCRP levels in brain capillaries. Here, we extend this strategy by repurposing the FDA-approved PI3K/Akt inhibitors alpelisib/miltefosine to improve brain uptake of anticancer drugs in GBM resection models.

Abstract 15

Basic Science

Fatty Acid Synthase in the Regulation of Stemness in Colorectal Cancer

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Background: Aberrant lipid metabolism is a universal characteristic in colorectal cancer (CRC). High expression of Fatty acid synthase (FASN), a vital enzyme of *de novo* lipogenesis, has been associated with a poor clinical outcome in CRC. CD166, a transmembrane glycoprotein protein and a marker of CRC stem cells, has also been associated with poor clinical outcome in CRC. However, the mechanisms of how CD166 contributes to carcinogenesis are not well understood. Our preliminary data show that downregulation of FASN leads to a decrease in CD166 expression in the transgenic mice models, mouse organoids and human CRC cells. Therefore, the purpose of this study is to elucidate the mechanisms of how FASN regulates expression of CD166 (I) and define the functional significance of CD166 upregulation during CRC initiation and progression (II).

Methods: Expression of FASN and CD166 were assessed in adenomas from Apc/Cre, Apc/FASN$^{\Delta/+}$/Cre, and Apc/FASN$^{\Delta/\Delta}$/Cre mice and in our novel mouse models Apc^{Min} and Apc/Cre-ERT2 with inducible hetero- and homozygous deletion of FASN. The effect of shRNA-mediated knockdown of FASN and CD166 in human established and primary CRC cell lines on cell signaling was assessed using qRT-PCR and Western blot analysis. Normal mouse intestinal and tumor organoids were utilized to further assess FASN regulation on stemness. TVB-3664, a novel FASN inhibitor, was used to assess the effect of pharmacological inhibition of FASN on organoid's growth. The LIVE/DEAD™ Viability/Cytotoxicity Cell Viability Kit, Cell Titer-Glo® 3D Cell Viability Assay, RayBio® Human ALCAM ELISA Kit and CytoSelect™ 48-Well Cell Adhesion Assay were used for functional studies.

Results: Downregulation of FASN expression and its pharmacological inhibition result in a decrease in normal intestinal and tumor organoid viability and size. Both genetic deletion and pharmacological inhibition of FASN decreases expression of stem cell markers such as CD133, CD166, and CD44 at the mRNA and protein levels in transgenic mouse models and CRC cell lines. Interestingly, shRNA-mediated downregulation of FASN in primary CRC cells leads to an increase in CD166 shedding. Furthermore, shRNA-mediated downregulation of CD166 results in increase of cell proliferation and cell adhesion to collagen IV in 2D culture, while decreasing colony formation in 3D.

Conclusion: Our data suggests that a decrease in FASN expression is associated with a decrease in stem cell markers' expression and organoid's growth and viability. Additionally, a decrease in CD166 expression is associated with an increase in cell proliferation and cell adhesion to the ECM but decreases colony formation which highlights the dynamic functional role of CD166 in CRC. In summary, our findings support the crucial role of FASN in regulation of the stem cell niche and stemness in CRC. Delineating the role of FASN regulation of stemness via altered expression CD166 and other stem cell markers will provide the rationale for targeting FASN as a preventative or early-stage therapeutic approach in CRC.

Abstract 16

Basic Science

Programming Macrophage-Engineered Vesicles for Enhanced Macrophage Modulation and Drug Delivery

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Macrophages, the immune surveilling cells, exhibit diverse functional states allowing them to assume reversible phenotypes ranging from pro to anti-inflammatory. In the tumor microenvironment, macrophages can either contribute to tumor progression (M2 macrophages) or tumor suppression (M1 macrophages). The ability to re-polarize macrophages from M2 to M1 phenotype offers a potential approach for treating cancer. While several immunotherapeutic approaches including the use of small molecules, endogenous extracellular vesicles (EEVs) or cell derived vesicles have shown promise for re-modulating tumor-supportive TAMs (M2-like macrophages) into tumor-suppressing M1-like macrophages, each of these approaches suffers from unique challenges. We developed programmed nanovesicles as an immunomodulatory therapeutic platform with the capability to re-educate M2 macrophages to an M1-like phenotype and simultaneously deliver therapeutics to tumor cells. Mouse bone marrow-derived macrophages were first programmed to over-express membrane-bound ligands, polarized to an M1 phenotype, and then used to generate programmed nanovesicles. Nanovesicles that are decorated with specific membrane-bound ligands are efficiently taken up by target cells. Furthermore, programmed nanovesicles generated from M1 macrophages exhibit enhanced capability to modulate macrophage phenotype. Programmed nanovesicles loaded with therapeutics also exhibit increased cancer cell killing ability compared to loaded but nonprogrammed vesicles and therapeutics in solution.

Abstract 17

Clinical

Findings on Ovarian Cyst Resolution for the Practicing Physician Based on Cyst Diameter

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Objectives: The purpose of this study was to characterize the resolution of ovarian cysts for the practicing physician in terms of cyst diameter. Ovarian cysts frequently resolve on their own, but little information on this resolution based on cyst diameter is available for use by examining physicians. The objective of this study was to analyze the resolution of incident ovarian cysts in relation to cyst diameter, structure, age, body habitus, and menopausal status using univariate and multivariate analyses. These categorizations are important to examining physicians for decisions on whether to continue monitoring the cyst or intervene surgically.

Methods: 2,638 women with incident cysts who underwent 51,356 TVUS examinations were analyzed for over 31.2 years in the University of Kentucky Ovarian Cancer Screening Program. Prevalent cysts were excluded as they would be examined with an undefined course of first appearance when defining resolution time. Other exclusions included women with concurrent ovarian malignancies, cysts with solid components and cases where surgery interrupted the natural history of measured cysts. Out of all the women in the study, 3,897 cysts were collectively identified and studied in a univariant and multivariant analysis through a clustering of variables identified in the patients.

Results: 2,424 of the 3,897 cysts in the study did resolve; the remaining 1,473 cysts were treated as right censored observations in the analysis. When considering multivariate analysis, septated cysts were 53.6% more likely to resolve than unilocular cysts (HR=1.536, $P<0.05$). For every centimeter increase in diameter, the hazard for resolution decreased by 32.5% (HR=0.675, $P<0.05$). Resolution was 34.2% less likely for women using HRT when compared to those who were not users (HR=0.658, $P<0.05$). For every one year increase in age, the hazard for resolution decreased by 2.1% (HR=0.979, $P<0.05$). Univariate analysis revealed similar results for these four factors. With univariate analysis, post-menopausal women's cysts were 21.2% less likely to resolve than pre-menopausal women's cysts, however, this finding was not significant in multivariate analysis.

Conclusion: Septated cysts were significantly more likely to resolve than unilocular cysts in both univariate and multivariate analyses. As the cyst diameter increased, cysts were found to decrease in time to resolution in both univariate and multivariate analyses. With HRT use, cysts were significantly less likely to resolve in univariate analyses but were not significantly affected in multivariate analysis. As the patients aged, it was determined they were more likely to have cyst resolution in univariate and multivariate analyses. In univariate analysis, post-menopausal women were more likely to have cyst resolution, but menopausal status was not affected in multivariate analyse. BMI status and family history of ovarian cancer did not significantly affect cyst resolution in both univariate and multivariate analyses. For the examining physician, the study presented here establishes expectations for cyst resolution based on variables pertinent to the patient in a population where no malignancy was observed.

Abstract 18

Basic Science

Peroxiredoxins and the Regulation of Ferroptosis in Colorectal Cancer

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Ferroptosis, a new form of regulated cell death, is typically characterized by excessively iron-dependent accumulation of lipid peroxidation, which causes the breakage of plasma membrane. Dysregulation of ferroptotic cell death has been linked to the development of cancer, and inducing ferroptosis has been proposed as a potential strategy for cancer treatment. Although several important regulators involved in ferroptosis have been discovered, the molecular mechanism behind this process has yet to be completely understood. Members of the peroxiredoxin (Prx) family function as leading cellular antioxidants that react with hydrogen peroxide to keep redox homeostasis as well as contribute to oxidative signalling under both physiological and pathological conditions. In this study, we investigated the role of Prx family members in the regulation of ferroptosis in colorectal cancer (CRC) cells using loss-of-function and gain-of-function experiments. Firstly, immunoblotting was used to examine endogenous levels of Prx family members in different colorectal cancer cells and cells with high or low expression of Prxs were selected for study. Secondly, each of the endogenously expressed 2-Cys containing Prxs including Prx1, Prx2, Prx3 and Prx4 in representative CRC cell lines was depleted by stably expression of ShRNA targeting their coding regions. The consequences of Prx knockdown in these cells in response to ferroptosis inducers,erastin, were examined by cell viability and colonogenic assays. Cell death due to ferroptosis in these cells was demonstrated by the measurement of lipid peroxidation using the sensor BODIPY 581/591 C11 and differentiated with other modes of cell death such as apoptosis, autophagy and necrosis by the combined application of various inhibitors. Finally, overexpression and rescue experiments were used to further validate the results observed in Prx-depleted cells. In conclusion, we found that 2-Cys containing Prxs are widely expressed in CRC cell lines, and depletion of Prx1, Prx2 or Prx4 but not Prx3 sensitizes CRC cells to erastin induced cell death. However, only depletion of Prx4 but not other Prxs sensitizes CRC cells to ferroptotic cell death that is characterized by the accumulation of lipid peroxidation and can be rescued by the treatment with inhibitors of ferroptosis. In consistence, overexpression of Prx4 also leads to the resistance of CRC cell to ferroptosis. Thus, our findings reveal an essential role of Prx4 in protecting colorectal cancer against ferroptosis and provide a potential target to improve the antitumor activity of ferroptosis-based chemotherapy.

Abstract 19

Basic Science

Extracellular Vesicles and Acute Lymphoblastic Leukemia: An Insight into the Mechanism of Chemotherapy-Induced Cognitive Impairment

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Acute Lymphoblastic Leukemia (ALL) is the most common malignancy in pediatric oncology patients, with a median age of diagnosis of between 2 and 5 years. The survival rate for pediatric ALL patients is approximately 90%, but a third of pediatric ALL survivors show evidence of neurocognitive decline, highlighting the critical need for prevention of collateral damage to normal tissues following cancer therapy. Both direct effects from cancer treatment and the elevation of inflammatory cytokines have been implicated in contributing to this neurocognitive decline. We have recently reported that extracellular vesicles (EVs) are early indicators of damage to the brain following whole-brain irradiation. Here, we investigate the role of EVs in ALL patients who were only treated with chemotherapy. We collected serum and cerebrospinal fluid (CSF) from pediatric ALL patients treated at the Dance Blue Hematology & Oncology Clinic at five different time points. The first time point was pretreatment: fluid that was collected prior to the introduction of any chemotherapeutic agents. The second time point was at the conclusion of induction therapy, and the last three time points were during consolidation therapy. EVs isolated from the serum of these patients (using SmartSEC from Systems Bioscience) were used for quantification of: 1) The marker of oxidative stress, 4-hydroxy-2-nonenal (HNE) adducted protein levels using an antibody against HNE-adducted-proteins. HNE is a product of lipid peroxidation and can adduct to proteins leading to protein misfolding and malfunction. 2) Markers of neuronal injury and/or glial cell activation using ProteinSimple technology (Jess). We found that the levels of HNE-adducted proteins in EVs significantly decreased following the conclusion of induction therapy ($p<0.05$) but increased during consolidation ($p<0.05$). However, HNE-adducted protein levels in CSF did not change at any time points examined. The kinetics of changes in HNE-adducted protein in EVs are consistent with the presence and absence of cancer, and suggest the existence of normal tissue injury. Importantly, when compared to CSF, EVs were a more sensitive indicator of astrocyte activation, as indicated by an increase in glial fibrillary acidic protein (GFAP) in the consolidation phase ($p<0.05$). The increase in GFAP is consistent with the rise in HNE-adducted proteins in the consolidation phase, supporting the presence of neuronal injury. Finally, we assessed, using an in vitro model, whether leukemia-derived EVs contribute to neuronal injury by increasing the production of the pro-inflammatory cytokines IL-1B and TNF-α. We found that the addition of EVs without treatment from two pre-B cells ALL cell lines leads to an increase in both IL-1β ($p<0.05$) and TNF-α ($p<0.05$) production in a human macrophage cell line. Overall, the results support the use of EVs as an indicator of oxidative stress and neuronal injury while also revealing potential downstream effects of leukemia-derived EVs on activating immune cells to release cytokines leading to neuronal injury.

Abstract 20

Basic Science

PLK1 in Cr (VI)-Associated Lung Cancer Progression

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Hexavalent chromium (VI) has long been reported as the International Agency for Research on Cancer (IARC) class I human carcinogen, and the most prevalent cancer induced by Cr (VI) is lung cancer. Enough evidence suggests that lung epithelial cell transformation often accompanies with alterations on cell cycle and cellular energy metabolism. Polo-like kinase 1 (PLK1), a key cell cycle regulator, might also regulate several metabolic pathways. Upon comparison of expression levels of PLK1 in Cr (VI)-transformed bronchial epithelial cells (BEAS-2B) and normal BEAS-2B cells, we found that PLK1 level is highly upregulated in Cr-(VI) transformed cells (CrT). Interestingly, knockdown of PLK1 in Cr (VI)-transformed BEAS-2B cells triggers upregulation of oxidative phosphorylation, but decreases anchorage-independent cell growth, cell migration, invasion and xenograft tumor formation. Furthermore, we found that downregulation of PLK1 results in an elevated protein level of pyruvate dehydrogenase E1 subunit alpha 1 (PDHA1). PLK1-mediated PDHA1 downregulation leads to a lower level of mitochondrial ROS but a higher level of mitochondrial membrane potential (MMP). Mechanistically, we show that PDHA1 can be directly phosphorylated by PLK1 at T57 and that PLK1-mediated PDHA1 phosphorylation results in PDHA1 re-localization from mitochondria to cytosol and protein degradation. In agreement, transfection of PLK1 (wide type or constitutively active T210D mutant) results in activation of mitochondrial fission, mitophagy and PDHA1 protein degradation. On the contrary, inhibition of PLK1 kinase activity by both expression of kinase dead K82M dominant negative mutant and PLK1 inhibitor BI6727 decreases the level of mitochondrial fission and mitophagy activation. Thus, we hypothesize that PLK1 elevation during Cr (VI)-induced transformation causes hyper-phosphorylation of PDHA1-T57, resulting in its protein degradation and reduced oxidative phosphorylation. Downregulated oxidative phosphorylation results in a lower level of mitochondrial derived ROS, eventually inhibiting mitochondrial-mediated apoptotic response. Defining the role of PLK1 in metabolic reprogramming in Cr (VI)-associated tumorigenesis may give us a new perspective and a target to inhibit the Cr (VI)-induced cancer development. In addition, PLK1 inhibitors may be used to increase the chemo-sensitivity of Cr (VI)-transformed tumor cells through restoring normal function of mitochondria, thus alleviating the drug resistance caused by dysfunction and hyperpolarization of mitochondria.

Abstract 21

Clinical

SSTR-2 Expression in Solid Tumors: An Immunohistochemistry Analysis

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Background: Somatostatin receptor (SSTR) expression has been characterized in well-differentiated neuroendocrine tumors (NET). However, the understanding of receptor expression in various non-neuroendocrine solid tumors is limited. This study was performed to evaluate SSTR-2 in various cancers to provide a rational basis for SSTR-2 targeted anti-cancer therapies.

Methods: Formalin-fixed paraffin, paraffin-embedded tissue was obtained from pathology archives after institutional review board approval. Tumor blocks were prospectively stained with an anti-SSTR-2 antibody via immunohistochemistry (IHC). The following tumor types were studied: small cell carcinoma (Code 0; n=14), medullary thyroid cancer (Code 1, n=10), melanoma (Code 2, n=10), merkel cell carcinoma (Code 3, n=10), head and neck p16 positive squamous cell carcinoma (Code 4, n=10), well-differentiated NET (Code 5, n=10), paraganglioma and pheochromocytoma (Code 6, n=20), poorly differentiated neuroendocrine carcinoma (Code 7, n=9), and p16 negative squamous cell cancer (Code 8, n=4). IHC was scored as follows: SSTR2 Intensity (0=none, 1=weak, 2=moderate, 3=strong), SSTR2 Localization (1= membranous; 2=cytoplasmic; 3=mixed), SSTR2 % Positivity (5% increments).

Result: The frequency of SSTR2 intensity in groups Code 0 to Code 8 were noted as 14.43%, 10.31%, 10.31%, 10.31%, 10.31%, 10.31%, 20.62%, 9.28% and 4.12% consecutively. Similar pattern was noted in SSTR2 localization from Code 0 to Code 8 as 14.58%, 10.42%, 10.42%, 10.42%, 10.42%, 10.42%, 20.83%, 9.38%, 3.13% respectively. SSTR2 positivity in chronicity from Code 0 to Code 8 were noted as 14.43%, 10.31%, 10.31%, 10.31%, 10.31%, 10.31%, 20.62%, 9.28% and 4.12%. The detailed analysis of the result is depicted in an attached tabular format.

Conclusion: As expected, well-differentiated NET and paragangliomas/pheochromocytomas expressed very high SSTR-2 positivity with high intensity. However, a subset of small cell carcinoma and head and neck p16 positive squamous cell carcinoma were observed to express SSTR-2. Targeting SSTR-2 in small cell carcinoma and head and neck cancer with help of radiolabeled somatostatin analog (Lutetium 177 dotatate) could be a promising therapeutic approach. Based on our promising SSTR-2 IHC data, a prospective study of SSTR-2 assessment with help of gallium 68 dotatate PET imaging in small cell lung cancer patients is currently underway at Markey Cancer Center.

Abstract 22

Translational

Discovery of Orally Bioavailable Small Molecule Inhibitor of the DCN1-UBE2M Interaction as a Potential Treatment of Lung Cancer

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The cullin-RING ubiquitin ligases (CRLs) are ubquitin E3 enzymes that play a key role in controlling proteasomal degradation and are activated by neddylation. The NEDD8-activating enzyme (E1) inhibitor Pevonedistat prevents neddylation of the CRL's and has clinically validated the neddylation pathway for oncology. We previously reported inhibitors that target CRL activation by disrupting the interaction of the Defective in Cullin Neddylation 1 (DCN1), a CRL neddylation co-E3, and UBE2M, a neddylation E2. Our first-generation inhibitors possessed poor oral bioavailability and fairly rapid clearance that hindered the study of acute inhibition of DCN-controlled CRL activity in vivo. Herein, we report studies to improve pharmacokinetic performance of the pyrazolo-pyridone inhibitors of the DCN1-UBE2M interaction. X-ray co-structures of bound inhibitors directed specific structural modifications to both improve physiochemical properties and modestly improve DCN1 binding. Careful attention to structural constraints, sites of metabolism, and structure-property relationships allowed substantial improvement in murine oral bioavailability including increase in Cmax, decrease in Clint, and increase in AUC. The current best inhibitor, 40, inhibits the interaction of DCN1 and UBE2M, blocks NEDD8 transfer in biochemical assays, thermally stabilizes cellular DCN1, and inhibits anchorage independent growth in a DCN1 amplified squamous cell carcinoma cell line. Additionally, we demonstrate that a single oral 50 mg/kg dose sustains plasma exposures above the biochemical IC90 for 24 hours in mice.

Abstract 23

Basic Science

Co-Targeting Nucleus Accumbens-Associated Protein-1 (NAC1) and the NF-κB Signaling Pathway in Melanoma

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NF-κB signaling pathway has been identified as one of the predominantly upregulated pathways in melanoma. However, the effectiveness of targeting the NF-κB pathway as a treatment for melanoma remains elusive. Nucleus accumbens-associated protein-1 (NAC1) is a transcription co-regulator belonging to the BTB/POZ gene family and is highly expressed in several types of cancer including melanoma. Published studies including our own have shown that NAC1 not only bestows oncogenic potential but also undermines therapeutic outcomes through its transcription- dependent or -independent functions. The objective of this study was to determine the effect of NAC1 on NF-κB signaling and the potential of NAC1 as a therapeutic target in melanoma. We found that NAC1 exerted negative effect on NF-κB signaling, and silencing of NAC1 expression elevate the level of nuclear NF-κB in human melanoma cells. Notable, inhibition of NF-κB signaling could significantly potentiate the anti-neoplastic effect of the NAC1 inhibition. This study may identify a novel NAC1-NF-κB signaling axis in melanoma cells, thus offering a potential new therapeutic approach to treatment of melanoma.

Abstract 24

Basic Science

High-Dimensionality Reduction and Spatial Clustering of MALDI-MSI Datasets to Study Lung Cancer Metabolic Heterogeneity

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The tumor microenvironment contains a heterogeneous population of stromal and cancer cells that engage in metabolic crosstalk to ultimately promote tumor growth and progression. Due to heterogeneity within solid tumors, pooled mass spectrometry workflows are less sensitive at delineating unique metabolic perturbations between stromal and immune cell populations. Thus, there is a critical need to resolve spatial metabolism among heterogeneous cell types within the tumor microenvironment. We recently developed a multiplexed matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) workflow for simultaneous spatial profiling of complex carbohydrate metabolites in formalin-fixed paraffin-embedded (FFPE) tissues. Herein, we report a data analysis pipeline that takes advantage of the high-dimensionality nature of multiplexed MALDI-MSI datasets and uses supervised learning-based high-dimensionality reduction and spatial clustering (HDR-SC) to examine complex carbohydrate metabolism within the tumor microenvironment. Spatial distribution of complex carbohydrate features in human FFPE lung cancer tissues were visualized by UMAP plots and spatial heatmaps and matched to anatomical regions by adjacent hematoxylin and eosin (H&E) stained tissue sections, confirmed by pathologist annotation. In human lung adenocarcinoma tissues, HDR-SC accurately defined tumor cells, tumor-infiltrating lymphocytes, cancer-associated fibroblasts, and necrotic tissue, and in-depth pathway enrichment analyses revealed unique metabolic pathways are associated with each distinct pathological region. We observed heterogeneous glycogen accumulation within tumor cells. Further, regions comprised of cancer-associated fibroblasts or necrosis with no presence of tumor cells were enriched for core fucosylated complex biantennary N-linked glycans while high-mannose N-linked glycans were overrepresented in regions of immune infiltration. Collectively, our results demonstrate the promising potentials of HDR-SC of pixel-based complex carbohydrate analysis to study cell-type and regional-specific stromal signaling within the tumor microenvironment.

Abstract 25

Basic Science

Critical Role of Nucleus Accumbens Associated Protein 1 in Triple Negative Breast Cancer

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Triple negative breast cancer (TNBC) is a subtype of breast cancer characterized by lack of actionable target receptors, estrogen (ER), human epidermal growth factor receptor 2 (HER2) and progesterone (PR). One of the important features of TNBC is enrichness of stem-like cells that contribute substantially to the TNBC malignant phenotype. Nucleus accumbens associated protein 1 (NACC1), a unique member of the BTB-POZ family of transcription factors, is implicated in tumor development and progression of various types of cancer. Although NACC1 is known to have an important role in maintenance of induced-pluripotent and embryo cells' stem-like features, whether this transcription co-regulator can modulate tumor stemness is unknown. The objective of this study was to test the hypothesis that NACC1 plays a critical role in maintaining stemness and aggression of TNBC. In analysis of the breast invasive carcinoma cBioportal dataset consisting of 1084 breast cancer cases, we found a high NACC1 mRNA expression in 71% of the cases. Analysis of CPTAC database also revealed an increase of NACC1 protein in tumor samples. Furthermore, TNBC cell lines MDA-MB-231 and HCC-1806, had high NACC1 expressions as compared to MCF10A, a non-malignant mammary epithelial cell line. Hematoxylin-eosin staining of 450 human breast cancer tissues revealed the high NACC1 expression in different stages of tumor development. Further, we showed that depletion of NACC1 in TNBC cells decreased 3-D mammosphere formation, cell migration, invasion and altered cells EMT phenotype. Canonical TNBC stemness markers CD44 and ALDH1A1 were reduced in TNBC cells subjected to depletion of NACC1. RNA sequencing uncovered enrichment of JAK/STAT, PI3K/AKT, WNT, stemness and tumor progression-associated pathways in NACC1 depleted cells. Moreover, the cells with NACC1 depletion had reduced levels of factors associated with those enriched pathways, including phospho-STAT3-Y705, B-catenin, PDK1, cyclin D1, cMYC, IL-6 and phosphorylated PTEN. NACC1 immunoprecipitation showed physical association of NACC1 with STAT3 and CD44 in TNBC cell lysate. In pulse-chase experiments, STAT3 turnover was higher in the NACC1-depleted cells as compared to control cells (half-life: 5hrs vs 24hrs, respectively). STAT3 degradation in NACC1-depleted cells could be rescued by MG132, a proteasome inhibitor. Notably, NACC1-depleted cells showed a significantly reduced ability to initiate tumor growth and to sustain progression in vivo. Taken together, these results provide a potential rationale for targeting NACC1 as a potential therapeutic strategy to treatment of TNBC. Further studies are ongoing to investigate the molecular mechanism and pathway(s) by which NACC1 enhance TNBC hostility and its role in regulation of the tumor-immune niche.

Abstract 26

Basic Science

Neurotensin Contributes to Mitochondrial Dysfunction in Nonalcoholic Fatty Liver Disease

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Introduction: Neurotensin (NT) is a 13-amino acid peptide hormone released from the gut in response to high fat ingestion. Increased plasma levels of pro-NT (stable 117-amino acid NT precursor) significantly correlate with nonalcoholic fatty liver disease (NAFLD), a condition that affects nearly 25% of adult U.S population and is characterized by excess hepatic lipid accumulation (steatosis). Recent studies indicate that NAFLD is caused by mitochondrial dysfunction, however, the mechanism for this dysfunction remains elusive. We have previously reported that NT promotes fat absorption in the intestine and also induces hepatic steatosis, thereby playing an obligatory role in obesity. The purpose of the current study was to evaluate the mechanism of NT action in inducing lipid accumulation in liver and its potential role in causing mitochondrial dysfunction in NAFLD.
Methods: Hepatocytes were isolated from male or female: i) NT wild type (WT) ($Nt^{+/+}$) and knockout (KO) ($Nt^{-/-}$) mice fed a low fat diet (LFD,10% kcal from fat) or high fat diet (HFD, 60% kcal from fat) for 23 weeks, and ii) NT receptor 1 WT ($Ntr1^{+/+}$) and KO ($Ntr1^{-/-}$) mice. Hepatic lipid metabolism and signaling pathways, in the presence of exogenous NT and palmitic acid (PA), were analyzed via western blot and qPCR and verified in whole liver extracts. Mitochondrial oxidative phosphorylation (OXPHOS) protein abundance was measured by western blot. Mitochondrial function and ROS generation were measured by Seahorse - Cell Mito Stress Test and Mitotracker and MitoSOX dyes in isolated hepatocytes.
Results: NT promoted lipid (BODIPY-C16) uptake in hepatocytes. Moreover, NT deficiency improved mitochondrial respiratory capacity in mice with either Nt or Ntr1 deletion. NT treatment significantly decreased mitochondrial activity (Mitotracker Deep Red staining) and ROS generation (MitoSOX staining) in hepatocytes isolated from $Ntr1^{+/+}$ mice, which was attenuated in $Ntr1^{-/-}$ hepatocytes. Mitochondrial fractionation showed that NT treatment reduced OXPHOS protein abundance and related mitochondrial ROS generated by oxidation of fatty acids. Mechanistically, NT inhibited the mitochondrial biogenesis protein, PGC1α, and its transcriptional targets (such as nrf1, tfam, hnf4α) in livers of HFD-fed $Nt^{+/+}$ but not $NT^{-/-}$ mice. NT treatment also decreased PGC1α protein expression in hepatocytes, suggesting that NT/NTR1 signaling directly regulates PGC1α activity.
Conclusions: NT promotes hepatic steatosis by two mechanisms: i) increasing lipid uptake, and ii) decreasing mitochondrial function. Our findings demonstrate that NT/NTR1 signaling negatively regulates PGC1α activity, thereby contributing to mitochondrial dysfunction in NAFLD under obese conditions.

Abstract 27

Basic Science

Induction of cAMP in the Skin after UV Exposure Enhances Photolesion Clearance

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Past work has demonstrated that topical application of forskolin, a derivative of the Plectranthus barbatus plant root, enhances melanogenesis and the clearance of UV-generated DNA-photoproducts by pharmacologically activating adenylyl cyclase and producing intracellular cAMP in the skin. Thus, the cAMP signaling axis serves to protect the skin against UV damage and mutagenesis. To date, these observations were limited to mouse and cellular models wherein cAMP was induced prior to UV exposure, which implied that this approach might be translationally useful when applied before sun exposure. We investigated whether inducing cAMP after exposure would offer any UV-protective benefit. Using an amelanotic animal model that mimics human skin, we compared the clearance of photoproducts in the skin when either vehicle or forskolin was applied only after UV application. Mice provided forskolin demonstrated lower quantities of 6,4-photoproducts and cyclopyrimidine dimers (UV-induced DNA lesions) at time points subsequent to UV exposure as compared to controls. We further investigated the impact of post-UV application of forskolin on an A375 melanoma cell line in vitro. While cells treated with forskolin did seem to clear 6,4-photoproducts at an enhanced rate to controls, the relationship was not statistically significant. Together, these data suggest that cAMP induction might be useful even after UV exposure, and that enhancement of UV damage by cAMP may rely on cell-non-autonomous mechanisms in the skin. Further understanding of how cAMP impacts repair of UV photodamage is needed to develop translationally useful UV-protective strategies.

Abstract 28

Basic Science

Inhibition of De Novo and Salvage Pathways for dNTP Synthesis Enhances Sensitivity to Ionizing Radiation in Pancreatic Neuroendocrine Tumor Cells

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Introduction: The treatment of gastroenteropancreatic neuroendocrine tumors (GEP-NET) with ionizing radiation (IR) is a promising treatment modality for a disease that carries a poor prognosis. However, GEP-NETs confer high levels of radioresistance via the repair of DNA damaged by IR. Deoxynucleoside triphosphates (dNTPs) are needed for DNA repair and are produced by two pathways: de novo and salvage, which depend on ribonucleotide reductase (RNR) and deoxycytidine kinase (dCK), respectively. Ataxia telangiectasia and Rad3-related protein (ATR) activates dCK and can be targeted with inhibitors. We hypothesized that blocking both pathways for dNTP synthesis with an ATR inhibitor (ATRi) and the RNR inhibitor (RNRi), triapine, will sensitize GEP-NETs to IR in vitro.

Methods: (i) The CellTiter-Glo (Promega) luminescent cell viability assay established drug sensitivities of two pancreatic NET cell lines (BON and QGP-1) for triapine and three ATRi (AZD6738, VX-970, BAY1895344). Assays were performed with at least three replicates. Data were fit using a four-parameter log-logistic model, and IC50 values were calculated with R statistical software. (ii) Loewe synergy models were used to calculate the two-drug combination effects in both cell lines with synergy scores greater than 0 indicating synergistic effects and scores less than 0 representative of antagonistic effects. (iii) Clonogenic assays were performed and analyzed via colorimetry with sulforhodamine B to assess the effect of each drug alone and in combination with IR. (iv) Immunoblots were used to assess apoptosis and ATR activation following IR, RNRi, and ATRi treatments.

Results: (i) Cell viability assays showed lower IC50 values in the BON cell line compared to the QGP-1 cell line (BON cell IC50 : triapine 2.8 μM ± 0.6 μM, AZD6738 0.9 μM ± 0.1 μM, VX-970 239nM ± 18nM, BAY1895344 81nM ± 18nM; IC50 in QGP-1 cells: triapine 6.3 μM ± 1.4 μM, AZD6738 6.1 μM ± 1.3 μM, VX-970 2.4 μM ± 0.6 μM, BAY1895344 1.1 μM ± 0.4 μM). (ii) Loewe synergy models estimated the combination of BAY1895344 and triapine to be synergistic with scores greater than 0 in both cell lines (BON 9.89, p-value 4.07e-11; QGP-1 15.91, p-value 1.96e-7). (iii) Clonogenic assays showed an increase in efficacy of BAY1895344 when combined with IR in both BON (IC50 s: 150nM ± 5.7nM without radiation versus 19nM ± 37nM with 2Gy) and QGP-1 cells (IC50 values: 277nM ± 33nM without radiation versus 93nM ± 2.6nM with 2Gy). (iv) Western blot analysis showed strong activation of the ATR pathway with IR and triapine alone with subsequent inhibition by an ATRi as well as greater apoptosis with combination treatment compared to individual treatments.

Conclusion: Our findings show that treatment of GEP-NET cell lines with inhibitors that block the de novo and salvage pathways for dNTP production markedly sensitize these cells to subsequent IR. The combination of IR with both an ATRi and an RNRi has the potential to be an effective treatment modality for GEP-NETs.

Abstract 29

Basic Science

PLK1 Functions as an Oncogene to Promote Progression and Metastasis of Melanoma

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Melanoma is one of the most frequently diagnosed cancers in the caucasian population of both genders. The survival rate of advanced melanoma patients will be exceedingly poor, as low as 27.3%. BRAF-V600E is the most common mutation found clinically, resulting in the constitutive activation of the MAPK signaling pathway. PLX-4032, a specific inhibitor of mutant BRAF, has shown an impressive response in phase 3 clinical trials and has been approved by FDA in 2011. However, due to the rapid development of resistance, the duration of response under the single treatment is frequently short, highlighting the urgent requirement for novel therapy in melanoma treatment.

Polo-like kinase 1 (PLK1), a crucial cell cycle regulator, participates in multiple mitotic processes, including centrosome maturation, mitotic entry, spindle assembly, sister chromatid segregation, mitotic exit, and cytokinesis. Compared to the normal tissues, the expression level of PLK1 is significantly elevated in multiple cancers. Most importantly, the expression level of PLK1 negatively correlates with the melanoma patients' survival rate based on the TCGA database. Furthermore, PLK1 has been identified as an oncogene to promote proliferation, motility, and resistance to various drugs. In short, accumulating evidence has indicated PLK1 as a potent and promising target in cancer treatment.

In the present study, we have validated the strong synergy between PLK1 inhibitor BI6727 and PLX-4032 in the treatment of melanoma carrying BRAF-V600E. Our in vitro and in vivo experiments have shown an improved efficacy of this combined therapy on inhibition of cell proliferation, induction of cell death, and suppression of cell metastasis compared to mono-treatment. Moreover, we found that overexpression of PLK1 promotes tumor growth and metastasis of melanoma in the BrafCA/Ptenloxp mouse model, whereas knocking out PLK1 would dramatically suppress melanoma progression.

Abstract 30

Basic Science

Taxol-Elevated PLK1 Overcomes BETi Resistant via Phosphorylating and Triggering Degradation of BRD4 in Prostate Cancer

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Background: Prostate Cancer (PCa) is the first diagnosis of cancer among men in America. The bromodomain and extra-terminal (BET) family of protein 4 (BRD4) has been widely studied and pursued as an attractive therapeutic target of PCa. However, recent studies revealed that SPOP mutant-related BRD4 stabilization is correlated with its inhibitor (JQ1) resistance. Therefore, to reveal the mechanistic pathways that control BRD4 stability may improve the clinical response rate and efficacy of BRD4-targeted therapy in PCa patients.

Result: Firstly, our results indicated that BRD4 is decreased along with PLK1 increasing during the cell cycle. Enforced overexpression PLK1 promoted BRD4 dramatically reduced. However, upon treatment with RO3306 or BI6727, the degradation trend was totally blocked. Ubiquitination of BRD4 obviously enhanced under PLK1 attending while applying of PLK1 inhibitor attenuated BRD4 ubiquitination.

Secondly, we found PLK1 interacts with BRD4 in vitro and in vivo. The further assay revealed PBD domain of PLK1 is responsible for interacting with CTD of BRD4.

Thirdly, we found PLK1 directly phosphorylated BRD4 at Ser24 and S1100. We generated an antibody specifically against p-BRD4 S24. We found that p-BRD4 S24 is at its peak once the cells were arrested at M-phase in which PLK1 was also at its peak. Along with cell cycle releasing from M-phase, both p-BRD4 S24 and PLK1 are decreasing while total BRD4 increased.

Furthermore, Regarding the translational study, WB and IHC results released that elevating PLK1 negatively correlated with BRD4 in PCa patients' samples. Moreover, Taxol-induced elevation of PLK1 promotes BRD4 decreasing in SPOP WT and mutant cells. The combination of Taxol and JQ1 resulted in significant inhibition of proliferation, colony formation and PDX tumor.

Conclusion: Collectively, our results suggested that PLK1 triggered BRD4 degradation during M-phase in a PLK1 kinase activity-dependent manner. Taxol-induced PLK1 overexpression overcomes SPOP mutant-related BRD4 inhibitors resistant in prostate cancer.

Abstract 31

Translational

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

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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 PCa ranking as the second leading cause of cancer-related deaths in USA 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 B-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 ROCK is depleted or depleted ROCK cells are treated with ENZ, there is a significant hindering of cell migration and invasion. Also, utilizing a combination therapy of ROCK 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 goal of this study was to define the mechanism of PORCN in ENZ-resistant CRPC and develop a therapeutic approach to combat this disease. My research has determined that Porcupine (PORCN) is associated with CRPC progression to ENZ-resistance, and that an inhibition or loss of PORCN has resulted in the regain of ENZ sensitivity in ENZ-resistant models. This model has also demonstrated that PORCN and Wnt signaling engage in a paramount role contributing to AR activation, promoting CRPC progression, and the development of ENZ resistance.

Abstract 32

Basic Science

NMR Determination of the Enantiomeric Form of 2-Hydroxyglutarate in Renal Cell Carcinoma with an Isocitrate Dehydrogenase 2 Mutation

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Biologically important 2-hydroxy carboxylates such as lactate, malate and 2-hydroxyglutarate (2HG) exist in two enantiomeric forms (D and L) with different biological origins and functions and cannot be distinguished under achiral conditions. In order to identify them, we have optimized a derivatization technique using diacetyl-L-tartaric anhydride (DATAN) to produce diastereomers from 2-hydroxy carboxylates enantiomers and applied 1D and 2D NMR methods directly to distinguish the enantiomers without the need for chromatography. We also demonstrated the application of this method on the ex-vivo media and tissue extract of a human renal cell carcinoma and showed the quantitative conversion to the diastereomers, enabling the determination of the enantiomeric ratio of 2-hydroxycarboxylic acids with high precision in various biological samples.

Abstract 33

Basic Science

ATR-I774Yfs*5 Promotes Genomic Instability through Micronuclei Formation

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Although mismatch repair (MMR) defects are associated with high risk of malignancy, the specific oncogenic drivers pertinent to MMR-affected cancers are poorly characterized. The heterozygous ATR-I774Yfs*5 mutation, the result of strand slippage in a poly-A tract of the Ataxia Telangiectasia and Rad3 related (ATR) gene, is overexpressed in MMR-defective malignancies including colorectal carcinoma (CRC) and is the most common ATR mutation in cancer. Here, we explore the contribution of ATR-I774Yfs*5 to genomic integrity. Using heterozygous ATR-I774Yfs*5 HCT-116 cells to mimic the native mutation, we found this mutation reduced ATR activity as measured by damage-induced Chk1 phosphorylation at S317 and ATR autophosphorylation ATR at T1989. ATR-I774Yfs*5 expression impaired genomic stability as visualized by the appearance of micronuclei in two stable expression models as well as in cell lines transfected with ATR-I774Yfs*5. Micronucleus development was dependent on replication and independent of ATR copy number. ATR-I774Yfs*5 expression did not alter cellular viability, cell cycle progression, or replicative rate, suggesting this mutation is well-tolerated despite its destabilizing effect on the genome. Taken together, these data suggest that the ATR-I774Yfs*5, whose development is favored in the context of MMR deficiency, may represent an important driver of a mutator phenotype by promoting genomic instability.

Abstract 34

Population-Based/Behavioral

Structural Equation Modeling of Healthcare Access Dimensions with Ovarian Cancer Treatment: Analysis of the Ovarian Cancer Epidemiology, Healthcare Access and Disparities (ORCHiD) Study

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Background: Racial disparities have been documented in various measures of healthcare access (HCA). Here, we conducted structural equation modeling (SEM) to define healthcare affordability–the ability to afford care, availability-- the type, quality, and quantity of healthcare resources, and accessibility-- the geographic location of healthcare resources and evaluated the direct and indirect associations with ovarian cancer treatment quality measures.

Methods: Black and Non-black patients with primary ovarian cancer of any histologic type ages 65 years or older diagnosed between 2008-2015 were selected from the SEER-Medicare linked dataset. Factor analysis for the three HCA dimensions measurable in SEER-Medicare was used to define HCA latent variables, with factor loadings higher than 0.4 summarized into HCA scores for each dimension. Reliability tests were performed using composite reliability (Ω; 0.7 or greater) and average variance extracted (AVE; 0.5 or greater). Structural Equation Models were performed using Mplus 8 to evaluate total, direct, and indirect associations of race and HCA dimensions with ovarian cancer treatment quality measures.

Results: A total of 8,987 patients with ovarian cancer were included in the analysis; about 7% were Black. Final SEM was conducted with 20 variables and three HCA latent values. The Affordability (Ω: 0.876; AVE=0.689), Availability (Ω: 0.848; AVE=0.636), and Accessibility (Ω: 0.798; AVE=0.634) latent variables showed high composite reliability. Black patients were less likely to visit a gynecologic oncologist (45% vs. 54%, p-value <0.0001) and less likely to receive any ovarian cancer-related surgery in the 12 months following diagnosis (50% vs. 62%, p-value <0.0001). Black patients had lower Affordability and Availability on average, both of which were associated with a lower likelihood of seeing a gynecologic oncologist, which in turn was associated with a lower likelihood of receiving surgery.

Abstract 35

Clinical

Association between CD47 Expression and Clinicopathologic Characteristics and Survival Outcomes in MIBC

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Introduction: CD47 is an antiphagocytic molecule that plays a critical role in immune surveillance. A variety of malignancies have been shown to evade the immune system by increasing the expression of CD47 on the cell surface. As a result, anti-CD47 therapy is under clinical investigation for these. CD47 overexpression is associated with negative clinical outcomes in lung and gastric cancers; however, the expression and functional significance of CD47 in bladder cancer is not fully understood.

Materials and Methods: We retrospectively studied patients with muscle invasion bladder cancer (MIBC) on transurethral resection of bladder tumor (TURBT) who subsequently underwent radical cystectomy (RC) with or without neoadjuvant chemotherapy (NAC). CD47 expression was examined by immunohistochemistry in both TURBT and matched RC specimens. Expression levels $\geq$ 1 % were considered positive. The difference between CD47 expression levels between TURBT and RC were also compared. The association of CD47 levels (TURBT) with clinicopathological parameters and survival outcomes were evaluated by Person's chi-squared test and Kaplan-Meier method respectively.

Results: A total of 87 MIBC patients were included. The median age was 66 (39-84) years. The majority of patients were Caucasian (95%), male (79%), and age >60 (63%). Most patients (75%) underwent NAC prior to RC. Of those who received NAC, 35% were responders and 64% were non-responders. Responders includes those with a pathologic complete response (T0) or partial response (Tis or T1); non-responders included those with stage $\geq$ T2. The final reported stages for all patients were as follows: stage 0 (32%), stage I (1%), stage II (20%), stage III (43%), and stage IVA (5%). 60% of patients were alive, 40% died from bladder cancer, and 30% had disease recurrence at a median follow-up of 3.1 (0.2-14.2) years. CD47 levels were detectable in 38 (44%) TURBT samples. There was no association between CD47 levels and clinicopathological parameters such as age, gender, race, NAC, final stage, disease recurrence, and overall survival (OS). Patients >60 (p=0.006), non-responders (p=0.002), stage $\geq$ III (p <0.001) were associated with worse OS by univariate analysis. There was no significance with multivariate analysis. The median OS for positive CD47 levels was 4 years (p=0.7). There was a slight positive trend for decreased CD47 levels between TURBT and RC in patients who received NAC (p=0.5), though this did not reach statistical significance.

Conclusion: CD47 expression is not a prognostic marker for MIBC patients. However, expression of CD47 was detected in nearly half of MIBCs, and future studies are needed to explore a potential role for anti-CD47 therapy in these patients. Furthermore, there was a slight positive trend between decreased CD47 levels (from TURBT to RC) in patients receiving NAC. More research is needed to understand how NAC may or may not modify immune surveillance mechanisms in MIBC.

Abstract 36

Basic Science

Ethanol Exposure Up-Regulates PD-L1/PD-1 Immune Checkpoint Pathway and Promotes Mammary Tumorigenesis

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Background: Around 5,000,000 new cases of breast cancer are reported annually in the United States. Evidence suggests that alcohol consumption, even moderate drinking, is associated with an increased risk of breast cancer. Epidemiological data also show that breast cancer risk does not vary by beverage type indicating that ethanol is the main causal factor. Mammary tumor development is a complex pathobiological process. The dynamic interaction between tumor cells and the tumor microenvironment may lead to disruption of tissue homeostasis, tumor growth, and progression. CD8+ cytotoxic T cells play a critical role in host anti-tumor immunity against breast tumorigenesis, which can be regulated by immune checkpoint pathways. The goal of the current study is to examine the role of the major immune checkpoint PD-1/PD-L1 pathway in ethanol-enhance mammary tumorigenicity.

Methods: FVB.Cg-Tg(Wnt1)1Hev/J transgenic mice develop spontaneous mammary tumors starting around 2-3 months old and have been widely-used mouse models for breast cancer research. In this study, female mice at the age of 5 weeks were randomly assigned into seven groups: control, ethanol exposure, PD-L1 or PD-1 antibody injection, ethanol exposure plus PD-L1 or PD-1 antibody injection, and IgG isotype control group. Mice were exposed to ethanol by feeding with an ethanol liquid diet (Bio-Serv, Flemington, NJ), while mice without the ethanol treatment were fed with an isocaloric liquid diet in which maltose was used to substitute isocalorically for ethanol. The ethanol concentration in the ethanol diet was increased with up-titration by the following: week 1, 2% ethanol; week 2, 4% ethanol; weeks 3 and on: 6.7% ethanol (which resulted in an average blood ethanol concentration of 80.17 ± 9.06 mg/dl in the early morning). Diet was provided ad libitum for the experimental period. For inhibition of PD-L1 or PD-1, rat anti-mouse PD-L1 antibody or PD-1 antibody were administered by intraperitoneal injection at the dose of 200 μg /mouse at the 6th, 8th, and 10th week. Rat IgG2a and IgG2b at the same dosage was used as isotype/injection control. During the 22-week experiment period, the body weights of mice and general health conditions were evaluated and recorded regularly.

Results: Ethanol exposure increased mammary tumorigenicity in the mice, which was ameliorated by the administration of PD-L1 or PD-1 antibodies. In addition, PD-L1 was up-regulated by ethanol on mammary tumor cells. Ethanol exposure also increased tumor-infiltrated PD-1+CD8+ T cells. Further, CD8+ T cells' cytotoxicity functions were compromised by ethanol exposure, which was mitigated by treatment of PD-L1 or PD-1 antibody.

Conclusion: Long-term ethanol exposure enhances mammary tumorigenesis accompanied with up-regulation of PD-1/PD-L1 immune checkpoint pathway and inhibition of the cytotoxic effector function of CD8+ T cells, which was mitigated by co-treatment of PD-1 or PD-L1 antibodies. The data indicate that PD-1/PD-L1-mediated inhibition of T cell anti-tumor function contributes to ethanol-enhanced mammary tumorigenesis.

Abstract 37

Basic Science

Pharmaceutical Inhibition of Latexin by a Newly Discovered Latexin Inhibitor Does Not Promote Leukemia Stem Cell (LSC) Proliferation and Expansion

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Leukemia is a hematopoietic malignancy caused by mutations in bone marrow hematopoietic stem cell (HSCs). Leukemia cells have been reported to possess the ability to modify the cellular microenvironment, further promoting their expansion at the expense of normal HSC function. Thus, except for therapies specifically targeting leukemic cells, restoration of hematopoietic homeostasis is important for leukemia treatment. Latexin (Lxn), a canonical carboxypeptidase A inhibitor, has been demonstrated to play important roles in the maintenance of hematopoietic homeostasis. Our published and ongoing studies have shown that genetic deletion of Lxn could: 1) enhance HSC survival and promote hematopoietic homeostasis; 2) mitigate radio- and chemotherapy-induced blood and HSC suppression; and 3) supress leukemic stem cells (LSCs). A fine tune of Lxn level could be a pivotal key for leukemia treatment. We thus developed small molecule screenings to search for Lxn inhibitors. A top candidate inhibitor was identified. Its PubChem CID number in NIH PubChem library is "58030856" ($C_{13}H_{11}N_5O_4S$, MW 333). We have found that it increases HSC function. We thus investigated the effect of Lxn inhibition on LSC growth and proliferation in vitro by treating LSCs and a leukemic cell line with this inhibitor. We made LSCs by transfecting HSCs with acute myeloid leukemia (AML)-specific MLL-AF9 and chronic myeloid leukemia (CML)-specific BCR-ABL oncogenic proteins. We treated both AML-LSCs, CML-LSCs and MOLM-13 (AML cell line) with different concentrations of Latexin inhibitor (20 μM, 50 μM, and 100 μM). We found that Lxn inhibitor in vitro inhibited growth of leukemia cells (MOLM-13) by 23% at 100 μM concentration and did not promote LSCs proliferation and expansion. Thus, Lxn inhibition not only enhances HSC function, but also inhibits LSCs and leukemic cells, indicating a two birds one stone effect. Thus, our newly discovered Lxn inhibitor could be a potential clinical treatment of leukemia. In the future, we will further evaluate the clinical value of this Lxn inhibitor in vivo and preclinical leukemia models with the long-term goal to translate these findings into the clinical setting.

Abstract 38

Basic Science

Mitochondrial Superoxide Targets Energy Metabolism to Modulate Epigenetic Regulation of NRF2-Mediated Transcription

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Mitochondria are central to the metabolic circuitry that generates superoxide radicals/anions ($O_2^{\bullet-}$) as a by-product of oxygen metabolism. By regulating superoxide levels, manganese superoxide dismutase plays important roles in numerous biochemical and molecular events essential for the survival of aerobic life. In this study, we used MitoParaquat (mPQ) to generate mitochondria-specific $O_2^{\bullet-}$ and stable isotope-resolved metabolomics tracing in primary human epidermal keratinocytes to investigate how $O_2^{\bullet-}$ generated in mitochondria regulates gene expression. The results reveal that isocitrate is blocked from conversion to α-ketoglutarate and that acetyl-coenzyme A (CoA) accumulates, which is consistent with a reduction in oxygen consumption rate and inactivation of isocitrate dehydrogenase (IDH) activity. Since acetyl-CoA is linked to histone acetylation and gene regulation, we determined the effect of mPQ on histone acetylation. The results demonstrate an increase in histone H3 acetylation at lysines 9 and 14. Suppression of IDH increased histone acetylation, providing a direct link between metabolism and epigenetic alterations. The activity of histone acetyltransferase p300 increased after mPQ treatment, which is consistent with histone acetylation. Importantly, mPQ selectively increased the nuclear levels and activity of the oxidative stress-sensitive nuclear factor erythroid 2-related factor 2. Together, the results establish a new paradigm that recognizes $O_2^{\bullet-}$ as an initiator of metabolic reprogramming that activates epigenetic regulation of gene transcription in response to mitochondrial dysfunction. DOI: 10.1016/j.freeradbiomed.2021.12.309

Abstract 39

Basic Science

NEDD4L Promotes the Ubiquitination and Internalization of PTPRF to Inhibit Wnt Signaling

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PTPRF belongs to a family of receptor-type protein tyrosine phosphatases. Our previous studies have identified PTPRF as a positive regulator of the Wnt signaling pathway. However, little is known on how the expression and localization of PTPRF is regulated. In this study, we show that NEDD4L, an E3 ubiquitin ligase, controls PTPRF protein stability and membrane localization. Overexpression of NEDD4L decreases the half-life of PTPRF whereas knockdown of NEDD4L has the opposite effect. Interestingly, NEDD4L utilizes the K29 and K63 linkage to ubiquitinate PTPRF, a process that also relies on the phosphatase activity of PTPRF. In addition, treating cells with endocytosis inhibitors has no effect on PTPRF ubiquitination, suggesting that NEDD4L modulates PTPRF ubiquitination at the membrane. Moreover, NEDD4L-dependent ubiquitination promotes PTPRF internalization and trafficking to multivesicular bodies as indicated by colocalization with Hrs. Functionally, NEDD4L blocks the ability of PTPRF to promote Wnt activation as a result of removing PTPRF from the plasma membrane. Taken together, our study identifies NEDD4L-dependent ubiquitination of PTPRF as a novel mechanism that fine-tunes the regulation of Wnt signaling.

Abstract 40

Basic Science

Development and Validation of Nanobodies Specific to the Oncogenic Phosphatase Protein Tyrosine Phosphatase 4A3 (PTP4A3 or PRL-3)

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Protein Tyrosine Phosphatase 4A3 (PTP4A3 or PRL-3) is an oncogenic dual-specificity phosphatase that drives tumor metastasis, promotes cancer cell survival, and is correlated with poor patient prognosis in a variety of solid tumors and leukemias. The mechanisms that drive PRL-3's oncogenic functions are not well understood, in part due to a lack of research tools available to study this protein. The development of such tools has proven difficult, as the PRL family is ~80% homologous and the PRL catalytic binding pocket is shallow and hydrophobic. Currently available small molecules do not exhibit binding specificity for PRL-3 over PRL family members, and the only research antibody specific for PRL-3 can only recognize denatured protein.

To address the lack of tools available to study PRL-3, we have developed alpaca-derived single domain antibodies, or nanobodies, targeting PRL-3. Nanobodies have emerged as a valuable research tool and show promise as cancer therapeutics as they are ~15kD and lack light chains, allowing them to reach cavities within active sites that conventional antibodies cannot normally reach. Nanobodies also maintain high specificity and affinity for their antigens.

We identified seven unique nanobodies that bind to PRL-3 with no activity towards PRL-1 and PRL-2, making our nanobodies one of the first tools to selectively target PRL-3 in its native state. We used biolayer interferometry and found the nanobody binding affinity for PRL-3 to be within a KD of 30 - 300 nM, similar to that of antibodies currently on the market. We identified PRL-3:nanobody interactions with hydrogen-deuterium exchange mass spectrometry (HDX-MS) and showed binding outside the active site. These data were confirmed by analyzing the effects of nanobodies on PRL-3 phosphatase activity and substrate binding. Our anti-PRL-3 nanobodies specifically pulled down PRL-3 over PRL-1/-2 in immunoprecipitation experiments. Finally, we used these nanobodies to analyze PRL-3 localization in fixed immunofluorescence experiments in human cancer cells. We found that a C-terminal tag on PRL-3, such as FLAG or GFP, enhanced PRL-3 localization to the membrane, compared to untagged protein, which may have confounded previous PRL-3 functional studies.

We are currently utilizing these nanobodies in two ways to understand PRL-3's role in cancer. First, we are assessing PRL-3 function and trafficking during various cancer processes, such as proliferation, invasion, and stress, to determine how PRL-3 localization contributes to cancer progression. Secondly, we are utilizing these nanobodies to validate potential interacting partners of PRL-3, that may be responsible for trafficking PRL-3 throughout the cell under these conditions. In total, our nanobodies will allow us to decipher a more specific role for PRL-3 in cancer that could be targetable in the future.

Abstract 41

Core Resources (Informational and not judged)

Patient-Oriented and Population (POP) Science Shared Resource Facility of Markey Cancer Center

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Markey Cancer Center's Patient-Oriented and Population Sciences Shared Resource Facility (POP Sciences SRF) plays a key role in helping Markey Cancer Center (MCC) clinician-scientists and researchers conduct research on the psychosocial, behavioral, and epidemiologic aspects of cancer prevention and control. The POP Sciences SRF provides a range of Pre-Award and Post-Award services to researchers. Pre-award services include scientific consultation regarding various aspects of the research process, such as selection of patient-reported outcome measures; measuring health behavior change and related processes; selecting the best platform for data collection; modifying recruitment strategies and informed consent procedures to streamline enrollment; refining a study design to capitalize on MCC, institutional, and community resources; linking investigators with MCC clinics, other SRFs, and relevant campus resources; fostering collaborations within and across MCC. Post-award services include accruing participants into observational studies or clinical trials; collecting data via survey research or qualitative methods; performing select qualitative research activities such as transcription and thematic analysis; coordinating with investigative team members, MCC clinics and providers, community partners and other SRFs. The POP Sciences SRF research collaborations are facilitated with help from the Kentucky Cancer Program, Kentucky Cancer Consortium, MCC Community Impact Office, MCC Research and Affiliate Networks plus other established partners in the community. The POP Science Shared Research Facility is staffed by three full-time research associates, a full-time manager, and a graduate research assistant and maintains a library of tools needed to support its research enterprise (e.g., audio recorders, iPads, qualitative data analysis software, PRO measures).

We welcome the opportunity to collaborate with you and support your cancer-relevant research.

Abstract 42

Core Resources (Informational and not judged)

The Cancer Research Informatics (CRI) Shared Resource Facility of the Markey Cancer Center

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The Cancer Research Informatics (CRI) Shared Resource Facility is a cancer center-managed shared resource that provides comprehensive informatics support across basic, clinical, translational, and population-based research at the Markey Cancer Center (MCC). The mission of CRI is to facilitate collaborative research among MCC members through the optimal application of informatics methods and technologies that maximize the accessibility and usability of data, information and knowledge in research. A key function of the CRI is the seamless integration of clinical, multi-omics, socio-economic, environmental, and outcome data into the Cancer Research Data Commons (CRDC) to support collaborative research by MCC research program members. Our data resources encompass a wide spectrum from population-based cancer data from the National Cancer Institute (NCI) Surveillance Epidemiology and End Results (SEER) Kentucky Cancer Registry to the mutational variants and unique phenotypes of individual cancer patients.

CRI resources, expertise, and services that are available to Cancer Center Members are categorized broadly into the following:

1. Curate, harmonize, integrate, and deliver clinical and molecular data for scientific discovery
2. Provide innovative high throughput computational methods in drug discovery
3. Support AI methods (deep learning and natural language processing) for social media, clinical, and molecular data mining

Our newest service is specifically focused on integrating molecular tumor profiles with SEER registry data that are made available in Markey's deployment of cBioPortal.

The CRI SRF is currently comprised of faculty with extensive cancer-specific experience encompassing basic, clinical, population-based and translational studies across all MCC research programs. Expertise includes patient cohort discovery, data integration of molecular and clinical data, natural language processing, machine learning, social media data mining, and in silico modeling and drug discovery. Professional staff include master's level managers responsible for software development and research computing systems, and professional staff with expertise in software development, mobile-app development, database management, data curation and biospecimen data management. Expertise in secure high-performance computational resources and multi-omics data storage is another focus area for the CRISRF.

Please visit the CRI website at https://ukhealthcare.uky.edu/markey-cancer-center/research/srf/cri.

Abstract 43

Basic Science

ABL1/2 Drives a Pro-Tumorigenic Microenvironment During Melanoma Resistance to BRAF/MEK Inhibitor Treatment

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Despite the development of newer therapeutic agents, metastatic melanoma remains an incurable disease for many patients (5-year survival rate; 27% in 2021). While immunotherapy is curative for some patients, others (@50%) are resistant to its effects or cannot tolerate the therapy. In contrast, targeted, BRAF/MEK inhibitor (BRAFi/MEKi) combination therapy is effective in reducing metastatic burden for most patients whose melanomas harbor BRAF V600 mutations; however, for the vast majority, resistance develops after 12-13 months, which results in rapid disease progression. Thus, new drug combinations are needed for patients that cannot tolerate or develop resistance to the above therapies. ABL family non-receptor tyrosine kinases (ABL1, ABL2) are most known for their involvement in human leukemia. Recently, we showed that they are critical drivers of melanoma acquired resistance to BRAFi/MEKi (dabrafenib/trametinib; D/T), and induce reactivation of MEK/ERK signaling via a novel pathway (Nat Comm, 2020). Moreover, nilotinib, a 2nd generation ABL1/2 inhibitor, dramatically reversed BRAFi/MEKi resistance in immune-deficient xenograft models. These data led to the development of a MCC Phase I clinical trial to examine the safety and initial efficacy of combining nilotinib with D/T for patients with BRAFi/MEKi-resistant disease.

Here, we extend our findings by demonstrating that nilotinib also significantly delays/prevents and reverses BRAFi/MEKi resistance in immune-proficient, syngeneic and genetically-engineered melanoma mouse (BRAF/PTEN) models. Moreover, D/T+nilotinib not only inhibits melanoma growth, in vivo, but also impacts the microenvironment. Using flow cytometry and single-cell RNA sequencing, we demonstrate that after only four days of treatment, tumors from D/T+nilotinib-treated mice have significantly increased anti-tumorigenic immune cell infiltration (e.g. CD8+ T cells, gdT cells, NK cells) and reduced numbers of pro-tumorigenic, immunosuppressive immune cells (e.g. MDSCs, TAMs) compared to tumors from mice treated with D/T alone. Bulk RNA sequencing reveals upregulation of mRNAs encoding chemokines and cytokines known to increase pro-tumorigenic immune cell infiltration in BRAFi/MEKi-resistant cells compared with their parental counterparts. Moreover, treatment with D/T+nilotinib blocks upregulation of some of these secreted factors (as compared to treatment with D/T alone). In addition to dramatically affecting immune cell infiltration, D/T+nilotinib also induces expansion of fibroblasts and endothelial cells (ECs; relative to D/T alone) within the tumors. Ongoing bioinformatics analyses is aimed at identifying the type of ECs and fibroblasts that are expanded, and future functional experiments will assess the contribution of the immune populations and associated chemokines/cytokines as well as ECs/fibroblasts to nilotinib's ability to reverse and prevent D/T resistance. In summary, our data indicate that ABL1/2 likely drive resistance not only by impacting the melanoma cells themselves (intrinsic) but also by affecting the melanoma microenvironment (extrinsic).

Abstract 44

Basic Science

Role of Prx4 in Prostate Cancer Development and Radiation Resistance

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Introduction & Objective: The peroxiredoxin (Prx) family of proteins functions as major cellular antioxidants that mediate oxidative signaling under physiological conditions as well as scavenge extra hydrogen peroxide in the context of oxidative stress. Among them, Prx4 is encoded by PRDX4 gene on X chromosome and has been found to contribute to the development of male reproduction system. Previous studies indicate that Prxs are frequently upregulated in various types of human cancer. However, the role of Prx4 in prostate cancer has not been well defined. The purpose of this study is to examine the expression of Prx4 in prostate cancer and to explore its functional significance in cancer radiation resistance and recurrence.

Methods: Bioinformatic tools were used to evaluate genetic alterations of PRDX4 gene as well as the levels of its transcripts in prostate normal and cancer populations. Kaplan-Meier survival analysis was used to explore the association of Prx4 levels with the prognosis of prostate cancer patients. Western blot and immunohistochemistry were used to evaluate the expression of Prx4 protein in cell lines and patient specimens. Loss of Prx4 in cells was achieved by knock down using lentiviral shRNA or knockout using CRISPR-Cas9 techniques. Cell proliferation, survival, migration and protein profiler kinase arrays were used to examine the differences between control and Prx4-depleted cells with or without ionizing radiation. In vivo studies were performed to further identify the function of Prx4 in prostate cancer development and radiation resistance.

Results: We demonstrated that PRDX4 gene is frequently amplified in prostate cancer and the level of its transcript is highly elevated. Patients with Prx4 at higher quartile have significantly reduced probability of survival compared with those in lower quartile. Prostate cancer cells express much higher levels of Prx4 than normal epithelial cells. Moreover, Prx4 is upregulated by the activation of AR-dependent signaling, and depletion of Prx4 sensitizes prostate cancer cells to radiation-induced cell death. Mechanistically, Prx4 contributes to prostate cancer radiation resistance through the activation of PI3K/AKT signaling pathway.

Conclusions: A combination of bioinformatic, histochemical, cellular, and molecular methods reveals that Prx4 plays a critical role in prostate cancer cell proliferation, radioresistance and reoccurrence.

Abstract 45

Basic Science

ABL and DDR Tyrosine Kinases Cooperate to Drive MEKi Resistance in NRAS-Mutant Melanomas

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Melanomas of the skin are among the most prevalent cancers in the U.S., and, unlike other cancers, their incidence rates have continued to rise over the past 30 years. NRAS mutations drive a particularly aggressive and deadly subtype of melanomas. First-line treatment for patients with metastatic NRAS-driven melanomas is immunotherapy, but only a subset of patients can tolerate the treatment and respond to their effects. Second-line regimens are limited to cytotoxic agents that are ineffective and have many adverse effects. Importantly, there are no FDA-approved targeted therapies for these patients because drugs that target the downstream RAF/MEK/ERK pathway, which are effective for patients with mutant BRAF-driven melanomas, do not increase progression-free survival (PFS) in patients with NRAS-driven melanomas due to intrinsic and acquired resistance. Here, we demonstrate that ABL1/2 and DDR tyrosine kinases cooperate to drive acquired resistance to MEK inhibitors (MEKi) in NRAS-mutant melanomas. Previous research shows that ABL1/2 play a critical role in driving the development of leukemia, and both ABL1/2 and DDR1/2 have oncogenic roles in melanoma. Using cell lines that we engineered to develop resistance to the MEKi, trametinib, we show that targeting ABL/DDR in combination with trametinib prevents survival and clonogenicity of resistant cells, induces apoptosis, and efficiently blocks BRAF/CRAF activation of MEK/ERK/MYC signaling during resistance. The signaling pathway we identified is clinically relevant as ABL2 and MYC mRNA expression are correlated in samples from patients harboring NRAS-mutant melanomas treated with MEKi. Furthermore, targeting ABL/DDR with nilotinib, an FDA-approved anti-leukemia drug, significantly delays the onset of MEKi resistance, in vivo. These results suggest that combining nilotinib and MEKi may be effective for treating patients with NRAS-driven melanomas, a highly aggressive subtype with few treatment options.

Abstract 46

Basic Science

Gender Differences in Hematopoietic Stem Cell and Niche

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Hematopoietic stem cells (HSCs) persist throughout the lifespan of an organism. One HSC can form a whole-blood system. The balance between HSCs differentiation and self-renewal is very important. If the balance is broken, it will lead to serious diseases such as leukemia, which is the fifth leading cause of cancer-related death. In SEER cancer statistic data, women consistently exhibit a lower incidence of hematological cancers compared to men. Under normal conditions, the number of circulating progenitor cells in women is lower than in men. Moreover, HSC division in female mice is more frequent compared to male mice. A dramatic gender difference exists in leukemia patients and also in the normal hematopoietic systems. However, very little is known about the sex differences and the underlying mechanisms in hematopoiesis and leukemia. To explore whether intrinsic or extrinsic factors contribute to gender differences, we set up two transplantation models. For the first transplantation model, we injected male bone marrow (BM) cells into both male and female recipients and also injected female BM cells into both male and female recipients. We found that the donors, no matter from male or female mice, transplanted to male recipients have better engraftment than female recipients. To further confirm and explore the intrinsic role in transplantation, we set up another model. We mixed equal numbers of male and female BM cells together, then injected the mixer into male and female recipients separately. We found that the transplantation of male donors to male recipients has the highest efficiency of engraftment. Thus, in our study, both intrinsic and extrinsic factors can contribute to gender differences. Bone marrow cells engrafted better in male recipients than female recipients. By conducting the single-cell analysis of the HSC niche, we found males have more MSCs (Mesenchymal Stem Cells) than females. Cxcl12 is a secretion protein, which can maintain HSC and induce mobilization. We found that male MSCs express more Cxcl12 than female MSCs. It may partially explain why the male niche supports HSC engraftment better. KDM5C (Lysine-specific demethylase 5C), which is located in the X chromosome, escapes from X chromosome inactivation. Thus its expression is double dose in female cells compared to male cells. KDM5C specifically catalyzes H3K4me3/me2 demethylation and inhibits gene transcription by decreasing H3K4 methylation. We have found that the Cxcl12 promoter has H3K4me3 binding sites, suggesting that KDM5C may contribute to the gender-specific differential expression of Cxcl12 through H3K4 modification. Knockdown KDM5C will up-regulate the expression of Cxcl12 in MSCs, and lead to the higher expression of Cxcl12 in male MSCs. In conclusion, the lower level of KDM5C in male MSCs leads to the higher expression of Cxcl12 which provides better support for HSC engraftment and maintenance.

Abstract 47

Clinical

Impact of Cytologic Rapid On-Site Evaluation on Pancreatic Biopsy Diagnostic Rate

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Background: Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) has become the standard biopsy technique for pancreatic lesions. Unfortunately, the non-diagnostic rate on cytologic evaluation may be significant (averaging 20-25%). Rapid On-Site Evaluation (ROSE) by cytopathologists is sometimes performed during the procedure in an attempt to assess specimen adequacy at the time of collection. In this study, we compared diagnostic rates of EUS-FNA biopsies of pancreatic lesions that were obtained with and without ROSE.

Methods: All of the pancreatic EUS-FNA specimens collected over a 2.5 year period (January 2019-June 2021) and submitted to the University of Kentucky cytopathology laboratory were included in the study. Of 436 total cases, 356 had ROSE performed by cytopathology faculty. The remaining 80 FNA specimens without ROSE were rinsed in plasmalyte and sent directly to the cytopathology lab for processing and interpretation. The diagnostic rate was assessed in two different ways: 1) The diagnostic rate was defined as a cytologic diagnosis that was clinically actionable, meaning that it was reflective of the needle being in the targeted lesion, and that it provided information that informed appropriate next steps in patient management, and 2) The diagnostic rate was defined as any final diagnosis rendered by a cytopathologist other than "non-diagnostic."

Results: For method #1, the diagnostic rate of pancreatic EUS-FNA specimen both with and without ROSE was 77%. For method #2, the diagnostic rate for biopsies obtained with ROSE was 95%, while the rate of those obtained without ROSE was 91%.

Conclusion: The data suggests that no matter how the diagnostic rate is determined, ROSE assessment has minimal if any impact in obtaining a diagnostic specimen. As this procedure is time-consuming for the practitioners and has an added financial cost for the patient, this practice should be re-assessed. Potential problems with this study include possible performer bias (i.e. more experienced endoscopists tend to request less ROSE procedures) and relatively small case numbers for the aspirates not assessed by ROSE.

Abstract 48

Basic Science

Biological and Toxicological Effects of Filtered Cigars

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Exposure to tobacco smoke is a risk factor in greater than 85% of lung cancers and contributes to other cancers as well as respiratory diseases such as chronic obstructive pulmonary disease. Kentucky has the highest incidence of lung cancer in the U.S., almost assuredly due to high rates of using cigarettes and other tobacco products. Filtered cigars are specialized tobacco products that in many ways are like cigarettes including their visual appearance and, more importantly and unlike other cigars, active inhalation of their smoke into the lungs. However, filtered cigars have not been the subject of extensive research as to their possible health effects. In this project, we have examined biological and toxicological properties of filtered cigars in comparison to conventional cigarettes, using reference products available from the Center for Tobacco Products in the UK College of Agriculture. Smoke condensates from filtered cigars and cigarettes were used to treat human lung and oral epithelial cell models as well as a specialized cell model engineered to be a gene reporter system for the Arylhydrocarbon Receptor (AHR) pathway that responds to many xenobiotic compounds including polyaromatic hydrocarbons present in tobacco smoke. Furthermore, we also compared how different smoking regimens affected the chemical composition and toxicity of the filtered cigars. For certain experiments, comparisons were performed based on one cigarette versus one filtered cigar, equivalent total particulate matter (TPM), or equivalent tobacco weight. For most of our comparisons, condensates from filtered cigars showed even higher toxicity than conventional cigarettes. Importantly, filtered cigars produced much more total particulate matter per rod than cigarettes. Additionally, filtered cigar smoke condensates induced stronger activation of the AHR pathway than cigarette smoke condensates. Although their cytotoxicity was comparable when measured based on equivalent TPM, the cytotoxicity of filtered cigar condensates would be much higher based on a product-to-product or equivalent tobacco weight comparisons. Also, condensates generated using the Canadian Intense smoking regimen produced even higher TPM levels and elicited much stronger induction of the AHR pathway than other smoking regimens. Ongoing studies are examining the toxicity of a variety of commercial filtered cigar products to determine if results are comparable to reference filtered cigars. If so, our findings would suggest that filtered cigars may be more harmful than conventional cigarettes, especially if they are consumed similarly to cigarettes.

Abstract 49

Translational

Evolution of Radio-Resistance in H3K27M-Driven Diffuse Midline Gliomas

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H3K27M-driven Diffuse Midline Gliomas are a subset of malignant pediatric gliomas that have a >90% mortality rate within 5 years of diagnosis and no current cure. They arise from the transformation of neural progenitor cells and are commonly resistant to radiotherapy and multiple types of chemotherapies. Recently Vinci et al. (2018) sequenced 142 patient samples and determined that the bulk of patient tumors are multi-clonal and each subclone in a patient tumor can have distinct mutational and gene expression profiles. Along with this intratumoral heterogeneity, intertumoral heterogeneity in H3K27M driven pediatric DMGs is common across patient populations with various p53 loss of function mutations and several other driver mutations. Despite this widespread heterogeneity, every H3K27M-driven DMG will become resistant to radiation, the current standard of care. This innate resistance suggests these tumors have a common survival mechanism that is inherent to the biology of this tumor type.
Our approach aims to understand the mechanisms of radio-resistance in H3K27M-driven DMG. We have isolated single clones from patient naive samples, and found that, even within the same patient tumor, resistance to radiotherapy varies by clone. Susceptible clones are currently being evolved to radio-resistance to determine what pathways are necessary for clone survival and tumor repopulation. To gain temporal resolution of radiation induced cell death and cell senescence, we have established clones that express a genetically encoded death indicator (GEDI) and a live cell cycle indicator (FUCCI). These tools will allow us to identify clones that have intrinsic adaptive mechanisms that confer radio-resistance as well as determine whether these adaptive mechanisms are tied to cell senescence or stem/progenitor cell phenotypes. We will also analyze how the metabolic program of clones change as they become resistant to treatment--tumor cell metabolic reprogramming often determines whether a tumor cell will become proliferative or go into apoptosis. Finally, we will examine how resistant clones might promote survival of susceptible clones via exosome secretion. Taken together, this project will provide new mechanistic insights into how H3K27M-driven DMG survives treatment and may uncover new therapeutic strategies for this cancer.

Abstract 50

Basic Science

Impact of Post-Translational Modifications on the Stability and Function of the Oncogenic Phosphatase PRL-3

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The oncogenic phosphatase PRL-3 has been implicated in the process of cancer metastasis for over 30 years and there is still no available means to inhibit this protein. The high expression of PRL-3 has been correlated with a poor patient prognosis in numerous cancers including colorectal, gastric, breast, glioma, ovarian, melanoma, prostate, and liver cancers. The connection is obvious: the more PRL-3 in a tumor, the more likely the cancer will spread and the more likely the patient will have a relapse after treatment. Within the cell, PRL-3 is known to bind to the magnesium transport protein CNNM3 to prevent magnesium efflux and this interaction has been shown to promote tumor metastasis. Despite the clear correlation between high PRL-3 expression and poor patient outcome; there are no available therapeutic options to target this phosphatase and its mechanisms of regulation remain unknown. By understanding how the cell controls PRL-3 we can take advantage of these systems to disrupt this oncogenic phosphatase and inhibit metastatic activity.

One-way cells control proteins is through the process of post-translational modification (PTM). Protein PTMs can have a variety of impacts including controlling the stability, localization, and interactions with other proteins. Understanding the PTMs of a protein is critical for the process of drug design and has frequently led to the development of alternative therapeutic strategies. We used mass spectrometry approaches to determine sites of PRL-3 PTM and identified several novel phosphorylation, SUMOlyation, and ubiquitination sites on PRL-3. Most impactful was the described phosphorylation at S143. Our screen encompassed all three members of the PRL family and S143 was the only residue found to be phosphorylated in all three members. S143 and the adjacent residues were highly evolutionarily conserved in virtually all NCBI available eukaryotic PRL-3, suggesting this PTM may play a significant role in PRL-3 function.

I hypothesize phosphorylation at S143 is essential for PRL-3 to bind the magnesium transport protein CNNM3 to promote cell proliferation and migration. I found that the over-expression of PRL-3 in a human cell line (HEK293) significantly increases both the proliferation rates and migratory capabilities of these cells. In contrast, the over-expression of the S143A, the phopho-dead mutant, does not promote the change; which suggests the phosphorylation of PRL-3 at S143 is critical for PRL-3 function. I am further testing my hypothesis by utilizing a phospho-mimetic mutant (S143D) to test the hypothesis that a constitutively phosphorylated PRL-3 will enhance proliferation and migration to a greater extent than that of the wild type.

My future directions include moving into mouse xenograft using wild-type or mutant PRL-3 tumors to investigate the impact of PRL-3 phosphorylation on invasion and metastasis. In addition, I want to investigate the protein network that is controlling this phosphorylation event in the pursuit of an alternative therapeutic target to block PRL-3 signaling by altering its phosphorylation profile.

Abstract 51

Translational

Targeting NRF2 Regulated Pathways in Combination with Artesunate in KEAP1 Loss of Function Non-Small Cell Lung Cancer

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Background: Lung cancer is the leading cause of cancer related deaths globally. Artesunate (ART) is an anti-malaria treatment with demonstrated in vitro anti-cancer activity in several cancer types. However, lung cancer cell lines with loss of function Kelch-like ECH-associated protein 1 (KEAP1) mutations are resistant to ART. KEAP1 negatively regulates nuclear factor-erythroid 2-related factor 2 (NRF2), an anti-oxidative stress response transcription factor. KEAP1 loss or NRF2 gain of function mutations in non-small cell lung cancer (NSCLC) confers chemoresistance and poor survival outcomes. Our group previously showed that ART and an NRF2 inhibitor have synergistic activity in KEAP1 loss of function NSCLC. We hypothesize that combining ART with drugs targeting pathways regulated by NRF2 can overcome ART resistance in KEAP1 loss of function NSCLC.

Objective: The objective is to identify anti-cancer compounds with synergistic activity in combination with artesunate in KEAP1 loss of function mutation NSCLC cell line.

Methods: A549 and H1299 lung cancer cell lines were maintained in RPMI-1640 with 10% fetal bovine serum. KEAP1 loss of function mutation is present in A549 but not H1299. Cells were seeded at 3000 cells per well with complete growth media in 96 well plates for 24 hours to allow for cell adherence. After 24 hours, media were removed and drug added to 96 well plate. Dose response assays were conducted with a range of 12 drug dilution concentrations. IC50 values were normalized to 0.1% DMSO control and analyzed in GraphPad Prism (v5.01). Synergy assays were conducted using a 6 by 6 checkerboard method normalized to 0.2% DMSO control coupled with Loewe synergy score. Loewe synergy scores were analyzed with synergyfinder (v3.0.13) package in R statistical software (v4.1.1). Cell viability for drug response and synergy assays were assessed after 72 hours of treatment by adding CellTiter-Glo 2.0 and analyzed with Varioskan microplate reader.

Results: Ten anti-cancer compounds and ART combinations were screened. Navitoclax (BCL-2, BCL-XL and BCL-W inhibitor) and telaglenastat (CB-839), a glutaminase inhibitor, were identified as having significant synergy with ART. Mean IC50 values of navitoclax were 6.28 μM (95% CI 4.94 – 7.97) and 14.3 μM (95% CI 13.5 – 15.2) in A549 and H1299, respectively. Mean IC50 values of CB-839 were 758 μM (95% CI 48.4 – 11.9 x 103) and 330mM (95% CI 1.61 – 67.6 x 103) in A549 and H1299, respectively. Mean Loewe synergy scores of navitoclax and ART combination were 4.9 ($p = 0.0055$) and 4.89 ($p = 0.02$) in A549 and H1299, respectively. Mean Loewe synergy scores of CB-839 and ART combination were 8.88 ($p < 0.001$) and 24.6 ($p < 0.001$) in A549 and H1299, respectively. Synergistic effects of ART and standard of care platinum agent in NSCLC were also assessed. Mean Loewe synergy scores of cisplatin and ART combination were 5.32 ($p = 0.127$) and 3.88 ($p = 0.281$) in A549 and H1299, respectively. Despite limited single agent activity of CB-839 in both cell lines, there were highly significant synergistic effects when added to ART.

Conclusions: Inhibiting glutamate synthesis is synergistic with ART and is a promising treatment combination for KEAP1 loss NSCLC supporting further preclinical development of the compounds.

Abstract 52

Translational

Integrin α6B4 Signals through DNA Damage Response Pathway to Sensitize Breast Cancer Cells to Cisplatin

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Integrin α6B4 is highly expressed in triple negative breast cancer (TNBC) and drives its most aggressive traits; however, its impact on chemotherapeutic efficacy remains untested. We found that integrin α6B4 signaling promoted sensitivity to cisplatin but not to other chemotherapies tested. Mechanistic investigations revealed that integrin α6B4 stimulated the activation of ATM, p53, and 53BP1, which required the integrin B4 signaling domain. Genetic manipulation of gene expression demonstrated that mutant p53 cooperated with integrin α6B4 for cisplatin sensitivity and was necessary for downstream phosphorylation of 53BP1 and enhanced ATM activation. Additionally, we found that in response to cisplatin-induced DNA double strand break (DSB), integrin α6B4 suppressed the homologous recombination (HR) activity and enhanced non-homologous end joining (NHEJ) repair activity. Finally, we discovered that integrin α6B4 preferentially activated DNA-PKc, facilitated DNA-PKc-p53 and p53-53BP1 complex formation in response to cisplatin and required DNA-PKc to enhance ATM, 53BP1 and p53 activation as well as cisplatin sensitivity. In summary, we discovered a novel function of integrin α6B4 in promoting cisplatin sensitivity in TNBC through DNA damage response pathway.

Abstract 53

Basic Science

A Portable Multiparametric Intravital Microscopy Platform for Metabolic Imaging on Biological Tissues

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Cellular metabolism is highly dynamic and strongly influenced by its local vascular microenvironment, gaining a systems-level view of cell metabolism and vasculature in vivo is essential in understanding many critical biomedical problems in a broad range of disciplines including neuroscience, cardiovascular biology, diabetes, and cancer biology. Several tools with a variety of practical and scientific limitations are currently used to report on different endpoints to piece together a narrative on tissue metabolism or vasculature. Unfortunately, none of them can simultaneously quantify the major metabolic and vascular parameters in vivo in real-time with easy access, albeit it is vital to do so for both basic biology science and therapeutics studies. Furthermore, most of them are: (1) housed in core facilities that require transporting samples or animals to their site; (2) expensive to use (hundreds dollars /service), and (3) time-consuming (multiple days) due to special sample preparation and complicated data processing. These factors all limit their access for high frequency measurements in cancer research. To maximize the ease and accessibility in obtaining in vivo tissue metabolism and vasculature measurements, it is highly significant that we develop new multi-modal metabolic tools with low-cost and point-of-care footprints, allowing one to easily quantify tissue metabolic and vascular endpoints together in vivo in near real-time in the aim of advancing many critical biomedical inquires. Here we report a novel portable multiparametric intravital microscope for murine breast cancer models for in vivo analysis in small animal models. The 2-NBDG has been used in cancer cells to report glucose uptake, similar to the clinically available FDG-PET. TMRE has been utilized to quantify cell mitochondrial membrane potential (MMP) to study OXPHOS. By measuring SO2 along with MMP, one can discern between OXPHOS vs. non-metabolic proton gradient changes. We have adapted these key metabolic probes with our fluorescence microscopy techniques with a aim of simultaneously imaging the key metabolic endpoints of small tumors in vivo for cancer research. Here we report the system characterizations using tissue mimicking phantom studies. We envision that this novel optical technology will allow us to provide a holistic view for understanding cancer biology and be an integral part to facilitate the apprehension of major grey areas of- tumor radiation resistance, chemotherapy, and potential cancer therapeutics research.

Abstract 54

Basic Science

Optical Imaging Captures Metabolic Changes of Radioresistant and Radiosensitive Head and Neck Squamous Cell Carcinomas under Radiation Stress

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UK College of Engineering, Biomedical Engineering

Radiotherapy 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 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 at the tumor cells resistance acquisition to develop improved radiation for HNSCC patients. By using optical imaging techniques, we identified metabolic changes in the radio resistance 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 responded differently in metabolism changes under RT stress along with significant increase in HIF1a expressions on rSCC-61,. further HIF1a inhibition reversed these metabolic changes caused by RT. Our preliminary results suggested that the HIF1a over expression by radiation stress might be, at least in part, responsible for the metabolic changes and consequently the radio resistance acquisition. The present study demonstrated that the optical imaging technique is useful to observe metabolic changes at 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 55

Basic Science

Diffuse Reflectance Spectroscopic Technique for Rapid Quantification of Nanoparticle Concentrations in Tissue Mimicking Turbid Medium

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Nanoparticles have been extensively exploited for many biomedical applications such as targeted drug delivery, bioimaging, and cancer therapy. Their unique bio-friendly characteristics include a high surface-to-volume ratio, and superb magnetic or electrical properties make them attractive for these biomedical applications. However, there are some potential toxicity and side effect of nanoparticles to in vivo biological systems. Therefore, it is vital to maintain an effective but safe biological relevant dose for in vivo use of nanoparticles on biological models. To achieve this goal, quantification of the nanoparticle concentrations in biological models rapidly with high accuracy is necessary for precision nanomedicine. Here we reported a novel diffuse reflectance spectroscopic model for rapid and accurate quantification of nanoparticles in tissue-mimicking turbid medium. Fiber probe measured diffuse reflectance can be described with a simple analytical model by introducing an explicit dependence on the reduced scattering coefficient. We developed this model with proper wavelength pairs for rapid quantification of nanoparticle concentrations in biological tissues. Our tissue-mimicking phantom studies suggested that our technique could quantify nanoparticle concentrations in near real-time with high accuracies using only two narrow wavelengths. We envision that our method will generally be applicable to all nanoparticles concentrations measurements as long as the assumption that the scattering caused by nanoparticles is negligible to background tissue scattering levels can be made. The simplicity of the model lends itself to the enticing prospect that it could be applicable to wide-field applications in real-time quantification of nanoparticle concentrations on in vivo biological models using optical spectroscopy or imaging platforms, which will significantly advance translational biomedical research.

Abstract 56

Basic Science

A Potent Small Molecule that Inhibits RPS6KB1 and Survival of Diverse Treatment-Resistant Cancer Cells

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Lung cancer is the leading cause of cancer-related deaths worldwide. Oncogenic mutations in KRAS and EGFR are the most common drivers of lung cancer. Mutations in the tumor suppressor gene TP53 often co-occur with mutant KRAS or EGFR, and contribute to therapy resistance and poor prognosis. To identify small molecules that can overcome therapy resistance, we screened an FDA approved drug library of about 1400 compounds for inhibition of a mutant-KRAS / TP53-null lung cancer cell line. This unbiased screen led to the identification of Ebastine (EBS), a second-generation anti-histamine reported in literature to exhibit anti-cancer effects. However, the IC50 of Ebastine is upward of 10mM and the exact molecular mechanism by which it induces cell death is not clearly elucidated. Toward the goal of developing more potent analogs of Ebastine, we generated an aminoguanidine derivative that is far more potent than EBS in diverse tumor cell lines. This analog exerted growth inhibition in cancer cell lines resistant to standard-of-care treatments at concentrations significantly lower than Ebastine. We designated this analog as Super-Ebastine. PRISM analysis of 900+ cell lines at the Broad Institute, MA, indicated that diverse cancer cell types are sensitive to Super-Ebastine. Importantly, analysis of the PRISM data predicted the Ser/Thr kinase RPS6KB1 (p70-S6K, ribosomal protein S6 kinase B1) as a prospective target of Super-Ebastine at a concentration of 4nM. RPS6KB1, as well as 4E-BP1 are the two key substrates of the upstream kinase complex mTORC1 that regulate cancer cell survival. Interestingly, our validation studies indicated that phosphorylation of RPS6KB1 but not 4E-BP1 was inhibited by Super-Ebastine. Consistently, in silico molecular docking studies independently confirmed RPS6KB1 as a target of Super-Ebastine with binding affinity far superior than the conventional inhibitor of this protein. RPS6KB1 is generally associated with therapy resistance in ER-positive breast cancer, and with aggressive forms of prostate cancer and lung cancer with poor prognosis. Conversely, inhibition of RPS6KB1 results in inhibition of cell survival proteins, such as survivin and BAD, and induction of apoptosis. Thus, our studies identified a novel small molecule that inhibits the critical cell survival kinase RPS6KB1 in therapy-resistant cancer cells and induces cell death.

Abstract 57

Population-Based/Behavioral

Social Determinants of Palliative Care Knowledge, Barriers, and Facilitators among Advanced Stage Lung Cancer Patients

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Background: Integrating palliative care (PC) into cancer treatment helps manage symptoms and improves quality of life and, thus, is a standard of care for advanced stage lung cancer. However, PC is underutilized. Although PC use is low among the general population of advanced stage lung cancer patients, disparities likely exist along social determinants. The goal of this study was to examine the association between social determinants of health and patient-reported PC knowledge, barriers, and facilitators among advanced stage lung cancer patients.
Method: Between 2020 and 2021, advanced stage lung cancer patients receiving treatment at either Markey Cancer Center or a Markey Cancer Center Affiliate Network Research Site were recruited to complete a one-time survey assessing PC knowledge, barriers, and facilitators. Responses to barriers and facilitators were collapsed for analysis to 'yes' or 'no.' Social determinants of interest included patient: sex, age, treatment setting (community or academic medical center), and residence (rural vs. urban). The most commonly reported barriers and facilitators identified by the entire sample were further examined according to ?? by social determinants. Bivariate tests or one-way ANOVA compared patients within social determinant strata.
Results: 77 advanced stage lung cancer patients (M age = 64.69 ± 10.31; 96.1% White; 49.4% female; 62.3% rural; 58.4% treated in the community) completed the survey. Eighteen percent of patients stated they knew what PC was and could describe it. Seventeen percent mistakenly thought PC was the same as hospice. The most common barriers to PC were being unsure what PC would offer (65%), concern insurance would not cover PC (62%), and an oncologist not discussing PC (60%). The most common facilitators to PC involved managing uncontrolled pain (62%) and receiving an oncologist's recommendation (58%). Fifty three percent men vs. 34% of women had never heard of PC ($p = .052$). The average age of patients who had never heard of PC was 66.67 years vs. 60.36 years for those who knew what PC was ($p = .16$). Whether a patient mistakenly thought PC was the same as hospice did not differ by treatment setting, patient residence, or age; however, more men than women reported this misperception (43% of men vs. 16% of women, $p = .056$). Barriers to PC did not differ by treatment setting, patient residence, or age; however, more men than women reported being unsure what PC would offer them (81% of males vs. 62% of females, $p = .113$). 75% of patients treated at a community site vs. 52% of patients treated at Markey said an oncologist recommendation would facilitate PC ($p = .072$). The average age of patients who said an oncologist's recommendation would facilitate PC was 66.23 years vs. 59.54 years for those who said it would not ($p = .008$).
Conclusions: Advanced stage lung cancer patients have limited knowledge of PC. Educating patients about PC, clarifying PC's role in treatment, and facilitating PC discussions between oncologists and patients may increase PC utilization. PC implementation interventions should consider patient age, sex, and treatment setting when designing and evaluating effectiveness of intervention, as older, male, and community-treated patients may have unique information needs.

Abstract 58

Clinical

Volumetric Specimen Imager for Intraoperative Lumpectomy Specimen Assessment: A Perspective Study

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Purpose/Background: Two dimension (2D) radiographic imaging are commonly used by surgeons and radiologists for nonpalpable lesions during breast conserving surgery (BCS), but 2D image is inaccurate in assessing the specimen. Recently, a unique volumetric specimen imager (VSI) has been developed to provide full-3D and thin cross-sectional slicing images. We hypothesized that this VSI can be implemented in the operating room (OR) to improve the visualization and orientation of specimen margins. The purpose of our study was to evaluate the potential of this VSI for specimen assessment in the OR in a retrospective study.

Methods and Materials: 1. Evaluate the clinical application (equipment safety, imaging quality and informatic integration) of the VSI in the OR and radiology systems. 2. Assess preliminary results of the VSI in predicting the specimen in 100 cases. Patients who undergo BCT for newly diagnosed breast cancers are prospectively included. After excision, each specimen is marked for orientation and imaged using 2D radiography first and then 3D imaging using VSI during surgery. One radiologist retrospectively analyzes 3D images. The lesion maximal dimension is measured and compared with the diagnostic images and pathology reports. The effect of breast density, lesion type and size on the prediction will be analyzed. Statistical analyses of the present study are performed using SPSS software.

Results: 1. Clinical integration of this new VSI is feasible in the OR and radiology systems. This VSI is safe and easy to operate in the OR. The imaging processing and viewing software are user friendly. The imaging acquisition and processing time is less than 10 minutes and can be integrated into the OR without interruption. 2. Currently, the 3D specimen images in 90 patients have been collected. Giving that this is ongoing project, we haven't analyzed the collected data. Based on our experience, we will be able to collect 100 samples in April and analyze them before the final presentation.

Conclusions: The new VSI is a promising new technique that could potentially be used for specimen assessment during BCS without interrupting the workflow. The results of this ongoing perspective study will show the accuracy of this VSI in predicting specimen margins in the final poster.

Clinical Relevance/Application: This new VSI provides full-3D and thin-slice cross-sectional images and has potential to improve the intraoperative specimen assessment. The protocol established from this study will help guide a perspective clinical trial to evaluate the clinical application of this new technique.

Abstract 59

Basic Science

A Gene Cluster Associated with Radiation Sensitivity of Oral Squamous Cell Carcinoma

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Oral squamous cell carcinoma (OSCC) is the sixth most common cancer worldwide. More than 60% of OSCC patients who undergo radiotherapy find the tumors recur with acquired resistance to ionizing radiation (ARR) and a significantly lower survival rate. Genomic alteration, a hallmark of carcinogenesis, makes individual tumor tissues unique, including copy number variations (CNV). Although CNVs occur quite frequently in tumors, whether particular CNV is associated with ARR has not been elucidated.

We observed that CNV in chromosome 14 11q12, a few mega bp gene cluster here named gcARR, influences ARR of OSCC. The gcARR harbors genes encoding key DNA repair/damage response proteins PRMT5 and APE1. We found that expressions of PRMT5 and APE1 are highly correlated in OSCC tissues but not in normal tissues, and together affect the outcome of OSCC radiotherapy. Moreover, we have evidence that simultaneous knockdown of PRMT5 and APE1 synergistically sensitizes OSCC in vitro and in vivo. Further genomic analysis reveals that CNV in gcARR is a key determinant of the high expression of these genes. The central hypothesis of this project is that the level of expression of genes in gcARR is an accurate predictor of the outcome of radiotherapy. A clinical ramification is that profiling CNV, transcription and protein levels of genes in gcARR in the OSCC tissues will help improve prognosis for radiotherapy.

To achieve the long-term translational goals, we propose to 1) test the synergistic effect of PRMT5 and APE1 on the OSCC sensitivity against IR, and 2) profile epigenetic signatures leading to co-regulation of gcARR genes, and 3) analyze the extent of gcARR CNV in pre-cancerous pathological tissues from oral mucosa.

We will identify signatures of genomic and epigenetic alterations in the PRMT5-APE1 region associated with DNA damage response, which will help us understand the genomic alteration behind ARR, and develop novel strategies to enhance radiotherapy efficacy.

Abstract 60

Translational

Human Macrophage-Engineered Vesicles for Utilization in Ovarian Cancer Treatment

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Background: Ovarian cancer is a deadly malignancy with high rates of recurrent and chemotherapy resistant disease. Tumor associated macrophages are a major component of the tumor microenvironment, with high levels of M2-protumor macrophages present, which contribute to chemoresistance and metastasis. M2 macrophages can be converted to M1 anti-tumor macrophages. Vesicles engineered from M1 macrophages (MEVs) retain the ability to convert M2 macrophages to the M1 type and are a novel therapeutic approach. **Objectives:** Establish that human M1 MEVs can repolarize M2 macrophages both in isolation and in co-culture with human ovarian cancer cells and assess anticancer effects. To determine if MEVs can localize to ovarian tumors in vivo. **Methods:** The ovarian adenocarcinoma lines: Caov-3 and OVCAR-3 and the murine macrophage line: RAW264.7, were cultured in appropriate media. Macrophages were isolated and cultured from human peripheral blood mononuclear cells obtained from the Kentucky Blood Center. Macrophages were stimulated to M1 or M2 phenotypes utilizing LPS/ IFN-γ and IL-4/IL-13, respectively. M1 MEVs were generated with nitrogen cavitation and ultracentrifugation. Ovarian cancer cells, macrophages and M1 MEVs were co-cultured and cytokine, PCR, and cell viability analysis were performed. Balb2/c SCID mice were injected with luciferase labeled CAOV3 cells transperitoneally and monitored according to appropriate IACUC protocols. Mice were injected with DiR labelled RAW.264.7 M1 MEVs via lateral tail vein injection and imaged 72hours post-injection for localization. **Results:** We observed high levels of TNF-α in M1 macrophages compared to M2 and controls and demonstrated an increase in TNF-α in M2 incubated with M1 MEVs (M1 vs M2: 2021 ± 383.8 vs 259.9 ± 133.7, $p<0.001$, M1 MEVs+M2 vs M2: 787.5 ± 298.3 vs 259.9 ± 133.7 $p<0.05$)Relative expression of CXCL8, a marker of M1 expression, demonstrated significant differences in M2+ M1 MEV versus M2 treated wells, but not compared to M1 wells ($p<0.0001$). This data suggests that M1 MEVs can repolarize M2 macrophages to an M1 state in vitro. Ovarian cancer and macrophage co-cultured cells treated with M1 MEVs show an increase in, TNF-α (M2+CAOV3+20% MEVs vs M2+CAOV3; 383.6 ± 120.4 vs. 0.1389 ± 20.03, $p<0.05$, M2+OVCAR3+20%MEVs vs M2+OVCAR3: 207.1±170.2 vs -45.65±55.35 $p=0.18$) suggesting that M1 MEVs convert M2 TAMs to an M1 phenotype in coculture. M1 MEVs at high concentrations also has an inhibitory effect on cell viability in both CAOV3 (100.0 ± 8.232 vs 82.27± 2.853 $p<0.0001$) and OVCAR-3 cell lines (100.0 ± 5.710 vs 87.69± 11.62$p<0.05$).

Table 1. Experimental TNF-α levels (pg/ml)

M1	M2	M2 + M1 MEVs	M2 + CAOV3 + 20% M1 MEVs	M2 + CAOV3	M2 + OVCAR3 + 20% M1 MEVs	M2+OVCAR3
2021 ± 383.8	259.9 ± 133.7	787.5 ± 298.3	383.6 ± 120.4	0.1389 ± 20.03	207.1 ± 170.2	-45.65 ± 55.35

Balb2/c SCID mice with CAOV3 tumor xenografts injected with DiR-labelled RAW.264.7 derived M1 MEVs display clear localization to visible tumor xenografts. **Conclusion:** Human derived M1 MEVs repolarize M2 macrophages both in isolation and in co-culture with ovarian cancer cells based on cytokine analysis and real time PCR markers. Additionally, repolarization displays an anti-cancer effect in vitro. Murine-derived M1 MEVs are able to localize to ovarian cancer xenografts in vivo. **Acknowledgements:** This research was funded by NIH Training Grant T32CA160003, Cancer Center Support Grant P30 CA 177558

Abstract 61

Core Resources (Informational and not judged)

The Sulfiredoxin-Peroxiredoxin Axis Promotes Urethane-Induced Lung Adenocarcinoma through the Regulation of the Tumor Microenvironment

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Lung adenocarcinoma is the most common type of lung cancer and has a strong association with tobacco smoking, which is well known to be a mix of carcinogens. Smoking leads to the accumulation of reactive oxygen species (ROS) and oxidative stress damages to macromolecules, which cause abnormal cell growth & proliferation, transformation, changes in cell metabolism, as well as a variety of other physiological functions. Accumulation of ROS only leads to increased expression of cellular antioxidants that contribute to tumorigenesis and cancer progression. Sulfiredoxin (Srx) is the only enzyme that reduces over-oxidized forms of typical 2-Cys Peroxiredoxins (Prxs). Compared to other Prx family members, Srx preferentially interacts with and has a higher binding affinity to Prx4. Our previous work demonstrated that the Srx-Prx4 axis enhances the RAS/RAF-MEK-ERK signaling cascade to promote lung cancer cell proliferation and metastasis. However, the role of the Srx-Prx axis in de novo lung tumorigenesis has not been explored.

In this study, $Prx4^{-/-}$ mice and $Srx^{-/-}Prx4^{-/-}$ mice were generated and mouse lung carcinogenesis was induced by a well-established urethane protocol. Briefly, wild-type and knockout mice at 8-week of age were injected intraperitoneally with 1g/kg of urethane once per week for three successive weeks. Ten weeks after the administration of urethane, all mice were euthanized and examined for tumors. Our results indicate that both knockout mice have significantly reduced rates of tumor incidence, multiplicity, and size. Compared with tumors from wild-type mice, the rate of cell proliferation in tumors from either knockout mice is significantly decreased. Macrophages are the most abundant immune cells identified in the tumor microenvironment of solid tumors and their presence correlates with reduced survival in most cancers. In addition to all other markers, we also compared the presence of two distinct states of polarized macrophages including the classically activated macrophage (M1) and the alternatively activated macrophage subsets (M2) in urethane-induced tumors using specific surface marker staining. We found that there are significantly increased numbers of M1 cells in tumors of either $Prx4^{-/-}$ or $Srx^{-/-}Prx4^{-/-}$ mice compared with those of wild-type mice. In contrast, there are significantly few numbers of M2 cells in tumors from either knockout mice. In vitro studies demonstrated that urethane could induce oxidative stress, stimulate expression of Srx, and secretion of Prx4 in normal lung epithelial cells. We also found that knockdown Prx4 or Srx could stimulate M2 polarization, but M1depolarization by coculturing macrophages and lung cancer cells.

It has been shown that M1 macrophages have immune-stimulatory properties through the expression of a series of pro-inflammatory cytokines, chemokines, and effector molecules, whereas M2 cells express a wide array of anti-inflammatory molecules. Therefore, the difference in tumor-associated macrophages caused by the loss of Prx4 or Srx and Prx4 may contribute to the reduced lung tumorigenesis observed in the mouse model. In addition, bioinformatic analysis shows that Prx4 is overexpressed in the majority of cancers, and the increased expression of Srx and Prx4 both show a strong correlation with poor prognosis in lung adenocarcinoma patients.

Abstract 62

Basic Science

The Role of Protein Tyrosine Kinases in DNA Mismatch Repair

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The DNA mismatch repair (MMR) pathway and its regulation are necessary for genomic stability. Cells deficient in MMR exhibit increased expansion and contraction of short repeat sequences in the genome, termed microsatellite instability (MSI). Loss of MMR also results in resistance to certain classes of DNA damaging chemotherapeutics. The standard treatment option for most patients includes chemotherapy, which has toxic side effects. Immunotherapy has recently been utilized as a treatment for various cancers. MSI-high cancers respond better to immunotherapy treatment compared to microsatellite stable (MSS) cancers. This increased response is believed to be due to the higher mutation frequencies associated with the MSI-high tumors, which lead to greater neoantigen production. The primary proteins required for MMR are known, however regulation of the pathway is not well understood. The MLH1 protein is critical for MMR and is required for the excision licensing of the daughter DNA strand. Loss or mutation of MLH1 leads to defective MMR, increased mutation frequency, and MSI. Recent studies by our laboratory and others have shown that treatment with a tyrosine kinase inhibitor, Imatinib, leads to decreased MLH1 protein expression, and resistance to MMR-dependent apoptosis. These observations suggest a link between Imatinib-sensitive protein tyrosine kinases (PTKs) and the regulation of MMR. We show that MLH1 protein reduction is not occurring at the mRNA level and is thought to be post-transcriptional. We have identified the Imatinib sensitive target most likely responsible for the MLH1 reduction phenotype. We show that the target protein and MLH1 have a physical interaction that likely prevents degradation targeting of MLH1 by the Hsp70 chaperone. A detailed understanding of MMR regulation by PTKs would significantly advance the knowledge in the field of MMR and have important implications for immunotherapy strategies.

Abstract 63

Basic Science

HLTF and SHPRH in Mismatch Repair and Cancer

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Colorectal cancer (CRC) is one of the leading causes of cancer deaths, with an increasing rate of CRC diagnosis in younger individuals. MMR is the DNA repair mechanism that repairs DNA mispairs and small insertions or deletions remaining after replication. MMR is also required for apoptosis after certain types of exogenous DNA damage that result in damage-associated mispairs. MMR defects are the underlying cause of Lynch syndrome, a familial cancer predisposition syndrome that increases susceptibility to multiple cancers, specifically CRC. MMR defects are also commonly found in sporadic CRCs. Model systems such as Saccharomyces cerevisiae, Escherichia coli, and human cell lines have been used to study the MMR proteins and pathways. The basics of the MMR mechanism are fairly well understood; however, proteins associated with MMR are still being identified, and the roles of these proteins are largely unknown. We have identified the yeast protein Rad5 as an interactor with the yeast MMR proteins Msh2 and Mlh1. Rad5 is a helicase and an E3 ubiquitin ligase which is involved in post-replicative repair and damage tolerance. However, to date, Rad5 has no known role in MMR. We have determined that these interactions are conserved through evolution to human Rad5 homologs, HLTF and SHPRH. The Rad5 interactions with Mlh1 and Msh2 appear to be split between the human homologs with human MSH2 interacting with HLTF and human MLH1 interacting with SHPRH. We have found that loss of SHPRH induces resistance to MMR-mediated apoptosis. Current experiments are in progress to determine the binding sites between these proteins. We are also investigating what functional impact the Rad5 homologs have on mutation rate and MMR-induced apoptosis and how the interactions affect the roles of MMR. Together this will allow for a deeper understanding of how accessory proteins may influence canonical and non-canonical MMR. Since MMR status is currently used to determine patient treatment, understanding how commonly mutated accessory factors interact is critical.

Abstract 64

Basic Science

Short-Term Exposure to Cigarette Smoke Activates an Epithelial-to-Mesenchymal Like Reprogramming in Human Bronchial Epithelial Cells

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Cigarette smoke is one of the main risk factors for lung cancer, which comes at the top of the list of cancer-related deaths in the United States in 2021. Cigarette smoke contains a mixture of more than 7000 chemicals, of which 250 have been identified as harmful and 70 carcinogenic. While some of these compounds cause DNA damage and mutations that contribute to carcinogenesis, there are other effects of exposure to tobacco smoke on lung physiology that are not as well understood and need to be investigated in more depth. The broad objective of our study is to investigate the individual and combined effects of tobacco constituents on cellular and molecular endpoints that have been observed in non-transformed human bronchial epithelial cells (HBEC). These HBEC cell lines (HBEC2, HBEC3KT, and HBEC14) were used as excellent precursor models for lung cancers and perhaps other lung diseases in humans. Cigarette smoke condensate (CSC) was derived from new-generation reference cigarette 1R6F from the Kentucky Tobacco Research and Development Center in the College of Agriculture. In one series of experiments, the HBEC cell lines were exposed to different CSC concentrations over a short time frame, and a change in cell morphology was observed. Using high concentrations of CSC, the cells started to elongate after 24hr, while with low concentrations, the more pronounced elongation was observed after 48hr. The observed morphological change may reflect a reprogramming process called epithelial to mesenchymal transition (EMT). EMT is relevant to cancer progression where cells get elongated and acquire invasive and metastatic capacity and to remodeling of lung tissue related to other lung diseases. The effect of CSC is not only time-dependent but is also dose-dependent, where it showed degrees of conversion into elongation in relation to the dose. With single treatments, doses of > 40 ug/ml were becoming cytotoxic, and the cells got rounded and died, while doses less than 5 ug/ml were too low to induce an observable change in cell appearance. At intermediate doses between 5-40 ug/m, all the cell lines showed the exact morphology change after exposure to CSC, but with different ratios; however, HBEC3KT was more sensitive to CSC and demonstrated a more uniform morphology of more than 90% of the treated cells. Repetitive treatment using low CSC concentration showed a more uniform effect after 72hr which makes it more physiologically relevant because it mimics the chronic exposure of smokers to cigarette smoke. This alteration could be reversed when the CSC is washed out and the cells subjected to fresh media at an early stage of the transition. Since EMT is a reversible process, this finding supports our theory in that such a transition might be an EMT. We hypothesize that short-term exposure of bronchial epithelial cells to CSC and cigarette smoke, in general, can trigger a cellular reprogramming resembling EMT and that this may be important in the development and progression of cancers and other diseases of the lung. Ongoing studies are underway to further examine the mechanism underlying it and whether it is EMT or not.

Abstract 65

Basic Science

Epigenetic Regulation of Wnt Signaling by Carboxamide-Substituted Benzhydryl Amines That Function as Histone Demethylase Inhibitors

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Aberrant activation of Wnt signaling triggered by mutations in either Adenomatous Polyposis Coli (APC) or CTNNB1 (b-catenin) is a hallmark of colorectal cancers (CRC). As part of a program to develop epigenetic regulators for cancer therapy, we developed carboxamide-substituted benzhydryl amines (CBAs) bearing either aryl or heteroaryl groups that selectively targeted histone lysine demethylases (KDM) and functioned as inhibitors of the Wnt pathway. A biotinylated variant of N-((5-chloro-8-hydroxyquinolin-7-yl)(4-(diethylamino)phenyl)-methyl)butyramide (CBA-1) identified KDM3A as a binding partner. KDM3A is a Jumonji (JmjC) domain-containing demethylase that is significantly upregulated in CRC. KDM3A regulates the demethylation of histone H3's lysine 9 (H3K9Me2), a repressive marker for transcription. Inhibiting KDM3 increased H3K9Me2 levels, repressed Wnt target genes and curtailed in vitro CRC cell proliferation. CBA-1 also exhibited in vivo inhibition of Wnt signaling in a Zebrafish model without displaying in vivo toxicity.

Abstract 66

Basic Science

CagC Gating Mechanisms Control Effector Passage through the Helicobacter Pylori cag T4SS Translocation Channel

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Gastric adenocarcinoma is the fourth leading cause of cancer-related deaths world-wide (>700,000 deaths annually), and the bacterial carcinogen Helicobacter pylori is the strongest known risk factor for stomach malignancy. H. pylori chronically colonizes the gastric mucosa of more than half of the global population and accounts for approximately 75% of the global gastric cancer burden and 5.5% of all malignancies. Strains of H. pylori associated with a significantly increased risk for developing stomach cancer exploit cag type IV secretion system (cag T4SS) activity to alter the mucosal microenvironment by delivering diverse immunostimulatory cargo, including the oncoprotein CagA and microbial DNA, into gastric epithelial cells. In response to host cell contact, H. pylori assembles cag T4SS-associated pilus-like structures that are hypothesized to facilitate effector translocation. CagC, a T4SS component orthologous to VirB2/TraA subunits in conjugative pili, is required for substrate transfer, but not cag T4SS pilus biogenesis, raising the hypothesis that CagC plays an important role in regulating cargo delivery to target cells. To determine whether translocation channel integrity is dependent on CagC, we used colony immunoblots to monitor the unregulated release of cag T4SS effector molecules in the absence of host cell contact. Inactivation of cagC resulted in aberrant mislocalization of protein and DNA effectors to the bacterial cell surface, suggesting that CagC governs substrate passage and/or release across the outer membrane. Using live cell assays to detect cell surface exposed DNA, we demonstrate that significantly more H. pylori cagC were immunocaptured by dsDNA monoclonal antibodies compared to WT and the corresponding complemented strain, or a strain lacking the cag T4SS core complex component CagY. Treatment of bacterial cultures with DNase prior to incubation with dsDNA antibody restored immunocaptured cagC bacteria to WT levels. Using reporter cell lines to analyze cag T4SS-dependent TLR9 activation, we show that cagC is required for DNA translocation to host cells, and demonstrate that TLR9 activation is significantly decreased when DNase I or dsDNase, but not RNase or exonuclease I, was added to co-cultures concomitantly with H. pylori, suggesting that dsDNA cargo is exposed to the extracellular milieu prior to entering host cells. In support of a two-step DNA secretion model, TLR9 activation was significantly impaired in the presence of dsDNA antibodies compared to co-cultures treated with isotype control or antibodies directed against DNA:RNA hybrids. Modeling by AlphaFold2 suggests that CagC adopts a helical structure similar to TraA pilins and is predicted to assemble into a gate-like mechanism within the cag T4SS apparatus. Collectively, these data suggest that CagC orchestrates the passage of effector molecules through the secretion apparatus and support a model whereby cag T4SS cargo is transported to an extracellular site prior to delivery to the gastric cell cytoplasm.

Abstract 67

Basic Science

NT Contributes to Defective Mitochondrial Function of Small Intestinal Epithelial Cells Associated with Obesity

Jun Song

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Withdrawn.

Abstract 68

Basic Science

Tumor-Secreted G-CSF Delays Neutrophil Apoptosis and Promotes Metastasis

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Neutrophils, a type of white blood cells, have been regarded as first line of defense in the innate immune system. Circulating neutrophils normally have a very short half-life and constitutively undergo apoptosis. An elevated neutrophil to lymphocyte ratio (NLR) has been associated with adverse breast cancer (BCa) prognosis and survival. However, the role of neutrophils in cancer progression, particularly in metastasis is controversial. Additionally, the mechanism regarding neutrophil-involved cancer progression remain to be defined. In several triple-negative breast cancer (TNBCa) mouse models, we observed that peripheral blood (PB) and spleen (SP) neutrophil apoptosis was dramatically delayed in tumor-bearing (TB) mice comparing to tumor free (TF) mice. Using enzyme-linked immunoassay (ELISA), we found that granulocyte colony-stimulating factor (G-CSF), which can stimulate granulopoiesis and mobilize hematopoietic stem cell, was dramatically increased in blood plasma of TB mice and 4T1 breast cancer conditioned medium (4T1-CM). In addition, both 4T1-CM and G-CSF can promote neutrophil survival via increased in the cellular levels of Mcl-1, an anti-apoptotic protein of the Bcl-2 family that blocked the proapoptotic action of Bak (and/or Bim) at the outer mitochondrial membrane in vitro. Moreover, metastasis was reduced in TB mice implanted with G-CSF-knockdown 4T1 cells. Thus, our data support a new role of tumor-secreted G-CSF, which delays neutrophil apoptosis and promotes metastasis.

Abstract 69

Translational

Diaminobutoxy-Substituted Isoflavonoid (DBI-1) Enhances the Therapeutic Efficacy of GLUT1 Inhibitor BAY-876 by Modulating Metabolic Pathways in Colon Cancer Cells

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Cancer cells undergo significant "metabolic remodeling" to provide sufficient ATP to maintain cell survival and to promote rapid growth. In colorectal cancer (CRC) cells, ATP is produced by mitochondrial oxidative phosphorylation (OXPHOS) and by substantially elevated cytoplasmic glucose fermentation (i.e., the Warburg effect). Glucose transporter 1 (GLUT1) expression is significantly increased in CRC cells, and GLUT1 inhibitors block glucose uptake and hence glycolysis crucial for cancer cell growth. In addition to ATP, these metabolic pathways also provide macromolecule building blocks and signaling molecules required for tumor growth. In this study, we identify a diaminobutoxy-substituted isoflavonoid (DBI-1) that inhibits mitochondrial complex I and deprives rapidly growing cancer cells of energy needed for growth. DBI-1 and the GLUT1 inhibitor, BAY-876, synergistically inhibit CRC cell growth in vitro and in vivo. This study suggests that an electron transport chain (ETC) inhibitor (i.e. DBI-1) and a glucose transport inhibitor, (i.e. BAY-876) are potentially effective combination for CRC treatment.

Abstract 70

Basic Science

Isolation and Characterization of Immune Cells from Murine and Human Glioblastoma

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Glioblastoma (GBM) is a brain cancer with limited response to presently available treatments. Radiation plus temozolomide combination therapy given after surgery is currently approved, which extends survival by 15-24 months. In addition to cancer-associated adverse effects, patients also experience cognitive impairment due to cancer and therapy-related neurotoxicity, which affects their quality of life. We hypothesize that these cognitive defects could be due to neuronal damage caused by pro-inflammatory cytokines induced by extracellular vesicles (EVs) produced by GBM. GBM is associated with a unique type of immune suppression which cannot be overcome with current checkpoint therapies that are highly successful in some other types of cancers, nor do they respond to chimeric antigen receptor T cell therapies. However, GBM tumors contain some immune cells which are mainly myeloid in nature. Our studies focused on characterizing the immune cell populations that play a role in GBM using both a mouse model and samples acquired via Cavitron Ultrasonic Surgical Aspirator (CUSA) used in some GBM surgeries. The CUSA samples were provided either as filtrate that was already in single cell suspension or as solid tissue samples that were dissociated via Dounce homogenizer. Percoll gradients were used to isolate mononuclear cells from these samples, and CD45 nanobeads were used to select for CD45+ lymphocytes from the mononuclear cells. There were very few CD45+ immune cells within the CUSA samples, but many microglial cells. In vitro cultures demonstrated robust amounts of IL-6 production in most samples. Only a small fraction of the samples displayed IFN-Œ≥ production following three-day stimulation in culture with T cell stimulatory signals. Future evaluation of CUSA samples will focus upon improved methods to isolate CD45+ cells due to the low efficiency of the nanobead isolations attempted so far. To generate a mouse model, 10,000 GL261 GBM tumor cells were implanted into J:NU mice and monitored via MRI. Mice were euthanized at two and three weeks post-injection, and brain tissue immune cells were isolated by Percoll gradients and cultured and analyzed via flow cytometry. 4-hour culture of GBM brain cells from mice displayed a larger presence of CD45+ cells than sham mice in both unstimulated and PMA + Ionomycin cultures. The immune cells responded robustly to stimulation with anti-CD3+anti-CD28, demonstrating the presence of significant numbers of T cells. Upon stimulation with GBM-derived EVs, these cells produced less TNF-α and more IL-10 compared to unstimulated cells. This may relate to known immune suppression in GBM. Altogether, it is clear that lymphocyte populations show differences in the brains of GBM patients compared to healthy individuals.

Abstract 71

Basic Science

Optical Metabolic Imaging to Capture Metabolic Changes in Breast Cancer Cell Lines with Different Radiosensitivity

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Breast cancer is the most common cancer among women around the world. Radiotherapy has been commonly used as an important adjuvant treatment after surgical removal of tumors in breast cancer patients. However, the occurrence of radiation resistance is a huge clinical challenge compromising the effectiveness of breast cancer treatments. An increasing number of studies demonstrated that metabolic reprogramming is closely associated with the acquisition of radiation resistance in breast cancer post-radiotherapy. Specifically, both glycolysis and mitochondrial metabolism changes have been shown to be involved in the development of radiation resistance. Here we employed optical imaging techniques to detect metabolic changes in breast cancer cell lines MDA231 and MCF-7 to understand the correlation between radiation resistance and metabolic reprogramming. We used fluorescent glucose analog 2-NBDG (2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl) Amino)-2-Deoxyglucose) and fluorescent staining TMRE (Tetramethylrhodamine, ethyl ester) to visualize and quantity tumor cell glucose uptake and mitochondrial membrane potential. By direct visualization of metabolic dynamic changes in breast cancer cell models, we may provide a comprehensive view of metabolic alterations in radiation resistance development in real-time and non-destructive conditions. We could potentially employ this optical strategy to monitor tumor cell therapeutic responses based on the metabolic alterations quantified by multiple metabolic endpoints.

Abstract 72

Basic Science

Decreased Expression of Antioxidant MnSOD Associated with Mesenchymal GBM with Radioresistant Phenotype

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Glioblastoma multiforme (GBM) ranks as the most prevalent cancer among malignant brain and central nervous system tumors that is also accompanied by an unfavorable prognosis with only 40% of patients surviving the first twelve months following diagnosis. Current treatment employs a multifaceted approach involving surgical resectioning followed by postoperative radiotherapy. Despite the varied approach, GBM often reoccurs with radioresistance. To further investigate the mechanisms that impart radioresistance in GBM, our lab has developed radiation resistant glioblastoma cells that are resistant to clinically relevant doses of radiation using the mesenchymal GBM LN18 cell line. Interestingly, radiation resistant cells (LN18-RR-60Gy) grew at a faster rate under two-dimensional cell culture, whereas the parental strain (LN18) replicated at a faster rate compared to the radioresistant line in three-dimensional cultures. Despite the LN18 cell's ability to replicate faster in a three-dimensional culture, the LN18-RR-60Gy cell survived radiation treatment at a greater percentage. Due to an elongated morphology and an increased ability to survive radiation-mediated ROS observed in LN18-RR-60Gy, we quantified the expression levels of epithelial-mesenchymal transition markers and antioxidant proteins. We found that LN18-RR-60Gy had increased expression of select EMT makers, including Snail and Slug; which support the phenotypic changes observed. On the contrary, LN18-RR-60Gy cells displayed a decreased expression of the antioxidant manganese superoxide dismutase (MnSOD). Based on the unanticipated reduction of MnSOD expression, our next steps for this project are 1) to determine the advantage of low MnSOD expression and 2) investigate the underlying mechanism(s) that regulate MnSOD expression in our GBM model. Because MnSOD scavenges mitochondrial superoxide radicals to form hydrogen peroxide and water, we propose that LN18-RR-60Gy suppresses MnSOD expression for their survival from overloading of H2O2 production, potentially by releasing MnSOD as extracellular vesicle's cargo upon radiation exposure.

Abstract 73

Biostatistics/Bioinformatics

Identification of a Long Non-Coding RNA–Protein Coding Gene Signature Associated with Poor Prognosis in Triple-Negative Breast Cancer

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UK College of Medicine, Toxicology & Cancer Biology

Emerging evidence has demonstrated that long non-coding RNAs (lncRNAs), which represent 33% of the human transcriptome and contribute substantially to the regulation of protein-coding genes, are involved in the development and progression of various types of neoplasms including triple-negative breast cancer (TNBC). TNBC is associated with a high risk of tumor recurrence, invasion and metastasis, leading to an aggressive phenotype and poor prognosis. Up to date, there are no well-established prognostic markers for various breast cancer subtypes including TNBC. In this study, we aimed to assess the prognostic potential of subtype-specific lncRNAs and their highly correlated protein-coding genes (PGGs) in TNBC. Using TCGA Breast Invasive Carcinoma dataset (PanCancer Atlas; 1084 cases) and employing Limma-voom, we identified the differentially expressed genes and lncRNAs (> 2-fold change) in all classified breast cancer subtypes (Basal-like, Luminal A, Luminal B, HER2) in comparison to 114 healthy subjects ($p < 0.05$). Among them, upregulation of 815 genes and 15 lncRNAs and downregulation of 748 genes and 10 lncRNAs were uniquely identified in basal-like phenotype. The differential expressions of the shortlisted genes and lncRNAs were validated on independent datasets using UALCAN and cBioportal tools. Further lncRNAs-PGGs co-expression and correlation ($R2 > 0.7$) analysis, functional annotation, association with clinicopathological factors, and risk analysis, were performed. We uncovered a signature of 7-lncRNA (FIRRE, LINC00937, DSCR9, LINC00896, SNHG1, TSIX, AFAP1-AS1; $p < 0.02$) and a 6-PCGs (NOP2, PRCC, NOP58, DKC1, DNAJC2, NPM3; $p < 0.0001$, FDR = 0.01) that have predictive prognosis values in patients with TNBC, and this signature could divide the TNBC patients into high and low-risk groups. In summary, we have identified a signature of lncRNAs and their co-expressed PCGs, which could be exploited as an independent prognostic factor for TNBC. Functional and mechanistic studies are ongoing to further validate these important bioinformatics results with potential clinical implications.

Abstract 74

Biostatistics/Bioinformatics

SYNE1 Mutation Incidence among Ovarian Cancer Patients in Kentucky

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Objective: Ovarian cancer (OC) is the fifth most common cause of cancer related death in the United States. Somatic tumor sequencing reveals the genomic hallmarks of ovarian cancers noting frequent mutations in TP53, PIK3CA, KRAS, and ARID1A. A less frequently mutated gene, SYNE1, demonstrates alterations in 10% of gynecologic malignancies and 5% of epithelial ovarian cancers. Previous studies identified Nesprin-1, the protein product of SYNE1, as an important component for mismatch repair. Nesprin-1 deficiency is associated with elevated tumor mutational burden (TMB) and improved response to immunotherapy (IO) in other malignancies. This study assessed the frequency of SYNE1 mutations in our population and the association with other tumor biomarkers.

Methods: Bioinformatic analyses were performed using information from the The Cancer Genome Atlas (TCGA) and the Oncology Research Information Exchange Network (ORIEN). Data were abstracted with clinical information through the Kentucky Cancer Registry (KCR). The frequency of SYNE1 mutations, association with biomarkers, and patient outcomes were assessed.

Results: SYNE1 mutations were significantly increased in the Kentucky population compared to TCGA. Frequency of SYNE1 co-mutation with KMT2D and USH2A is significant. The Kentucky ovarian cancer population appears to have elevated frequency of genetic mutations compared to the nationwide cohort; however the biomarker data is still pending.

Conclusions: SYNE1 is a novel genetic alteration in cancer with a presumed role in DNA repair and cellular stability. Given the association with TMB and improved response to IO, SYNE1 could represent a valuable biomarker to predict immunogenicity in ovarian cancer.

Abstract 75

Basic Science

Development of Iron Oxide Nanoparticles for Cancer ROS Therapy

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Introduction: Iron oxide nanoparticles (IONPs) have manifested great potential for targeted drug delivery, thermal therapy, and molecular imaging. Recent studies showed that IONPs have intrinsic peroxidase like-activities that generate reactive oxygen species (ROS) including hydroxyl and hydroperoxyl free radicals via Fenton reactions. ROS are highly reactive molecules and play an important role in physiological and pathological processes. The moderate level of ROS improves cell proliferation and differentiation, but the excessive level of ROS induces severe cell damage and apoptosis via damaging DNA and enzymes and oxidizing fatty acid. This study aims to investigate is to develop an IONP-based nanoplatform for effective cancer ROS therapy.

Materials and Methods: Magnetite and Wüstite nanoparticles were synthesized by thermo-decomposition of iron acetylacetonate. Maghemite nanoparticles were synthesized by oxidation of magnetite nanoparticles. The size distribution and crystal structure of the nanocrystals was measured by TEM and XRD. As-synthesized nanocrystals are covered by a layer of hydrophobic oleic acid and oleylamine. In order to change surface properties from hydrophobicity to hydrophilicity, the nanocrystals were coated with DSPE-mPEG2000 through hydrophobic interaction using a dual solvent exchange method. To measure the $OH^{\cdot}$ and $OOH^{\cdot}$ generation by IONPs, IONPs were mixed with TMB and hydrogen peroxide solution in a 96-well microplate. The reaction was measured by light absorbance at 655 nm.

Results: Compared with the catalytic activity of 6, 10 and 15nm magnetite nanoparticles, we found that the ROS generation increased as the size of nanoparticles decreased. This is because at the same concentration, the amount of smaller IONPs is higher than the amount of bigger NPs, which means they have a larger surface area to happen Fenton reaction. In addition, compared with $OH^{\cdot}$ and $OOH^{\cdot}$ generation on magnetite, maghemite and ferumoxytol (FDA-approved iron oxide nanomaterials), Wüstite nanoparticles have a stronger ability to produce $OH^{\cdot}$ and $OOH^{\cdot}$. Finally, we observed the peroxidase-like activities of Wüstite nanoparticles and horseradish peroxidase (HRP) under different PH conditions. Iron oxide nanoparticles have better peroxidase-like activities when PH is around 4, but HRP can generate more $OH^{\cdot}$ and $OOH^{\cdot}$ under neutral conditions.

Conclusions: Wüstite nanoparticles synthesized by thermo-decomposition show higher catalytic activities compared with other iron oxide nanoparticles. Moreover, the catalytic activities are size and composition-dependent. In the end, free radical generation only occurs at lower pH. IONPs have the best catalytic activity when pH =4.

Abstract 76

Basic Science

Neutrophil-Derived S100A8/A9 Co-Operates with Tumor-Secreted G-CSF to Promote "Emergency" Granulopoiesis and Metastasis

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UK College of Medicine, Toxicology & Cancer Biology

In response to inflammation circulating neutrophils are significantly elevated, in a process termed "emergency" granulopoiesis (EG), which leads to enhanced mobilization of immature neutrophils from bone marrow (BM) into circulation. In addition, many metastatic cancer patients with poor overall survival have elevated circulating neutrophil counts. However, knowledge about the connection between EG and metastasis is lacking. In several metastatic breast cancer (BCa) mouse models, we observed that the tumor induced a systemic inflammation environment accompanied by EG and neutrophilia. Using cytokine array and enzyme-linked immunoassay assays (ELISA), we found that granulocyte colony-stimulating factor (G-CSF), a well-known hematopoietic cytokine regulating granulopoiesis, was dramatically increased in blood plasma but not bone marrow (BM) of tumor-bearing (TB) mice implanted with G-CSF-expressing BCa cells, suggesting other factors in BM are likely involved in cancer-induced EG. Further proteomics and ELISA analysis showed that S100A8/A9 protein—abundantly present in neutrophils—was significantly upregulated in the BM fluids and blood of TB mice compared to tumor-free (TF) mice. In addition, S100A8/A9 promoted colony-forming unit-granulocyte (CFU-G) formation in vitro and granulocyte/ macrophage progenitor (GMP) proliferation in vivo and in vitro. Moreover, tumor-secreted G-CSF directly increased S100A8/A9 release from neutrophils. By single-cell RNA sequencing (scRNA-seq) analysis, we identified several subsets of neutrophils from spleens of metastatic stage TB mice displaying similar gene signatures as BM immature neutrophils. Consistently, most circulating neutrophils from metastatic stage TB mice showed immature morphology. Importantly, elevated immature neutrophil counts and higher plasma G-CSF and S100A8 levels were frequently observed in metastatic cancer patients. Thus, our data support a new role of S100A8/A9 in cooperation with tumor-secreted G-CSF in driving cancer-related EG, which expands immature neutrophils and promotes metastasis.

Abstract 77

Basic Science

Formation of Regulatory Complexes Controlling Translation of the PD-L1 Message in KRAS-Active Non-Small Cell Lung Cancer

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Background: Lung cancer is the most common cancer worldwide, with 2.21 million cases and 1.8 million deaths in 2020. The current standard of care consists of surgery, radiation therapy, chemotherapeutic agents, targeted therapy, and immune checkpoint inhibition. Although immune checkpoint inhibitors (ICI) have a remarkable efficacy, long-lasting response, and low toxicity, only a portion of the patients respond well to the treatment. Our group seeks to understand response to therapy in non-small cell lung cancer using cell line model systems.

Unpublished work from our group demonstrated that SHP2, a tyrosine phosphatase, negatively regulates PD-L1 expression, a target of ICI. Using immunoprecipitation of tagged-SHP2 ectopically expressed in H460 cells, mass spectrometry identified DDX3 as a potential partner of SHP2. Other proteins were identified, including eukaryotic translation initiation factor 1a (eIF-1a), that suggested SHP2 participated in a larger complex that can regulate translation initiation. DDX3x also harbors a tyrosine residue at position 104 that could serve as an interacting site for SHP2. We hypothesize that SHP2 forms a complex with DDX3x and eIF-1a on newly transcribed mRNA to negatively regulate PD-L1 expression.

Materials and Methods: Tagged-SHP2 was transfected into H460 cells and subsequently immunoprecipitated with an anti-Myc antibody. The immunoprecipitated complexes were separated by SDS-PAGE. Excised gel slices were treated with dithiothreitol, iodoacetamide, digested with trypsin, and subjected to liquid chromatography-mass spectrometry (LC-MS/MS) analysis. MS data sets were searched in MASCOT software against a database from UniProt to determine interacting proteins. To confirm SHP2 interactions, a pull-down assay was performed using both A549 and H460 extracts with immobilized his-tagged, DDX3X purified from bacteria, and pull-downs were also completed using immobilized his-tagged SHP2. Immunoprecipitation of endogenous DDX3X and SHP2 was conducted in A549 and H460 cell extracts. Western blot analysis was performed to detect interacting proteins.

Results: DDX3X is the highest ranked protein by percent coverage from the IP- MS assay following SHP2 immunoprecipitation. DDX3X purified from bacterial expression systems was able to pulldown SHP2 protein from both A549 cells and H460 cells, but the reverse was not observed. Immunoprecipitation of endogenous SHP2 and DDX3X are being optimized to detect co-precipitating proteins.

Conclusion/Future Direction: DDX3X has the potential to form a complex with SHP2 that may regulate the expression of PD-L1 in concert with eukaryotic translation initiation factors. It is possible the phosphorylation of either or both DDX3X and SHP2 are necessary for complex formation given the results from the bacterially-expressed protein pulldowns. Further studies will be needed to identify the components and activity of this complex in conjunction with the PD-L1 mRNA.

Abstract 78

Population-Based/Behavioral

Preparing an Online Data Mapping Resource for Social Determinants of Health and Cancer Data

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Health professionals and researchers are interested in the links between Social Determinant of Health (SDOH) and Health Outcomes. Health policy professionals, local and gubernatorial policy makers, and health policy advocate groups also want to base their initiatives and campaign with data; specifically, they would like to have a visual product of the data for their communities. To address the needs of these players in the health policy and health research area, several cancer centers or universities have created websites that collate, distribute and visualize the data for health outcomes and SDOH. However, they often do not share source code, lack API for developers, or require manual updates of the data, which can result in delays in updates. The Community Impact Office at Markey Cancer Center has set up a generalizable system that periodically updates data, allows users to download data, and visualize the data on the webpage. In the poster presentation, we will present how the system is designed and architectured to collect, manage, and visualize data for public use.

Abstract 79

Population-Based/Behavioral

Prioritizing Cancer Needs across Kentucky: A Community-Engaged Concept Mapping Project

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UK Markey Cancer Center, Community Impact Office

Background: In 2021, the UK MCC Community Impact Office (CIO) collaborated with a steering committee of partners to conduct a new statewide Kentucky Community Cancer Needs Assessment (CNA). The CIO manages and co-leads the Kentucky Cancer Consortium (KCC), the state's cancer control coalition, and the Kentucky Cancer Program (KCP), Kentucky's statewide cancer prevention and control program. In conjunction with KCC and KCP, the goal of this study was to gather community input on priorities for cancer-related needs in Kentucky, including prevention/risk reduction, screening, treatment, and follow-up/survivorship. This study built upon the CNA quantitative data and focus group findings.
Methods: We recruited 162 participants to take part in concept mapping, a participatory mixed method used to explore connections between concepts and to identify key areas of focus. Fifty-one community members and 111 organizational partners identified by KCC and KCP participated in online survey-based activities followed by a series of discussion groups. First, we invited participants to participate in online sorting and rating activities utilizing 80 items identified as important for prioritization. Using multidimensional scaling and hierarchical cluster analysis, we generated concept maps, which we utilized in a series of guided discussions in which we explored strategies for addressing challenges and key cancer topic areas in Kentucky communities.
Results: With the collected data, we generated point and cluster maps displaying spatial representations of the perceived similarity of the items. We identified a six-cluster solution, indicating six thematic topics within the items. In the discussion groups, participants named these thematic clusters: Proactive Behaviors for Improved Health; Outreach, Education, & Integrative Support; Equitable Accessibility; Concerns, Beliefs, & Stigmas; "Kentucky Uglies:" Current Status of Cancer & Risk Factors; and Disadvantages in Appalachia, Black, and Hispanic Communities. Additionally, participants commonly identified 3 key areas for continued and future efforts: 1) lung cancer screening and smoking rates; 2) HPV vaccination rates; and 3) disparities among rural, Appalachian, Black, and Hispanic Kentuckians, including those due to poverty and social determinants. Participants also identified key strategies for cancer-focused efforts, including continuing and expanding: 1) the use of health communication strategies for information on risks, screening guidelines, use of insurance benefits, and partnering with local organizations; 2) the promotion and expansion of patient navigation strategies; 3) the provision of accessible, culturally appropriate information about treatment and supports self-efficacy in treatment decisions; 4) ways to increase access to care, through financial or travel assistance as well as mobile clinics and at-home screening options; and 5) additional strategies to improve patient-provider trust and communication.
Conclusions: Using concept mapping, we prioritized the wide-array of CNA findings into 6 thematic concepts, 3 commonly discussed focus areas, and 5 potential strategies for next steps. These findings suggest the prioritization of strategies centered on lung cancer risk reduction and screening, including a focus on high smoking rates. Essential to addressing lung cancer and many other forms of cancer, issues of health equity, including factors affecting Black and Hispanic Kentuckians and those who live in rural and low-income communities, must continue to be a priority. Moving forward, researchers, service providers, and healthcare professionals dedicated to improving cancer in Kentucky should consider ways to build upon the community member and partner prioritized strategies.

Abstract 80

Core Resources (Informational and not judged)

MCC Research Communications Office: Helping Markey Researchers with Editing, Graphics and Grants

Donna Gilbreath, Cathy Anthony, Marcia Ballard, Jennifer Bybee, Megan Eder, Terry L. Keys, Phillip Strunk, Amy Beisel

UK College of Medicine, Markey Cancer Center

The UK Markey Cancer Center Research Communications Office (RCO) was created in 2009 to help cancer researchers obtain grant funding, publish material in support of their research initiatives and to facilitate opportunities for continuing education.

The RCO staff includes professional editors, graphic designers, project managers, grant specialists, an event and seminar coordinator, and a website specialist available to help all cancer researchers at the University of Kentucky–free of charge! We can help with:

Research dissemination
– Editing journal articles, manuscripts and book chapters
– Preparing conference posters and presentations
– Creating graphs, charts, maps and other figures for publication or presentation

Grant management
– Editing grant proposals
– Tracking, evaluating, and reporting on pilot awards and funded projects
– Assisting with proposal development, budgeting, ensuring adherence to sponsor guidelines, and the coordination of large grants

Communication
– Facilitating surveys and coordinating messages for the Markey Listserv
– Serving as the key point of contact for Markey website and Markey Connect content
– Writeing and distributing the Markey Minute newsletter and Markey Cancer Connections

Events and seminars
– Coordinating the weekly Markey Research Seminar and the annual Markey Cancer Center Research Day
– Promoting and coordinating other meetings, seminars, and special events
– Facilitating Markey's Patient Advisory Group

RCO works with faculty, staff, grant agencies, and medical and scientific publishers to assist Markey Cancer Center researchers in effectively communicating discoveries made at the University of Kentucky.

RCO is located in Pavilion CC (Ben F. Roach Building), rooms 415, 416, and 418.

Learn more about the RCO at https://ukhealthcare.uky.edu/markey-cancer-center/research/rco

Abstract 81

Core Resources (Informational and not judged)

Markey Cancer Center Research Network Coordinating Center

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Established in 2016, the Markey Cancer Center Research Network (MCCRN) is an alliance of physicians conducting clinical research studies in the prevention, early detection, and treatment of cancers. The MCCRN conducts studies initiated by our own physicians and scientists as well as national studies available through the Markey Cancer Center's membership in the National Cancer Institute's National Clinical Trials Network. The MCCRN serves as a liaison between the Markey Cancer Center and investigators throughout Markey's catchment area. The network provides innovative research studies, support and education for our network research centers, and thorough quality assurance so our studies meet the highest ethical standards. By allowing patients across Kentucky and beyond to participate in clinical trials close to home, the MCCRN supports Markey Cancer Center's mission of reducing cancer burden with a focus on Kentucky and its most vulnerable populations through research, prevention, treatment, education, and community engagement. **Research Collaborations & Development Opportunities:** Achieving our mission requires collaboration and leadership among our members. We assist physicians and research programs seeking to initiate or expand their research portfolios, selecting studies appropriate for their patient populations. The network is guided by the input of multidisciplinary healthcare professionals, including medical oncologists, radiation oncologists, radiologists, and surgeons involved in developing innovative approaches to cancer care. MCCRN provides expertise and guidance for MCC/MCCRN investigators and research teams. MCCRN offers education and training, study monitoring, budget and billing expertise, and regulatory support. **Markey Investigator-Initiated Trials:** Because of our collaborative relationships, our investigator-initiated studies are developed with a unique insight to operations of community-based sites in mind, while maintaining compliance and integrity of the project. Research studies are targeted to focus both on the areas with the highest rate of disease and the types of cancers that most affect these regions. **MCCRN Coordinating Center:** Services delivered by the MCCRN Coordinating Center include network membership and onboarding, needs assessment and program development, research education and training, monitoring and research oversight, data management, protocol development and site selection, project management, contract and budget negotiation, and centralized processing of site and subject payments. We also facilitate interaction with other Markey programs such as the Data and Safety Monitoring Committee, the Markey Quality Assurance Program, and the Molecular Tumor Board, to name just a few. Site Research Teams are provided with a variety of resources, including CRA mentoring, screening support, recruitment materials, audit support, and assistance with IRB submissions. **MCCRN Members:** Site membership requirements include regulatory, site assessment, research training, and submission of qualifying documents. A contractual relationship is established, and an onboarding process completed. Our members are dedicated clinicians qualified by relevant expertise and training, assuring quality conduct of clinical trials. **Research at Home:** MCCRN sites are located across the state of Kentucky and neighboring Appalachia, allowing patients to consider research opportunities while remaining at home under the direct care of their trusted local physicians. **Achievements:** MCCRN sites have enrolled 733 patients to studies in 40/120 counties (33% of state), with 12 additional counties outside the state. The MCCRN program contributes to removing barriers to research participation and reducing the burden of cancer care by bringing important research studies into the communities we serve.

Abstract 82

Basic Science

Leveraging Tumor Organoid Models to Understand the Roles of LKB1 in Lung Tumorigenesis

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

UK College of Medicine, Toxicology & Cancer Biology

Non-small cell lung cancers (NSCLC) is an umbrella term for the largest class of diagnosed lung cancers and includes the most common lung adenocarcinoma (LUAD), followed by lung squamous cell carcinoma (LUSC) and lung adenosquamous cell carcinomas. While LUADs typically have many targetable genetic mutations for therapy, LUSCs lack these mutations and are typically more difficult to treat. In LUADs, two of the most frequently mutated genes are KRAS and STK11 (a.k.a. LKB1) and tumors with both mutations have significantly lower survival than tumors with KRAS but no LKB1 mutation. In addition, these co-mutations are thought to diminish the efficacy of immunotherapies due to the loss of PD-L1 expression and interferon responsive pathways. Furthermore, LKB1 is frequently altered in lung adenosquamous cell carcinomas. Altogether, this indicates the importance of LKB1 in both lineage determination and therapy resistance of NSCLC. With this in mind, we have developed a murine organoid model (3D cell line) wherein Lkb1 can be deleted, and we compare this model to two Lkb1 mutant models of differing cell lineage states. The switching model is a bronchial LUAD (1203) that expresses Lkb1 until exposed to adeno-Cre virus, deleting both alleles of Lkb1. The other two models are one of LUSC histology (3650) and the other LUAD histology (3690) that are both Kras mutant and completely null for Lkb1. Unexpectedly, Lkb1 deletion in the 1203 organoids decreased gene expression of various LUSC markers (Sox2 and Krt5). This could indicate that deletion of Lkb1 in this model has shifted the lineage from bronchial adenocarcinoma to a more distinct form of LUAD. Alternatively, by both qPCR and immunohistochemistry, 3690 expresses both surfactant protein C (Spc) and club cell secretory protein (Ccsp), markers of distal lung, while 3650 is completely Krt5 positive and expresses Sox2, confirming the lineage identities of these models. Moving forward, further understanding of how deletion or rescue of Lkb1 in these models alters not only the genetic and histological landscape of the organoids but also their response to various therapies is critical. Our future plans with these organoids include investigating if switching their lineages offers increased efficacy of chemotherapies and immunotherapies, as well as examining mechanistic differences in major Lkb1 pathways (i.e. metabolism and proliferation). This knowledge will allow us to understand Lkb1's role in lineage determination of NSCLC and the efficacy of therapies in tumors with similar genotypes.

Abstract 83

Basic Science

Engineered M1 Macrophages for Targeted Delivery of Cisplatin Drug in Osteosarcoma Cells: An In Vitro Study

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Introduction: Osteosarcoma (OS) is the most common primary bone malignancy that affects children and young adults. In the US it is anticipated that 3,910 new cases will be diagnosed with 2,100 death in 2022. Treatment for the disease includes surgery and neoadjuvant chemotherapy including methotrexate, doxorubicin and cisplatin.

Rationale: Lung metastasis is the most significant prognostic marker and can only be eliminated with chemotherapy. Conventional drugs like cisplatin are highly toxic and can damage the normal cells. Therefore, a strategy to deliver these drugs with minimum toxicity would be clinically useful. The tumor microenvironment has tumor-associated macrophages (TAM) which are critical in development of metastasis in OS whereas M1 macrophages show anti-inflammatory properties. Use of vesicles engineered from M1 macrophages to target cancer cells and TAM, could be an advance and alternative approach to deliver cisplatin to the targeted cancer cells.

Materials and Methods: Human PBMCs were isolated from blood and stimulated to M1 macrophages (LPS, IFN-Ɣ) and were used to prepare the empty and the cisplatin loaded nanovesicles using Nitrogen cavitation. These vesicles were used at a starting percentage concentration of 20% and diluted 3-fold to obtain six different concentrations and plated on OS cells lines (HOS and 143B) and on human kidney cells (HEK). Free cisplatin was used as a positive control. The dose-response to calculate the IC50, as well as DNA damage, was examined on all treated cells to monitor the efficiency of various drugs.

Results: The cisplatin-loaded vesicles at starting 20% concentration as well as free cisplatin showed a cytotoxic effect on OS cells, whereas the empty vesicles did not show any cytotoxicity. The dose-response curve for 143B cells treated with cisplatin and cisplatin vesicles from three independent experiments showed a mean IC50 value of 3.735 ± 0.5 µM and 0.92 ± 0.7% respectively. In the case of HOS cells, the mean IC50 for cisplatin and cisplatin vesicles were found to be 5.43 ± 2.76 µM and 0.78 ± 1.0% respectively. HEK cells also showed cytotoxic effect after treatment with cisplatin and cisplatin vesicles with an IC50 of 2.62±1.9 µM and 1.6± 0.92% respectively. Cisplatin is known to induce DNA damage by phosphorylating the H2AX histone variant and converting it to Ɣ-H2AX and in the present study, we have observed higher intensity of Ɣ-H2AX staining in free cisplatin (33 µM) which was almost 13.2±7.9-fold high in 143B and 10.41±6.2-fold high in HOS cells as compared to normal control cells. Similarly, the DNA damage intensity using cisplatin vesicles (6.66%) was found to be 8.1±4.0-fold high in 143B and 11.2±3.89-fold high in HOS cells. The different concentrations of free cisplatin and cisplatin vesicles showed DNA damage intensity in dose-dependent manner.

Discussion: Cisplatin is the most versatile drug used to treat cancer patients but is toxic at higher doses and poses many side effects. TAMs can be targeted with engineered M1 macrophages loaded with cisplatin, representing a novel method to deliver cisplatin directly to the tumor. Our results had shown the efficacy of cisplatin-loaded vesicles on OS cells inducing cytotoxicity through DNA damage. Therefore, engineered M1 macrophages can be a promising strategy to control osteosarcoma in patients.

Abstract 84

Population-Based/Behavioral

Patient Recruitment for a Large Population-Based Study (ReCAPSE) Through the POP Sciences SRF

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Background: The ReCAPSE project (Recurrence from Claims And PROs for SEER Enhancement) is a NCI-funded project to develop a machine-learning algorithm to identify cancer recurrence for breast cancer and evaluate the feasibility and accuracy of collecting cancer recurrence directly from female breast cancer patients at a population level. The Patient-Oriented and Population Sciences Shared Resource Facility (POP Sciences SRF) is a Markey core facility that recruits participants, tracks enrollment, and provides data collection services for the ReCAPSE project.

Aim: This goal is to showcase the recruiting process used for the ReCAPSE project and the impact of recruiting by various methods.

Methods: The ReCAPSE study plans to contact around 6500 female breast cancer patients diagnosed between 2015-2017 with stage I-III disease. The Kentucky Cancer Registry (KCR) handles the initial contact of patients obtaining permission to release their information to the study. Once the POP Sciences SRF researcher receives patient information, they work to obtain patients' consent and have participants complete the two short study surveys in a six-month period.

Once KCR provides participant information, these individuals are first recruited by the POP Sciences SRF staff through email. A reminder email is sent a week after the first email if the participant does not respond to the online REDCap survey. After this two-week period, participants then receive multiple phone calls reminding them to complete the survey. A hardcopy package is sent to the participants as a last effort of recruitment. For those who don't have email, a hardcopy package is sent initially then follow-ups with phone calls begin two weeks later. Patients who receive the hardcopy package still have an option to complete the survey online. A QR code and survey link are included in the invitation letter for online completion, or participants can return the paper survey.

Results: With the current participants enrolled, email is a more popular method among participants for recruitment, with 69.7% of participants agreeing to complete via email compared with 28% requesting to complete the surveys on paper. Of participants who have completed the survey, 87.6% have completed it electronically, with 12.4% completing the survey on paper and returning it. (Updated results will be shown at the time of the presentation.)

Discussion: This study demonstrates how POP Sciences SRF can effectively help Markey researchers on study recruitment. This recruitment information can be utilized to determine the best methods for recruiting participants in future research studies.

Abstract 85

Core Resources (Informational and not judged)

Ability and Opportunity: Equitable Training Practices in Science and Medicine

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Underrepresented racial/ethnic minorities and underserved persons make up a small fraction of scientists and leadership in cancer research. Much of this disparity is a result of structural racism and classism in our educational system that has led to dramatic inequities in terms of opportunities, proactive mentorship and advancement in science-based careers. In an effort to address the increased awareness of the need of divergent perspectives as well as inclusive and equitable training, research training paradigms nationwide have been engineered specifically for those that are marginalized. The objective of the Markey Science Training in Research, Oncology, Networking and professional Growth (STRONG) Scholars Program is to increase the number of individuals who are from underrepresented and underserved groups in cancer research. STRONG programming consists of research and clinical experiences, cancer education, personalized mentoring as well as personal and professional development. Throughout the program, STRONG participants successively demonstrated increased acquaintance with, understanding of, and motivation to pursue careers in cancer research in addition to the adoption of positive science identities bolstered by a sense of academic belonging.

Abstract 86

Translational

Novel Pharmacogenetics Mobile Application as an Educational Intervention for Patients and Providers

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Introduction: Genetic polymorphisms contribute to inter-patient variation in disease predisposition and drug response, however implementation into clinical practice is lacking. We hypothesized a telephone app designed for patients to track and share their genomic information could enhance knowledge and use about personal genomic information. This prospective study was designed to evaluate the usability and clinical utility of a novel pharmacogenomics mobile phone application.

Methods: The application was designed by a multidisciplinary research team with specialties in pharmacy, precision medicine, behavioral sciences, biostatistics and computer science. Six individuals who had participated in IRB study #474986, received their genetic testing results from that study and had been found to have at least one polymorphism in a pharmacogenetically relevant gene were enrolled. The study coordinator presented the patient with a demo version of the application and conducted a semi-structured interview to obtain feedback on the apps usability and clinical utility. Participating patients completed surveys before and after the semi-structured interview which assessed baseline health behaviors and knowledge of pharmacogenetics and their experience using the app respectively.

Results: Of the patients who participated, five identified as female and one identified as male. Additionally, five patients identified as Caucasian and one identified as Black. All patients were over the age of 50 with four between the ages of 50-60, one between the ages of 60-70 and one between the ages of 70-80. Each of the patients had at least a college degree, with two patients additionally having completed a graduate degree. Data from the semi-structured interviews was assessed and used to guide revisions of the application. In general, the patients liked the idea of the app with four of six patients stating that they could see themselves using the app in the future while two said that it could be useful but they likely would not use the app personally. Patients noted that they liked the layout (2/6), graphics (1/6) and the search medications tool (3/6) but some offered feedback that more lay terms should be used (4/6). The majority of patients (5/6) responded that if they were to use the app without the study coordinator in the room with them, they would find an introduction or explanation of the app useful. All six patients had a difficult time understanding the modules that listed pharmacogenetic and medically actionable genes.

Conclusion: Based on the feedback provided, the two modules that listed pharmacogenetic and medically actionable genes were removed from the application completely. The language used throughout the app was revised to be more patient-friendly, with the majority of revisions made to the recommendations in the search medications tool. Additionally, an introductory video was created which covers an overview of pharmacogenetics and how a patient's genes can impact their medications, as well as information on how to navigate the modules within the app.

Abstract 87

Informatics/IT

How Hospitalized Bone Marrow Transplant Patients Use an Apple Watch App to Track Physical Activity and Report Symptoms? A Feasibility Study

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Background: Physical activity following a Bone Marrow Transplant (BMT) is beneficial for recovery. However, covid restrictions and the lack of formal exercise programs posed challenges for BMT patients to exercise during hospitalization. We reported feasibility data documenting participant's use of an Apple Watch application called "BMT Go!" in a pilot study.

Methods: Adults receiving BMT at the Markey Cancer Center from Fall 2021 to Spring 2022 were provided an Apple Watch with the BMT Go! app installed. Physical activity data (e.g., steps) is collected in the app and sent to the server when the app is active. This data was then retrieved and parsed using "python" and "pandas" computer languages. The change rates of the step counts and physical wellbeing (reported in the Functional Assessment of Cancer Therapy (FACT) survey) before and after transplant/intervention were reported. The patients also received a prompt on their watch to answer a survey reporting 11 symptoms on a scale of 1-10 up to twice a day if they have not submitted a symptom report on that day.

Results: Nine out of 10 patients completed the pre- and post-surveys and had their physical data recorded. On average, a 49% decrease in daily steps until the day of discharge occurred, corresponding to a 9% decrease in physical wellbeing. The app was actively used an average of 66% of the hospital days based on the data availability. 61% of patients completed the symptom survey at least once a day of their hospital stay while in the study. When limiting the view to high scores (greater than or equal to 5), loss of appetite (17), nausea (17), and tiredness (19) were reported the most and had the highest averages—6.23, 5.8, and 6.33—respectively.

Conclusions: The use of the survey to track self-reported symptoms is feasible as on average the daily symptom survey was completed over half of the time and gave an insight into the worst aspects of BMT. The app is also useful in this setting as it gave a convenient way to track physical aspects such as heart rate and steps while providing encouragement and motivation to patients. The data collected in the BMT Go! app was able to be analyzed and displayed on a web-based dashboard where providers can access and understand their patients' physical activities and reported symptoms.

Abstract 88

Basic Science

Lipid Metabolism Reprograming upon Spermine Synthase Inhibition as a Therapeutic Opportunity in Colorectal Cancer

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Dysregulation of polyamine metabolism has been linked to the development of colorectal cancer (CRC). Our recent work demonstrates that spermine synthase (SMS), a polyamine biosynthetic enzyme that converts spermidine to spermine, is overexpressed in CRC, which is required for balancing cellular spermidine levels to facilitate CRC tumorigenesis (Nat Commun 11:3243, 2020). Our findings reveal SMS as an attractive therapeutic target in CRC; yet, genetic depletion of SMS expression only shows a limited antitumor effect. Using unbiased metabolomics and transcriptomics analyses, we identified a lipid metabolism reprograming as among the most impacted metabolic change by SMS depletion in CRC cells. Specifically, SMS depletion significantly altered long-chain fatty acid, triacylglycerol and phospholipid metabolism. Furthermore, targeted inhibition of SMS significantly increased the number of lipid droplets and the levels of long-chain fatty acid acylcarnitines for oxidative phosphorylation in mitochondria, and upregulated expression of genes associated with increased mobilization of polyunsaturated fatty acids and the genes associated with lipid peroxidation for induction of ferroptosis, an iron-dependent form of nonapoptotic cell death. The glutathione peroxidase 4 (GPX4) is a key negative regulator of ferroptosis by neutralizing lipid peroxides. Notably, pharmacological inhibition of GPX4 or its upstream regulator system xc- in combination with genetic depletion or pharmacologic inhibition of SMS synergistically caused lipid peroxidation leading to ferroptosis induction and marked suppression of CRC cell growth. Collectively, these findings highlight lipid metabolism reprograming as an adaptive response to targeted inhibition of SMS to enable CRC cell survival, which represents an Achilles' heel that can be exploited for potential effective therapy for CRC.

Abstract 89

Translational

Potent Synergistic Effect on c-Myc Driven Colorectal Cancers Using a Novel Indole-Substituted Quinoline with a Plk1 Inhibitor

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Developing effective treatments for colorectal cancers through combinations of small-molecule approaches and immunotherapies present intriguing possibilities for managing these otherwise intractable cancers. During a broad-based, screening effort against multiple colorectal cancer cell lines, we identified indole-substituted quinolines (ISQs), such as N7,N7-dimethyl-3-(1-methyl-1H-indol-3-yl)quinoline-2,7-diamine (ISQ-1), as potent in vitro inhibitors of several cancer cell lines. We found that ISQ-1 inhibited Wnt signaling, a main driver in the pathway governing colorectal cancer development, and ISQ-1 also activated adenosine monophosphate kinase (AMPK), a cellular energy-homeostasis master regulator. We explored the effect of ISQs on cell metabolism. Seahorse assays measuring oxygen consumption rate (OCR) indicated that ISQ-1 inhibited complex I (i.e., NADH ubiquinone oxidoreductase) in the mitochondrial, electron transport chain (ETC). In addition, ISQ-1 treatment showed remarkable synergistic depletion of oncogenic c-Myc protein level in vitro and induced strong tumor remission in vivo when administered together with BI2536, a polo-like kinase-1 (Plk1) inhibitor. These studies point toward the potential value of dual drug therapies targeting the ETC and Plk-1 for the treatment of c-Myc-driven cancers.

Abstract 90

Basic Science

Redox Extracellular Vesicles Induce Neurotoxic Cytokine Production from Innate Immune Cells

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Background: Therapy induced cognition impairment (TICI) or chemobrain is a well-recognized side effect of cancer therapy, which reduces quality of life for cancer survivors. Most cancer therapies induce reactive oxygen species (ROS), which can lead to cancer cell death, but they also promote neuronal cell death both directly and indirectly leading to TICI. High grade gliomas are one of the cancers that respond poorly to therapy and are associated with development of chemobrain. Currently, the mechanisms underlying chemobrain are not well understood. The systemic side effects such as cachexia, fatigue, and cognitive impairment are associated with sustained elevation of inflammatory cytokines and microglial cell activation. However, the mechanisms by which these cytokines are produced and the key sources of such neuropathogenic cytokines are not well understood.

Hypothesis: Chemotherapy and radiation treatment of glioblastoma (GBM) leads to production of extracellular vesicles (EVs) with oxidized proteins that uniquely stimulate specific immune cells to produce cytokines like TNF-α which was shown to induce therapy induced cognition impairment (TICI) by causing neurotoxicity.

Methods: Mice were injected with Doxorubicin at 20mg/kg. After 72 hours, serum was collected and EVs were isolated using ExoQuick-ultra kit. GBM cells were irradiated at 6GY. EVs were isolated from supernatant after two-day culture with the ExoQuick-Ultra kit. Immune cells such as bone marrow derived macrophages (BMDM) were then treated with EVs for 24 hours. Supernatants were collected and assessed for inflammatory cytokines by ELISA. For in vivo experiment, redox EVs were injected intravenously into C57Bl6 mice, 48 hour later spleen cells were isolated, stained and analyzed by flow cytometry. Serum was analyzed for cytokines.

Results: Prior studies from our group showed that TNF-α has a major role in TICI, as it can cross the blood brain barrier and induce neurotoxicity. Here we made several novel observations that lead to a new paradigm about TICI. First, we found that chemotherapy or radiation induces production of EVs from cells and tissues that are targets of the drugs. Second, these EVs contain more proteins adducted to 4-hydorxy nonenal (HNE) compared to controls. Third, these EVs contain proteins released from tissues such as brain that are damaged by therapy. Fourth, the HNE adducted EVs are better than control EVs in inducing macrophages to produce the pro-inflammatory cytokines, TNF-α, and IL-6. In addition, murine microglial cells, an innate immune cell type found in brain, responded to EVs by producing TNF-α. Bone marrow derived dendritic cells produced IL-6 but very little TNF-α upon EV stimulation. Fifth, serum IL-6 levels and IL-6 producing cells were significantly increased in vivo when C57BL6 mice were injected with Redox EVs.

Abstract 91

Basic Science

Neurotensin Negatively Regulates White Adipose Tissue Lipolysis in Human, Mouse and 3T3-L1 Preadipocytes

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Recently, rapid economic developments and lifestyle changes associated with reduced physical activity, and increased consumption of high calorie diets resulted in a higher obesity prevalence, affecting around half of the middle-age adults. Obesity is mainly associated with impaired lipolysis which is defined as the hydrolysis of triacylglycerols (TAGs) stored in lipid droplets (LDs) to generate fatty acids (FAs) and glycerol to be used by other organs. Lipolysis is mediated by two main lipases including adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL). Our research group has previously shown that deficiency of neurotensin (NT), a tridecapeptide released from gut neuroendocrine cells, protected high-fat diet (HFD)-induced obesity by inhibiting fat absorption. Weather NT directly regulates fat metabolism in white adipose tissue (WAT) is yet to be explored. The aim of this study was to define the effects of NT on lipolytic pathways in human and mouse mesenteric adipose tissue and in 3T3-L1 cells. Methods. To determine whether NT contributes to WAT lipolysis during obesity, human mesenteric fat tissues provided by Markey Cancer Center Biospecimen and Tissue Procurement Shared Resource Facility, NT wild type (WT) ($Nt^{+/+}$) and knockout (KO) ($Nt^{-/-}$) aging mice (1.5-year-old), and Ntr1WT ($Ntr1^{+/+}$) and Ntr1KO ($Ntr1^{-/-}$) mice on normal chow were used. Explants, excised from human and mouse mesenteric fat pads were cultured and glycerol in the media measured as an assessment of lipolysis. The mouse 3T3-L1 preadipocytes were also used for mechanistic studies. In addition, qPCR, western blot and confocal microscopy were performed to evaluate gene and protein expression and colocalization. Results. NT treatment decreased lipolysis, transcriptional regulation of ATGL and HSL lipases, as well as LD-coating protein Perilipin1 in human mesenteric adipocytes. Compared with aged $Nt^{+/+}$ mice, lipolysis was increased in $Nt^{-/-}$ mesFat, in which, ATGL, HSL and Perilipin1 mRNA expression was higher. Lipolysis was also increased in $Ntr1^{-/-}$ mesFat and ATGL and HSL mRNA expression was also higher compare to the control. Furthermore, NT treatment decreased lipolysis as well as ATGL and p-HSL protein expression and their colocalization with Perilipin1 on the surfaces of LDs in 3T3-L1 mature adipocytes after differentiation. Conclusion. NT directly inhibits lipolytic gene expression thus decreasing lipolysis in human and mouse mesenteric adipose tissues and in mouse 3T3-L1 preadipocytes. This study provides new insight in our understanding of the contribution of NT to the abnormal WAT metabolism associated with obesity and aging, which leads to novel therapeutic strategies to target this hormone in a clinical setting to stop or prevent obesity.

Abstract 92

Basic Science

Characterization of the Immune Microenvironment of Pancreatic Ductal Adenocarcinoma

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Introduction: Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive cancer with a 5-year survival rate of 11%. Extremely poor prognosis is due to the fact that a large percentage of patients present with distant metastases. There have been minimal changes in chemotherapy regimens over the last decade with only marginal improvements in survival. There is evidence to show that chemotherapy resistance comes from PDAC's uniquely stromal-dominant tumor microenvironment, which may act as a physical or chemical barrier to drug. Stroma and immune components of the TME contribute to drug resistance. As such, advanced cancer models are needed to investigate the effects of the TME on therapeutic response. We hypothesized that 3D organoid cultures can be used for accurate assessment of the cancer to TME interface.

Methods: Under institutional review board approval, surgical specimens were allocated for our research use. We generated PDOs from the human tissue specimens and paraffin imbedded a subset of that allocated parental tissue. We characterized the immune components of the TME in both patient derived organoids (PDOs) and human clinical tissue specimens of PDAC in order to learn if organoids can recapitulate the parental tumor's immunophenotype. We performed immunofluorescence staining on whole mount PDOs and matched human tissue against stromal markers alpha smooth muscle actin (α-SMA) and fibroblast activation protein (FAP). Subsequently, we stained PDOs and matched human tissue against immune markers CD3, PD-1, CXCR4 and CD14, as well as early passage PDOs for the T-regulatory cell markers interleukin receptor 2-α (IL-2Rα) and forkhead box protein 3 (FOXP3). After immunostaining, slides were imaged on a Nikon Eclipse confocal microscope and images analyzed using Elements software provided by Nikon.

Results: We used organoids and paraffin imbedded parental tissue for a comparison of what portions of the immune components of the TME are preserved in PDOs. We identified both immune signaling markers and immune cell markers. In our PDOs we found α-SMA positive cells classified as cancer associated fibroblasts (CAFs), as well as cells expressing both α-SMA and FAP, a subvariant of CAFs known as myofibroblasts. α-SMA positive, cytokeratin 19 (CK19) negative cells were the dominant cell type in immunostained parental tissue sections. In early passage PDOs IL-2Rα and FOXP3 positive cells were detected. We observed CK19 negative, CD3 positive cells, as well as cells expressing the pan macrophage marker CD14 in human tissue sections. In both our human tissue specimens and their daughter PDOs we identified the expression of the immune signaling markers CXCR4 and PD-1 in CK19 positive PDAC cells.

Discussion: In conclusion, we have discovered that the TME of PDAC is diverse in immune marker expression, which is mirrored in organoids derived from parental tissues. Our future plans include generating better models of drug testing and personalized medicine based on individual tumor immunophenotypes.

Abstract 93

Basic Science

Plk1 Targets ASF1A

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Although ASF1A (Anti-Silencing Function 1A Histone Chaperone) is well-known as a histone chaperone that is involved in nucleosome assembly, accumulating evidence supports that ASF1A also plays a critical role in tumorigenesis. Herein, we identified ASF1A as a novel substrate of polo-like kinase 1 (Plk1), a critical regulator of cell cycle-related events, such as G2/M transition and sister chromatid segregation. We show that Plk1 phosphorylates S166 of ASF1A in mitosis and that Plk1 phosphorylation of ASF1A results in its proteasomal degradation. We will dissect the cellular consequence of Plk1 activity-associated ASF1A degradation. Considering the classical role of ASF1A in DNA replication, we will first ask whether Plk1 phosphorylation of ASF1A affects nucleosome assembly after DNA replication. ASF1 is also implicated in DNA damage response. Second, we will ask whether Plk1 phosphorylation of ASF1A affects G2 DNA damage checkpoint recovery, a process in which Plk1 is required. The expected results will likely reveal new mechanisms for therapy resistance.

Abstract 94

Translational

Withdrawn

Abstract 95

Basic Science

Targeting Mitochondrial Redox Capacity Coupled with Mitochondrial Protein Translation to Improve Radiation Efficacy

Luksana Chaiswing, Fang Fang Xu, Jon Thorson, Heidi Weiss, Rani Jayswal, Weixiong Zhong, Kristy Mayer, Wei Luo, William St. Clair, Daret St. Clair

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Radiation therapy (RT) is widely used to treat localized prostate cancer (PCa) and RT secondary mechanism is producing reactive oxygen species (ROS). To improve RT, there is a dire need to uncover cellular events that cause cells to become resistant to RT. In this study, we show that prostate cancer cells that survive and regrow after RT (termed radiation resistant prostate cancer, or RR-PCa) have an abundance of mitochondria characteristics. Elevation of mitochondrial mass/number, mitochondrial H2O2, and mitochondrial biogenesis markers have been identified in RR-PCa cells. Hence, our overarching hypothesis is that RT-activated mitochondrial biogenesis is an acquisition mechanism that drives PCa survival post-RT. Regardless of whether these mitochondria are pre-existing or acquired during RT, we propose that to kill RR-PCa with abundance mitochondria, we have to target both pre-existing and new mitochondria. To target pre-existing mitochondria, we propose to overload mitochondrial hydrogen peroxide (mtH2O2) and to target new mitochondria, we propose to inhibit the mitochondrial protein translation process. We screened FDA-approved drugs in search of compounds that have the ability to raise the level of mitochondrial hydrogen peroxide while blocking mitochondrial protein translation. We found azithromycin (AZM), a macrolide antibiotic, to be an effective prototype compound that possesses both properties. Our preliminary data demonstrate that AZM alone kills PCa and RRPCa both in vitro and in vivo. Treatment of AZM at the time of radiation further promotes the killing effect of RT, especially PCa with abundant mitochondria (RR-PCa), compared to AZM or RT alone. This proposed study provides a novel strategy by which targeting mitochondrial redox capacity (overload ROS) and concurrently targeting mitochondrial biogenesis can be used to improve RT efficacy of cancers, especially the ones with mitochondrial abundance phenotype.

Abstract 96

Core Resources (Informational and not judged)

The Biostatistics and Bioinformatics Shared Resource Facility, Markey Cancer Center

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The Biostatistics and Bioinformatics Shared Resource Facility (BB SRF) provides essential data science expertise in biostatistics and bioinformatics to catalyze and enable the Markey Cancer Center's (MCC) basic, clinical and population research. The BB SRF has exhibited continued growth and increased breadth of services over the last eight years of Cancer Center Support Grant (CCSG) funding and has been successfully integrated as team science members of MCC multi-disciplinary and translational research groups providing centralized, comprehensive, state-of-the-art and accessible services to ensure scientific rigor in the development and execution of cancer research at the MCC. BB SRF services encompass Biostatistics and Bioinformatics components covering 1) study planning, power and sample size calculations for grant applications; 2) statistical analyses, including interim and final analysis for the entire spectrum of cancer research studies; 3) bioinformatics methods for design and analysis of 'omics and high-throughput data; 4) design and implementation support for clinical trials 5) statistical programming for data quality control and data processing; and 6) mentoring, education and general consultation to MCC investigators. BB SRF services are coordinated with other MCC SRFs with hand-offs and integrated workflows to ensure comprehensive, seamless and non-overlapping support. During the current funding cycle, key technical strengths of the BB SRF include 1) innovative methods and new designs for MCC investigator-initiated trials including NCI CTEP clinical trials supporting the Translational Oncology program; 2) cutting-edge bioinformatics and integration of 'omics and high throughput platforms supportive of all programs; 3) data science methods for cancer population, surveillance and behavioral research supportive of the Cancer Prevention and Control program.

In the current funding cycle, the BB SRF supported196 unique users (64% peer-reviewed MCC members), were integrated as team science members supporting over 55 newly funded grants as co-investigators and over 45 investigator-initiated trials, engaged in novel methodological work as evidenced by statistical publications and BB SRF independent R21, R03, U and H grants; ultimately enhancing the science of MCC Research Programs. The BB SRF as a cancer center-managed facility receives significant personnel and resource investment and is governed by rigorous oversight from the Markey Cancer Center.

The BB SRF which received an Exceptional rating from the last CCSG renewal, continues to demonstrate value added and outstanding usage from members of all Research Programs; operated in an efficient, cost-effective and stable manner and was directed by strong leadership along with significant breadth of faculty technical expertise addressing the growth and emerging research directions of the MCC. The highly experienced personnel and diverse skill set of the BB SRF adds significant value to the execution of scientifically rigorous research at the MCC and demonstrates that the BB SRF is well positioned to support the MCC as a Comprehensive Cancer Center for the Commonwealth of Kentucky in this renewal application.

Abstract 97

Population-Based/Behavioral

Stylists against Skin Cancer: An Interventional Study

Cody D. Estep, Mike Fritz

UK College of Medicine

Background: Roosta et al. showed that many hairstylists do not have the confidence to examine a scalp for skin cancer.1 Furthermore, they showed that 15% of stylists do not refer their clients to physicians or have ever recognized a concerning skin lesion.1 The study found that 85% of the stylists do not know the ABCDEs of melanoma or that certain phototypes are more predisposed to skin cancer. However, it was shown that hairstylists desire to learn more about skin cancer screening and prevention.

Purpose: Our study aimed to determine the efficacy of a brief, one-hour lecture given to a population of hairstylists in various cities in the Midwest. This lecture was designed to teach hairstylists about risk factors and skin cancer recognition on the scalp. We hypothesize that the mean cumulative correct answers on our post-lecture quiz will be greater than the cumulative correct answers from the pre-lecture quiz.

Methods: Six separate cities in the Midwest were employed in this study. Geographical demographics ranged from urban to rural. All ethnicities and races were included in the study. The study population included hairstylist students that attended schools in any of the study regions. The students were given an anonymous 10 question pre-lecture multiple-choice quiz. The lecture was then conducted and followed by an identical post-lecture quiz. The percent correct answers were recorded for both the pre-and post-lecture quizzes. The mean percent correct answers of the pre-and post-lecture tests were compared for statistical significance using a one-sided independent t-test. Significance was set at p-value < 0.05. The students were also surveyed before and after the lecture with a question on their confidence to identify a lesion concerning for skin cancer (p-value < 0.05).

Results: 50 hair stylist students were surveyed in the greater Cincinnati, OH area. The mean cumulative pre-lecture percent correct score was 80% and the mean cumulative post-lecture percent correct score was 92%, yielding an improvement of 12% with a p-value of <0.0001. Non-response rate was zero. A pre-test Likert score of 2.8 was found for the question "how comfortable are you in identifying a lesion concerning for skin cancer on the scalp?" and a post-test Likert score of 4.1, showing increased comfortability in identifying skin lesions. No results have been reported for Eastern Kentucky sites at this time.

Discussion: A community-based, lecture-style intervention seems to effectively inform non-medical audiences, like hairstylist students, about skin cancer, including risk factors, prevention, and prognoses. The effectiveness in Eastern Kentucky still needs to be assessed. Previous studies have shown that other occupations, like tattoo artists, also see a role in screening for melanoma.3 Further interventions could implement a similar strategy in these professional environments.

Limitations: One limitation is that there was only one metropolitan area studied. Furthermore, there remains the ceiling effect.

Conclusions: A community-based lecture-style intervention seems efficacious in teaching hair stylist students about skin cancer on the scalp and improves comfortability in identifying concerning lesions. Sessions will be conducted in rural Kentucky to assess any discrepancies in the current data. Further studies should look at incorporating nail changes acral melanoma information and possibly experiment with different, maybe more difficult survey questions.

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