INDIANA UNIVERSITY
MELVIN AND BREN SIMON
COMPREHENSIVE CANCER CENTER

Cancer Research Day
2021 Abstract Book

POSTER #1

REGULATION OF STING BY HISTAMINE SIGNALING IN HUMAN INTRA- AND EXTRA-HEPATIC CHOLANGIOCARCINOMA AND 3D SPHEROIDS

Konstantina Kyritsi[1], Tianhao Zhou, Lindsey Kennedy, Vik Meadows, Nan Wu, Burcin Ekser, Shannon S Glaser, Gianfranco Alpini, Heather L Francis

[1] *Gastroenterology, Indianapolis, IN*

Email: *kkyritsi@iu.edu*

Background: Histamine (HA), synthesized by mast cells (MCs), exerts its effects through H1-H4 receptors (HRs). MC number increases in cholangiocarcinoma (CCA) and blocking MC-derived HA inhibits tumor growth. We have shown that the expression of STING (stimulator of interferon gene) expression increases in nude mice (nu/nu) treated with HA and decreases after treatment with cromolyn sodium, mepyramine (H1HR antagonist) or ranitidine (H2HR antagonist). **Aim:** To determine the role of HA-regulated STING in intra- and extra-hepatic CCA using 3D spheroid tumors. **Methods:** *In vitro*, we generated 3D spheroids with the CCA lines, Mz-ChA-1 (extrahepatic) and SG231 (intrahepatic), alone or in combination with human mast cells (hMCs), human hepatic stellate cells (hHSCs) or combination. After 48 hr of treatment with H-151 (STING inhibitor, 400 nM), HA (25 mM), cromolyn (25 mM), mepyramine (25 mM) or ranitidine (25 mM), we evaluated by: (i) *q*PCR the expression of STING, PCNA, VEGFA/C and the EMT markers S100A4 and vimentin; (ii) EIA kits HA and STING levels in culture media; (iii) immunofluorescence (IF) STING immunoreactivity in spheroid sections co-stained with chymase (MC marker), desmin (HSCs marker) and CK-19 (biliary marker).

The same parameters were measured in tumors from nu/nu mice injected with Mz-ChA-1 cells (3×10^6) and treated with HA, cromolyn or HR antagonists. In human CCA obtained from explants, STING expression in MCs, HSCs and cholangiocytes was evaluated by IF by co-staining for chymase, desmin or CK-19, respectively. **Results:** STING expression decreases in extrahepatic CCA, whereas expression increases in intrahepatic CCA shown by qPCR and western blot. 3D spheroids originated from Mz-ChA-1 or SG231 combined with hMCs, hHSCs or a combination and treated with HA had increased: (i) HA and STING levels; (ii) gene expression of STING, PCNA, angiogenesis and EMT markers. Inhibition of STING had no effect on Mz-ChA-1 CCA spheroid phenotypes, whereas blocking STING decreased SG231 CCA phenotypes. When HA/HR signaling was inhibited, both intra- and extra-hepatic 3D spheroid tumors had reduced (i) HA and STING levels; (ii) gene expression of STING, PCNA, angiogenesis and EMT markers. There was a colocalization of STING with MCs, cholangiocytes and HSCs in nu/nu tumors that increased after HA treatment, but decreased when the HA/HR axis was blocked. In human CCA explants, there was increased STING immunoreactivity in MCs, HSCs and CCA cells compared to controls. **Conclusion:** There is a dysregulation of STING signaling in CCA that is regulated by the HA/HR axis. 3D spheroids mimic human CCA tumors and demonstrate a novel technique to study CCA phenotypes.

Basic Science ***Assistant Scientist in Medicine***

POSTER #2

MITIGATION OF CANCER-INDUCED CACHEXIA WITH EXOGENOUS KETONE SUPPLEMENTATION

Thomas OConnell[1,3], David Logsdon[1], Angela Poff[2], Dominic D'Agostino[2], Maricel Soliven[2], Andrew Koutnik[2,4]

[1] *Otolaryngology, Head & Neck Surgery, Indianapolis, IN*
[2] *Molecular Pharmacology And Physiology, Morsani School Of Medicine, Tampa, FL*
[3] *Indiana Center For Musculoskeletal Health*
[4] *Florida Institute For Human And Machine Cognition*

Email: *thoconne@iu.edu*

The application of a ketogenic diet (KD) as an adjuvant cancer therapy has been a topic of research for several decades. A driving principle for the KD is the dramatic reduction in carbohydrate consumption to deprive tumors of their preferred energetic substrate, glucose. The high fat composition of the KD leads to the formation of ketone bodies which can serve as an alternative fuel for the rest of the body. A significant challenge for the therapeutic application of the KD is low patient compliance due to the strict dietary restrictions and individual intolerance to high fat diets, especially in the context of cancer patients. There is an increasing body of literature on the use of exogenous ketone (EK) supplements to mimic the benefits of the KD without the strict dietary restrictions. In a recent study, we reported that the exogenous ketone supplementation in a murine cancer model could significantly attenuate cachectic wasting. This study used the immunocompetent VM-M3 model which displays progressive tumor growth, metastatic spread and severe muscle wasting. Treatment with the ketone ester, 1,3-butandiol acetoacetate diester led to a significant preservation of muscle weight in the gastrocnemius, quadriceps, tibialis anterior along with preservation of intraperitoneal adipose tissue. To better understand the mechanisms by which EKs mitigated muscle wasting, we applied multi-platform metabolomics analysis to the mice used in this study, including (1) sham surgery with no tumor, standard chow, n = 6 (2) VM-M3 tumor hosts on standard chow, n = 7 and (3) VM-M3 tumor hosts on chow supplemented with 1,3-butandiol acetoacetate diester, n = 8. Metabolic profiles were collected from serum, muscle and liver tissue to provide a systems level evaluation of the effects of the cachexia and the EK supplements. The largest metabolic perturbations were associated with the presence of the tumor, but many significant differences were observed between the tumor bearing standard chow and EK groups. The use of exogenous ketones provides an alternative fuel source for skeletal muscle leading to a resuscitation of oxidative metabolism in the muscle along with a reduction in the catabolism of amino acids. Highly significant perturbations to several metabolites associated with one-carbon metabolism including glycine, methionine and sarcosine were also observed in the EK treated group. These novel finding suggests new mechanisms by which exogenous ketones attenuate cancer-induced cachexia.

Basic Science ***Faculty***

POSTER #3

MITOCHONDRIA-TARGETING AGENT MITOQ IMPROVES MUSCLE ATROPHY, WEAKNESS AND OXIDATIVE METABOLISM IN C26 TUMOR-BEARING MICE

Fabrizio Pin[1], Joshua R. Huot[1,2], Andrea Bonetto[1,2,3]

[1] *Anatomy, Cell Biology & Physiology*
[2] *Department Of Surgery*
[3] *Department Of Otolaryngology-Head & Neck Surgery*

Email: *fpin@iu.edu*

Cancer cachexia is a debilitating syndrome characterized by skeletal muscle wasting, weakness and fatigue. Several pathogenetic mechanisms can contribute to these muscle derangements. Mitochondrial alterations, altered metabolism and increased oxidative stress are known to promote muscle weakness and muscle catabolism. To the extent of improving cachexia, several drugs have been tested to stimulate mitochondrial function and normalize the redox balance. The aim of this study was to test the potential beneficial anti-cachectic effects of Mitoquinone Q (MitoQ), one of the most widely-used mitochondria-targeting antioxidant.

Here we show thatC2C12 murine myotubes exposed to 25% C26 colon cancer conditioned medium (CM) in combination with MitoQ (75 and 150 μM) exhibited decreased myotube atrophy (+25% vs. vehicle, $p<0.01$). In an *in vivo* experiment, daily MitoQ administration (25mg/kg in drinking water) was able to improve body weight loss (+9% vs. vehicle, $p<0.05$) in the C26 bearers, without affecting tumor size. Consistently, the C26 hosts displayed ameliorated skeletal muscle mass (+8 and 12% vs. vehicle for TA and GSN respectively, $p<0.05$) and strength (+37% vs. vehicle, $p<0.05$) upon treatment with MitoQ, whereas the white adipose tissue (WAT) was only partially preserved (+20% vs. vehicle, $p=0.06$ vs. vehicle). In line with improved skeletal muscle mass, the treatment with MitoQ was able to partially correct the expression of E3 ubiquitin ligases *Fbxo32* and *Trim63*.Assessment of genes involved in mitochondrial biogenesis and homeostasis showed altered levels of *Pgc1α*, *Opa1*, *Mitofusin-1* and *Pink1* in the tumor hosts, although only *Mitofusin-1* levels were significantly affected by the treatment. Interestingly, the levels of *Pdk4* and *CytB* genes involved in the regulation of mitochondrial function and metabolism, were partially corrected by MitoQ, also in line with modulation of hexokinase (HK), pyruvate dehydrogenase (PDH) and succinate dehydrogenase (SDH) enzymatic activities.

Overall, our data identify MitoQ as an effective treatment to improve skeletal muscle mass and function in tumor hosts and support studies aimed at testing the anti-cachectic properties of MitoQ in combination with routinely administered chemotherapy agents.

Basic Science ***Faculty***

POSTER #4

ERG EXPRESSION INFLUENCES CELL FATE DECISION

Saranya Rajendran[1], Peter Hollenhorst[1]

[1] *Biochemistry And Molecular Biology, School Of Medicine, Indiana University, Bloomington, IN*

Email: *sarajen@iu.edu*

Prostate cancer is the second most common cancer among men. The TMPRSS2-ERG re-arrangement occurs in ~50% of prostate cancers, resulting in aberrant ERG expression. ERG expression can influence cell fate decisions. In different models ERG can promote either luminal epithelial fates, or epithelial to mesenchymal transition. To determine how ERG promotes specific cell lineages, we performed single cell RNA sequencing of the immortalized normal RWPE-1 cell line. We saw at least four distinct cell type populations, including mesenchymal, basal, and luminal progenitor cells. Based on our previous data that ERG cooperates with mAKT to promote luminal differentiation, we suggest that ERG can promote luminal or mesenchymal transition, depending on AKT status. We found that TLR4 and VEGF pathways can regulate ERG phosphorylation. Inhibition of TLR4 inhibited ERG function in a basal cell line, while inhibition of VEGF inhibited ERG function in a luminal cell line, indicating ERG regulation is cell type dependent.

Basic Science ***Faculty***

POSTER #5

INTRINSIC DIFFERENCES IN TARGETED THERAPY-INDUCED FEEDBACK SIGNALING IN CANCER CELLS UNDER PHYSIOXIA VERSUS AMBIENT AIR

Adedeji Adebayo[1], Brijesh Kumar[2], Hal Broxmeyer[3], Harikrishna Nakshatri[4,5]

[1] *Department Of Biochemistry And Molecular Biology, Indiana University School Of Medicine, Indianapolis, IN*

[2] *Department Of Surgery, Indiana University School Of Medicine, Indianapolis, IN*

[3] *Department Of Microbiology And Immunology, Indiana University School Of Medicine, Indianapolis, IN*

[4] *Department Of Surgery, Indiana University School Of Medicine, Department Of Biochemistry And Molecular Biology, Indiana University School Of Medicine, Indianapolis, IN*

[5] *VA Roudebush Medical Center*

Email: *akadebay@iu.edu*

BACKGROUND: Preclinical studies in cancer research are typically carried out following processing and propagation of cancer cells in ambient air (~21% oxygen). However, cancer cells reside in microenvironments with significantly lower oxygen levels. Breast tumors for example, have a median oxygen concentration of about 1.3%. Therefore, there is a likelihood that initial exposure of cancer cells to excessive amounts of oxygen could impact their behavior and response to targeted therapies (even if they are subsequently cultured in hypoxic conditions). The goal of this study was to evaluate cancer cells collected and processed under physioxia (3-5% oxygen) and ambient air (~21% oxygen) in terms of their kinome, differential responses to targeted therapies and potential resistance mechanisms to these therapies.

METHODS: Tumor tissues from MMTV-PyMT and MMTV-Her2/Neu$^+$ mice were harvested in physioxia, chopped into bits and split into two portions. Each portion of the tumor tissues were processed under physioxia and ambient air respectively. Tumor fractions were derived from the same tissue to ensure that the differences observed are due solely to differences in oxygen tension. The processed tumor cells were then either lysed for Western blotting and phospho-receptor tyrosine kinase (RTK) assay or cultured at 5% oxygen for further studies, including drug screening assays.

RESULTS: The phospho-RTK array showed an increase in the phosphorylation status of EGFR in the cells collected and processed in ambient air compared to physioxia. This was validated via Western blotting for phospho-EGFR (Y1068), which also showed a phosphorylation status similar to the RTK array.PyMT and Her2/Neu$^+$ tumor cells collected and processed in physioxia were more resistant to Alpelisib (BYL719) and Lapatinib, which are PIK3CAα and EGFR inhibitors respectively. A time course treatment of PyMT and Her2/Neu$^+$ cells collected, processed and propagated in physioxia, with 2μM Alpelisib showed a significant resistance to PI3K inhibition as indicated by a sustained expression of phospho-AKT (S473), compared to the cells in ambient air which showed a consistent decline. In addition, phospho-ERK (T202/Y204) exhibited higher basal expression levels and significant resistance to PI3K inhibition under physioxia compared to the cells in ambient air; a finding that was more significant in the Her2/Neu$^+$ cells. DUSP5, a phosphatase specific for ERK1/2 with antitumor activity was found to exhibit higher basal levels in the Her2/Neu$^+$ cells in ambient air compared to physioxia. Furthermore, treatment with Alpelisib significantly induced DUSP5 levels at 2 hours of treatment, with sustained expression levels up to 24 hours of treatment in ambient air. A significant decrease in DUSP5 expression was observed under physioxia.

CONCLUSIONS: These results suggest that tumor cells collected, processed and cultured in physioxia exhibit resistance to PI3K inhibition potentially via an upregulation of AKT and ERK signaling, despite an observed decrease in EGFR activity.

Basic Science ***Graduate Student***

POSTER #6

ELUCIDATING THE MECHANISM OF THE FIBROBLAST GROWTH FACTOR RECEPTOR ROLE IN ALTERING IMMUNE CELL INFILTRATE AND EXPLORING THE COMBINATION OF FGFR INHIBITORS WITH IMMUNE CHECKPOINT INHIBITION

Mitchell Ayers[1], Michael Wendt[2]

[1] *Interdisciplinary Life Science Program (PULSe), Medicinal Chemistry And Molecular Pharmacology, Purdue University, W. Lafayette, IN*

[2] *Medicinal Chemistry And Molecular Pharmacology, Purdue University, W. Lafayette, IN*

Email: *ayersm@purdue.edu*

The success of immunotherapy demands that cytolytic immune cells be present and activated in a solid tumor mass. Exclusion and suppression of T-lymphocytes are drivers of immunotherapy failure. Our group and others have demonstrated that pharmacological inhibition of fibroblast growth factor receptor (FGFR) inhibits tumor growth through direct inhibition of tumor cell proliferation, increased recruitment of cytolytic T-cells, and decreased infiltration of suppressor cells. FGFR inhibition increases the efficacy of immunotherapy in tumor models of immune-cell exclusion. The mechanism by which FGFR alters the immune infiltrate remains elusive. We analyzed clinical gene expression data in tumors that bear constitutively activated forms of FGFR and demonstrate that FGFR activation changes T-cell infiltration into the tumor, while not effecting suppressor cells. We seek to functionally validate mechanisms by which FGFR signaling in tumor cells alters immune-cell infiltration. One such mechanism may utilize the T-cell chemokine CXCL16, which can promote T-cell migration into the tumor microenvironment. Additionally, it has been shown that the FDA approved FGFR inhibitor, Erdafitinib, has off target effects toward LCK, which is essential to proper T-cell receptor engagement and activation. Instead, we propose that the FDA approved FGFR inhibitor Pemigatinib, may provide better synergy with immune checkpoint inhibitors when compared to Erdafitinib. Understanding these mechanistic data and determining an FGFR inhibitor that does not hinder T-cell receptor activation is essential to optimize the combined use of FGFR inhibitors with immunotherapy.

Basic Science ***Graduate Student***

POSTER #7

PP2A REGULATED MACROPINOCYTOSIS AND THERAPEUTIC VULNERABILITIES IN PANCREATIC CANCER

Garima Baral[1,3], Brittany Allen-Petersen[1,3], Claire Pfeffer[2,3]

[1] *Biological Sciences, West Lafayette, IN*
[2] *PULSe, West Lafayette, IN*
[3] *Purdue University*

Email: *gbaral@purdue.edu*

Pancreatic Ductal Adenocarcinoma (PDAC) patients have the lowest five-year survival rate (10%) of all cancers. KRASis mutated in ~90% of patients and is the major driver for PDAC progression. Oncogenic KRAS signals downstream effectors for cell proliferation, migration, survival, and nutrition replenishment. Development of drugs against oncogenic KRAS has been very challenging and highlights the need for alternative strategies to target this signaling pathway.

Oncogenic KRAS promotes non-selective uptake of extracellular materials by macropinocytosis, an actin driven plasma membrane ruffling process. Targeting the process of KRAS driven macropinocytosis can be crucial in limiting nutrition to the progressing cells and preventing tumor growth. It is shown that core of the pancreatic tumors is low in crucial nutrients, including glutamine. Pancreatic tumors scavenge glutamine from the environment through induction of macropinosome formation. The process of macropinocytosis is primarily driven through induction of EGFR ligands and signaling by EGFR-Pak1 axis in PDAC. While the mechanism of macropinosome formation with ligand induction is understood, the regulation of phosphorylation pathways in macropinocytosis is still not clear. Pak1 is known to stabilize Protein phosphatase 2A (PP2A) at the cell membrane, suggesting its potential role in membrane dynamics and formation of macropinosomes.

PP2A is a heterotrimeric serine/threonine phosphatase that regulates the downstream targets of KRAS: AKT, ERK1/2 and c-Myc. The holoenzyme comprises of A, B and C subunits, where the regulatory B subunit provides substrate specificity to the enzyme. Therapeutic activation of PP2A by the Small Molecule Activator of PP2A (SMAP), DT-061 is shown to reduce tumor progression in KRAS driven tumors. To study the role of PP2A in macropinocytosis, we treated a panel of PDAC cell lines with DT-061 and found an induction in EGFR ligands expression, mimicking the glutamine deprived condition. We also see aberrant vacuole formation with DT-061 treatment, consistent with methuosis, a non-apoptotic cell death process driven by excessive macropinocytosis. This finding was confirmed with an increase in the uptake of high molecular weight Dextran by PDAC cells when treated with DT-061. Alternatively, stable knockdown of one of the B subunits of PP2A, B56α, showed attenuation of the EGFR ligand expression. These findings suggest that activation of PP2A-B56α in late stage PDAC promotes high levels of macropinocytosis, ultimately leading to cell death. Further study of metabolic reprogramming in PDAC progression in-vivo with B56α loss will be crucial for identification of potential targets in the future.

Our study shows a novel role of PP2A in nutrient scavenging and these pathways can be further exploited to identify potential therapeutics in Pancreatic Cancer.

Basic Science ***Graduate Student***

POSTER #8

VALIDATION OF SHP2 AS A MULTIFUNCTIONAL THERAPEUTIC TARGET TO METASTATIC BREAST CANCER

Hao Chen[1], Sarah Libring[2], Gregory Cresswell[3], Mitchell Ayers[1], Kasi Viswanatharaju Ruddraraju[1], Jinmin Miao[1], Zhong-Yin Zhang[1], Luis Solorio[2], Timothy Ratliff[3], Michael Wendt[4]

[1] *Medicinal Chemistry And Molecular Pharmacology, Purdue University, West Lafayette, IN*
[2] *Biomedical Engineering, Purdue University, West Lafayette, IN*
[3] *Comparative Pathobiology, Purdue University, West Lafayette, IN*
[4] *Purdue University, Medicinal Chemistry And Molecular Pharmacology, West Lafayette, IN*

Email: *chen2334@purdue.edu*

Despite the benefits achieved by recently developed therapeutics, Metastatic Breast Cancer (MBC) remains one of the toughest clinical challenges with an overall survival rate of ~23%. Thus, there is a critical need to find novel targets and develop the next-generation therapeutics to treat MBC. MBC cells not only alter internal signaling pathways, but also interact with external tumor microenvironment (TME) to support their growth, survival and immune escape. These signaling networks contribute to the heterogeneity and plasticity of MBC and lead to therapeutic failure of targeted therapies and immune checkpoint blockades (ICBs). Blocking the shared key nodes in these signaling networks is an efficient strategy to solve this problem. The aim of the project is to identify a multifunctional therapeutic target in both tumor and immune cells to enhance current therapies with combination strategies.

Src homology region 2 (SH2)-containing protein tyrosine phosphatase-2 (SHP2) is an oncogenic phosphatase with multiple roles in tumor cells and TME. In tumor cells, SHP2 is a key signaling scaffold protein, and it also interacts with PD-1 in T-cells to reduce cytotoxicity. SHP2 is a druggable target with distinct inhibitors, one with allosteric-binding mechanism (SHP099) and another one with an active-site mechanism (11a-1). However, there is still a mechanistic knowledge gap in understanding the signaling inputs, outputs and functions of SHP2 in the MBC-promoting signaling networks.

In this study, we used small molecular inhibitors to systemically target SHP2, and doxycycline inducible genetic depletion to manipulate SHP2 specifically in tumor cells. With the comparison between 2D and 3D culture, we found that extracellular matrix signaling activated by fibronectin is one of the signaling inputs of SHP2. Another branch of signaling inputs from multiple receptor tyrosine kinase signaling was also confirmed with ligand-dependent growth and signaling assays. These signaling inputs have differential activation mechanisms. Besides confirming ERK and AKT signaling as the traditional outputs of SHP2, we found that some immune checkpoints on the membrane of tumor cells depend on SHP2 with flow cytometry. To find better strategies in targeting SHP2, we used 4T1 and D2.A1 metastatic mouse model. SHP2 inhibitors decrease pulmonary metastasis and extend the survival of mice. They also synergize with the FGFR inhibitor and ICBs to delayed pulmonary metastasis.

In summary, our working model suggests that SHP2 in tumor cell is a multifunctional therapeutical target with multiple signaling inputs and outputs to facilitate metastatic growth and reprogram tumor microenvironment. The future directions aim to elucidate the detailed mechanisms by which SHP2 regulates tumor microenvironment, and evaluate the effects of new SHP2 inhibitors in clinical trials.

Basic Science ***Graduate Student***

POSTER #9

DETERMINING THE ROLE OF PA-PLG PATHWAY IN THE PROGRESSION OF PANCREATIC DUCTAL ADENOCARCINOMA

Nayela Chowdhury[1], Yi Yang[2,5], Yingnan Shen[3], Bumsoo Han[3], Alisa Wolberg[2,5], Matthew Flick[2,5], Melissa Fishel[4]

[1] *Pharmacology And Toxicology, Pediatrics, Indiana University School Of Medicine, Indianapolis, IN*
[2] *University Of North Carolina At Chapel Hill School Of Medicine, Chapel Hill, NC*
[3] *Purdue University, School Of Mechanical Engineering, West Lafayette, IN*
[4] *Pediatrics, Pharmacology And Toxicology, Indiana University School Of Medicine, Indianapolis, IN*
[5] *Blood Research Center*

Email: *nanachow@iu.edu*

Pancreatic Ductal Adenocarcinoma (PDAC) has the highest mortality among all cancers, with an estimated 60,430 new cases to be diagnosed in 2021, from which 48,220 people will die from the disease. PDAC patient tumors show high expression levels of multiple components of the coagulation system (e.g., tissue factor) and the fibrinolytic system, including urokinase plasminogen activator (uPA) and receptor (uPAR). PDAC is also associated with one of the highest rates of venous thromboembolism (VTE) compared to other solid cancers due to substantial tissue factor expression which drives the production of excess thrombin. In pancreatic cancer pathogenesis, thrombin can bind to PAR1 receptors on the cancer cells and can mediate crosstalk between coagulation and fibrinolysis through activation of the Plasminogen Activator (PA) pathway. This pathway mediates the activation of plasmin from its precursor, plasminogen (Plg), which then contributes to cancer metastasis. We hypothesized that targeting the PA (uPA/uPAR) system would disrupt tumor progression. Our group has been characterizing the effects of knocking out components of the PA pathway on tumor growth and metastasis. Using pancreatic cancer cell lines from the genetically engineered mouse model KPC (K-ras$^{LSL.G12D/+}$; p53$^{R172H/+}$; PdxCre), individual components of the fibrinolytic system (uPA and uPAR) have been eliminated using CRISPR-Cas9 technology. To investigate the contribution of uPA/uPAR system in the tumor, KPC cells depleted of uPA and uPAR were injected into mice. The knockout tumors were significantly smaller than Cas9 control tumors. To investigate the contribution of uPA/uPAR system in the microenvironment, we used plasminogen knockout mice (Plg$^{-/-}$) and Plg specific antisense oligonucleotide (Plg-ASO) treatment to eliminate or deplete plasminogen levels in mice. Orthotopic injection of KPC cells in Plg$^{-/-}$ mice had significantly attenuated tumor growth compared to WT mice. To analyze if our findings can be extended to models utilizing human cells, we tested our hypothesis using human derived Pa02C and Pa03C PDAC cells. Treatment with Plg-ASO significantly reduced tumor growth of two human Pa03C and Pa02C PDAC cells in a subcutaneous xenograft model. Using the Pa03C orthotopic model, we confirmed the significant decrease in tumor growth within the pancreas following treatment with Plg-ASO and bioluminescence imaging showed a significant decrease in metastatic burden compared to Control-ASO. In conclusion, our data suggests a mechanism involving the thrombin-PA system driving tumor progression and metastasis through uPA/uPAR via plasminogen. To clarify the role of Plg and PA in PDAC metastasis, we will analyze their expression in patient tumor samples and correlate expression with disease stage and incidence of VTE. We are collecting tissue biopsies of both male and female patients who are in various disease stages and corresponding blood samples from all these patients and analyzing the plasma for coagulation factors and VTE markers to examine the correlation between disease stage and therapy.

Basic Science ***Graduate Student***

POSTER #10

MIR-10A TARGETS CHEMORESISTANCE GENES IN OVARIAN CLEAR CELL CARCINOMA

Kaitlyn Collins[1], Xiyin Wang[2], Kenneth Nephew[3,4], Chad Creighton[5], Shannon Hawkins[1,2,4]

[1] *Department Of Biochemistry And Molecular Biology, Indiana University School Of Medicine*
[2] *Department Of Obstetrics And Gynecology, Indiana University School Of Medicine*
[3] *Department Of Cellular And Integrative Physiology, Indiana University*
[4] *Indiana University Melvin And Bren Simon Comprehensive Cancer Center*
[5] *Department Of Medicine, Baylor College Of Medicine*

Email: *kaicolli@indiana.edu*

Ovarian carcinomas are the most lethal gynecological malignancy for women in the United States. Women with high-grade serous ovarian carcinoma initially respond to standard platinum- and taxane-based chemotherapy but later develop chemotherapy-resistant disease, leading to death. Ominously, women with ovarian clear cell carcinoma (OCCC) begin with chemotherapy-resistant disease. Therefore, studies of chemoresistance in OCCC are crucial.

Endometriosis, a chronic inflammatory condition where endometrial-like tissue grows outside the uterus, is a significant risk factor and precursor lesion for OCCC. Small RNA sequencing on well-characterized human OCCC from women with concurrent endometriosis revealed that miR-10a, a key overexpressed miRNA in chemotherapy-resistant breast and cervical cancers, was 11-fold upregulated compared to endometriomas. *In vitro* carboplatin-chemoresistance testing in human OCCC cell lines identified a positive correlation between carboplatin's half-maximal inhibitory concentration (IC50) and basal miR-10a expression [R^2= 0.97].

MiR-10a expression was 3-fold higher in the platinum-resistant A2780 cell line, A2780CR5, compared to the non-platinum-resistant A2780 parent line. Carboplatin response in SMOV2 cells transduced to overexpress miR-10a demonstrated an increase in carboplatin IC50 from 18.6 µM to 31.6 µM, consistent with a functional role of miR-10a in chemotherapy resistance. MiRNA molecules function to decrease target gene expression through complementary binding to their seed sequences. Using a combination of bioinformatic analyses and hand annotation of miRNA:mRNA target prediction algorithms, 15 downregulated potential miR-10a target genes were identified, including Cell Adhesion Molecule L1 like (*CHL1*, log2fold change= -3.1, *P*<0.05) is a tumor suppressor gene and a crucial platinum-response gene in lung cancers; GATA Binding Protein 6 (*GATA6*, log2fold change= -3.7, *P*<0.05) is also a tumor suppressor and suggested treatment response marker in pancreatic ductal carcinoma; and Serpin Family E Member 1 (*SERPINE1*, log2fold change= -5.3, *P*<0.05) is implicated in chemotherapy response in colon and ovarian cancers.

Basic Science ***Graduate Student***

POSTER #11

TARGETING GCN2 REGULATION OF AMINO ACID HOMEOSTASIS IN PROSTATE CANCER

Ricardo Cordova[1], Jagannath Misra[1], Nur P. Damayanti[2], Parth H. Amin[1], Kenneth R. Carlson[1], Angela J Klunk[1], Andrew Elmendorf[1], Hyeong-Geug Geug[1], Emily T. Mirek[3], Marcus J. Miller[4], Tracy G. Anthony[3], Roberto Pili[5], Ronald C. Wek[1], Kirk A. Staschke[1]

[1] *Biochemistry And Molecular Biology, Medicine, Indiana University, Indianapolis, IN*
[2] *Medicine, Indiana University, Indianapolis, IN*
[3] *Nutritional Sciences, Rutgers, New Brunswick, NJ*
[4] *Clinical Medical & Molecular Genetics, Indiana University, Indianapolis, IN*
[5] *Medicine, Jacobs School Of Medicine & Biomedical Sciences, University At Buffalo, Buffalo, NY*

Email: *rcordov@iu.edu*

The Integrated Stress Response (ISR) plays a critical role in the adaptation and survival of tumor cells to various exogenous and endogenous stresses. The ISR features four protein kinases (PERK, GCN2, PKR, and HRI), each activated by different stresses, that phosphorylate the eukaryotic translation initiation factor eIF2, resulting in repression of global protein synthesis. Paradoxically, eIF2 phosphorylation also enhances translation of select gene transcripts, including the transcription factor ATF4, which is central for ISR-directed gene transcription. Therefore, the ISR directs translational and transcriptional control that is critical for cancer stress adaptation. Moreover, eIF2 phosphorylation and ATF4 have recently been suggested to play a role in prostate cancer (PCa) growth and survival; however, the specific function of eIF2 kinases, their mode of activation, and the mechanisms by which the ISR facilitate PCa progression are unknown.

We discovered that GCN2 is activated in a range of PCa cell lines, contributing to enhanced eIF2 phosphorylation and ATF4 expression. Genetic or pharmacological ablation of GCN2 or ATF4 inhibits growth in androgen-sensitive and castration-resistant PCa cell lines in culture and cell line-derived and patient-derived xenograft mouse models *in vivo*. Induction of GCN2 is accompanied by limitations of select amino acids and accumulation of cognate tRNAs that are reported to be activators of GCN2. A transcriptome analysis of PCa cells treated with a specific GCN2 small molecular inhibitor indicates that GCN2 is critical for expression of *SLC* genes, including amino acid transporters (ASCT1, ASCT2, 4F2, CAT1, LAT1, LAT3, and xCT). Using CRISPR-based phenotypic screens and genome-wide gene expression analyses of wild-type and GCN2-depleted PCa cells, we confirmed the importance of the transporter genes in PCa fitness. One transporter, SLC3A2 (4F2), is a key *SLC* gene induced by GCN2 and is essential for PCa proliferation. SLC3A2 engages with many nutrient transporters, allowing for their localization to the plasma membrane. Importantly, expression of SLC3A2 reduced GCN2 activation and rescued growth inhibition of GCN2-depleted PCa cells.

Our results indicate that select amino acid limitations activate GCN2 in PCa, resulting in the upregulation of key amino acid transporters, including 4F2 (SLC3A2), which provide for nutrient import to facilitate protein synthesis and metabolism required for PCa progression. We conclude that GCN2 and the ISR are promising therapeutic targets for both androgen-sensitive and castration-resistant prostate cancer.

Basic Science ***Graduate Student***

POSTER #12

DEFINING THE MECHANISMS OF TGLI1'S ONCOGENIC FUNCTIONS

Haddie DeHart[1], Richard Carpenter[2]

[1] *Cell, Molecular And Cancer Biology PhD Program , Indiana University School Of Medicine, Bloomington, IN*

[2] *Medical Sciences Program, Biochemistry And Molecular Biology, Indiana University School Of Medicine, Bloomington, IN*

Email: *hkdehart@iu.edu*

Truncated glioma-associated oncogene homolog 1 (TGLI1) is an alternative splice variant of GLI1 in which a portion of the N-terminal region is lost. Despite the loss of 41 amino acids, TGLI1 operates as a gain of function transcription factor with the ability to bind to and activate genes distinct from GLI1. In doing so, TGLI1 promotes invasion, migration, angiogenesis, and stemness in both breast cancer and glioblastoma (GBM). Currently, the mechanisms behind the differential binding of TGLI1 from GLI1 remain unknown. To address this knowledge gap, we investigated protein-protein interactions of TGLI1 and GLI1 using mass spectrometry. We found that TGLI1 and GLI1 have distinct protein interactions, which may mediate the unique gene binding profile of TGLI1. However, further investigation into changes in protein interactions and other mechanisms influencing TGLI1 DNA binding, is needed. The expression of TGLI1 in breast cancer and GBM drives cells towards a more stem like state. Recently, it was shown that breast cancer stem cells expressing TGLI1 are capable of activating astrocytes to promote breast cancer metastasis to the brain. While GBM stem cells (GSCs) have been shown to activate astrocytes, the role of TGLI1 in this activation, has not been investigated. Understanding TGLI1-expressing GSCs' activation of astrocytes is important as astrocyte activation has been linked to increased proliferation, invasion, immune evasion, and chemoresistance in GBM. As TGLI1 has been shown to drive a more aggressive cancer phenotype, these studies will be crucial in delineating the exact behaviors of the TGLI1 protein that promote cancer progression.

Basic Science ***Graduate Student***

POSTER #13

DISRUPTING THE FUNCTIONAL INTERACTIONS BETWEEN MEDIATOR SUBUNIT MED25 AND ONCOGENIC PEA3 TRANSCRIPTION FACTORS

Nicholas Downing[1], Asdf Asdf, Peter Hollenhorst[1]

[1] *Medical Sciences, Indiana University, Bloomington, IN*

Email: *nfdownin@iu.edu*

Mediator is an indispensable regulatory complex that bridges DNA-bound transcription factors to RNA Polymerase II (Pol II) for targeted gene expression. It's non-essential MED25 subunit can interact with ETV1/4/5 within the PEA3 subfamily of the E26 transformation-specific (ETS) transcription factors. These oncogenic transcription factors have established roles in both breast and prostate cancer. While others have demonstrated that the MED25-ETV5 interaction promotes ETV5 transactivation and that MED25 and ETV4 share transcriptional targets, disruption of these interactions have yet to be evaluated at a phenotypic level in prostate cancer. I have demonstrated that knockdown of MED25 protein does not influence cellular proliferation or clonogenic growth, but significantly reduces ETV1/4-induced migration *in vitro*.

Our lab has shown that exogenous expression of ETV1/4 promotes migratory phenotypes in the normal prostate RWPE1 cell line. To address the role of MED25-ETV interactions in promoting migration, CRISPR/Cas9-mediated knockout of MED25 will first be achieved in RWPE1 cells. This will be followed by rescue with mutant MED25, harboring mutations in its activator-interacting domain (ACID) that specifically prevent its interactions with ETV. I also plan to achieve MED25 knockout in PC3 prostate cancer cells with high expression of ETV4, as well as in COP1-KO LNCAP prostate cancer cells with stable ETV1 expression. Interactions will be evaluated by affinity pulldown using purified HIS-tagged ETS proteins and cell lysates from these DMED25 cells. Upon expression of ETV1/4/5 in DMED25 RPWE1 cells, ChIP-seq of ETV and Pol II will be implemented to evaluate if the MED25-ETV interactions are required for Pol II recruitment to sites of ETV-chromatin binding. If these results support the hypothesis that MED25-ETV interactions are required for expression of genes involved in cancer cell migration, I plan to evaluate the efficacy of norstictic acid in prostate cancer cell lines. This allosteric regulator of MED25 was recently studied for clinical use in triple negative breast cancer and is thought to act by binding the ACID domain of MED25. Extending this knowledge could provide therapeutic benefit to patients whose prostate tumors present high expression of ETV transcription factors.

Basic Science ***Graduate Student***

POSTER #14

ERG PARTICIPATES IN TWO DISTINCT INTERACTIONS WITH CO-ACTIVATOR EWS

Benjamin Greulich[1], Taylor Nicholas[2], Peter Hollenhorst[1]

[1] *Medical Sciences, School Of Medicine, Indiana University, Bloomington, IN*
[2] *Department Of Biology, Indiana University, Bloomington, IN*

Email: *bmgreuli@iu.edu*

Prostate cancer is responsible for the second most cancer-related deaths in American men. A hallmark of this disease is the gene fusion between the promoter of an androgen-driven gene and an ETS transcription factor. The most common fusion is *TMPRSS2-ERG*, which results in expression of ERG and subsequently increased migration and tumor growth. While ERG is the most common oncogenic ETS factor to become expressed in prostate cancer, accounting for approximately 50% of cases, fusions can also occur with ETV1, ETV4, and ETV5. Our lab has shown these four proteins share a unique ability among the ETS family to interact with a co-activator EWS. An understanding of this interaction can reveal new therapeutic targets to inhibit oncogenic ETS factors in prostate cancer.

Interaction assays using purified ERG and EWS fragments have shown a direct interaction between ERG and the N-terminus of EWS, and this interaction is dependent on ERG's proline 436. ERG can also interact with the chimeric protein EWS/FLI1, which is responsible for the development of Ewing's Sarcoma. However, ERG fails to interact with YS37 EWS/FLI1, in which 37 tyrosines are mutated to serine, thus abolishing its ability to phase separate. This indicates that this direct interaction is dependent on EWS' intrinsically disordered region and might occur within the context of a phase separated droplet.

A weaker interaction between ERG and the C-terminus of EWS (ctEWS) has also been observed in cell extracts but not with purified protein, indicating this interaction to be indirect. This ERG-ctEWS interaction is independent of ERG's proline 436, but may be dependent on RNA. This RNA-dependence seems logical, as ctEWS contains multiple nucleic acid binding domains. To determine additional factors that facilitate this indirect interaction, IP-mass spectrometry of ctEWS was performed and revealed multiple potential interacting partners that overlap with ERG IP-mass spectrometry data. One hit has been confirmed to interact with ERG and ctEWS via co-immunoprecipitation. In addition to ERG, ETV1 also appears to interact with this IP-mass spec hit. Ongoing experiments aim to determine if the interaction between this hit and ERG or ctEWS is direct and to determine which region of ERG is required for this interaction. Additionally, knockdown cell lines are being constructed to determine if the IP-mass spec hit is required for the ERG-ctEWS interaction or ERG function.

Taken together, these data have led us to hypothesize that ERG and EWS are involved in a higher order, potentially phase-separated structure that is required for activation of ERG transcriptional targets. Continued work to define this complex can provide new therapeutic targets or approaches to targeting ERG in prostate cancer.

Basic Science ***Graduate Student***

POSTER #15

INDUCED ENDOREPLICATION BY AURORA B KINASE INHIBITION AS A MODEL FOR TUMOR HETEROGENEITY

Kelly Hartsough[1], Robert Policastro[2], Michael Rotelli[2], Jason Zhang[2], Aryan Mishra[2], Shengyao Chen[2], Gabriel Zentner[2], Brian Calvi[2], Claire E. Walczak[1]

[1] *Medical Sciences, Bloomington, IN*
[2] *Biology, Bloomington, IN*

Email: *khartso@iu.edu*

Endoreplication is an alternative cell cycle wherein cells undergo alternating G and S phases without dividing. While endoreplication can occur naturally to generate polyploid cells in growth and development, it can also be co-opted by cancer cells, leading to genome instability, tumor evolution, and cancer relapse; however, the mechanisms leading to these outcomes are unknown. We developed a cell culture method to generate induced endoreplicating cells (iECs) from diploid RPE-1 cells by treating cells with cell cycle inhibitors to study how changes in cell cycle dynamics lead to genomic heterogeneity and how cells with increased ploidy return to division. We found that Aurora B kinase inhibition resulted in a subtype of endoreplication called endomitosis, wherein cells enter mitosis but do not divide. Cells treated with a high concentration of Aurora B inhibitor failed at the metaphase/anaphase transition, resulting in daughter cells with multi-lobed nuclei. In contrast, cells treated with a lower concentration of Aurora B inhibitor failed at cytokinesis and resulted in binucleate cells. We found that cells with multi-lobed nuclei preferentially undergo DNA synthesis, suggesting that nuclear phenotype and/or the level of Aurora B inhibition may play a critical role in determining whether a cell can bypass the tetraploid checkpoint to become polyploid. To understand the molecular pathways contributing to endoreplication, we performed RNA-seq on iECs and found that there was an increase in the senescence-associated secretory phenotype signature and core senescence genes. Using fluorescence microscopy, we found that some, but not all, cells were positive for senescence markers, suggesting the response to Aurora B inhibition is heterogeneous. To ask whether polyploid iECs can resume proliferation, iECs were flow-sorted based on DNA content and plated in the absence of inhibitor. After an initial lag phase, a subset of cells began to rapidly proliferate and form colonies of mitotically dividing cells. We are currently assessing why only some cells return to division, and if senescence is a determinant factor. We are also re-expressing genes downregulated in iECs to ask if we can establish a gene re-expression network that stimulates return to division. Our studies provide a potential model for tumor heterogeneity that will help define molecular mechanisms that lead to endoreplication and promote return to division, and may provide new insights into how cancer cells evade normal cell cycle control.

Basic Science ***Graduate Student***

POSTER #16

RATIONAL DESIGN AND IDENTIFICATION OF N-HETEROCYCLIC DYRK1A INHIBITORS AS POTENTIAL ANTICANCER THERAPEUTICS

Francisco Huizar[1,3], Harrison Hill[2], Emily Bacher[2], Kaitlyn Eckert[2], Eva Gulotty[2], Kevin Rodriguez[2], Zachary Tucker[2], Olaf Wiest[2], Jeremiah Zartman[1,3], Brandon Ashfeld[2,3]

[1] *Department Of Chemical And Biomolecular Engineering, University Of Notre Dame, South Bend, IN*
[2] *Department Of Chemistry And Biochemistry, University Of Notre Dame, South Bend, IN*
[3] *Harper Cancer Research Institute*

Email: *fhuizar@nd.edu*

Dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) has been shown to phosphorylate an array of proteins involved in apoptosis, proliferation, cell cycle regulation, and tumor cell senescence. Glioblastoma tumors have been found to overexpress *DYRK1A* wherein DYRK1A stabilized EGFR-dependent self-renewal of tumorigenic cells. In addition to this, *DYRK1A* phosphorylation of SIRT1 has been shown to induce deacetylation of p53 to promote cell survival in HEK293T cells. Conversely, knockdown of *DYRK1A* in osteosarcoma U2OS cells resulted in DNA damage-induced apoptosis, suggesting that *DYRK1A* confers cancer cell resistance by desensitizing cells to apoptosis signals. Furthermore, breast cancer transcriptome analysis has shown *DYRK1A* to be consistently and significantly deregulated in triple negative breast cancers. These findings suggest that development of a selective, potent DYRK1A inhibitor could have far reaching clinical applications in anticancer therapies. However, while attempts have been made to modulate DYRK1A activity using chemotherapeutics, a non-toxic, selective inhibitor remains elusive. Utilizing molecular modeling studies of the toxic DYRK1A inhibitor harmine, we developed new classes of *N*-heterocyclic DYRK1A active site inhibitors. Compound libraries were generated for seven core structures based on their predicted DYRK1A ATP-binding site interactions. We report the discovery of three new classes of DYRK1A inhibitors after in vitro and in vivo evaluation of the compound libraries. ELISA assays for human recombinant DYRK1A revealed that the core heterocyclic motifs benzofuranones, spirooxindoles, and pyrrolones, showed statistically significant DYRK1A inhibition. Utilization of a low cost, high-throughput functional genomic in vivo model system substantiated the in vitro results, and the resulting correspondence validates generated classes as architectural motifs that serve as potential DYRK1A inhibitors. The unique scaffold design of these DYRK1A inhibitors allows for modifiable sites to enable future exploration of attachment groups that confer high selectivity, high potency, and low toxicity. Further expansion and analysis of these core compound structures will allow discovery of safe, more effective chemical inhibitors of DYRK1A to serve as a new therapeutic approach for anticancer therapies.

Basic Science ***Graduate Student***

POSTER #17

CAN DEEP LEARNING UNCOVER LOST TRAJECTORIES FROM NOISY FLUORESCENT IMAGES?

Fadil Iqbal[1]

[1] *Department Of Physics, Indiana University-Purdue University Indianapolis, Indianapolis, IN*

Email: *fadiqbal@iu.edu*

Artificial Intelligence has a new member: Deep Learning (DL) – which is a powerful tool capable of recognizing patterns. It has shown promise in restoring noisy fluorescent images. Noisy fluorescent images are a result of low exposure time – where it is important to reduce the photo-toxicity when imaging cells. However noisy images are also present when performing particle tracking on fast moving molecules. The exact position of these molecules are required to construct a trajectory but as we go for higher imaging speeds, the exposure time reduces and noise increases. This makes it hard to do any tracking. Our study has verified the capability of two DL packages which are capable of restoration in two ways: supervised and unsupervised. We applied them to the following examples: 1. A synthetic bead model where noise can be added artificially – we were able to restore low SNR movies and restore their individual trajectories by both supervised and unsupervised methods. 2. Experimentally we applied these techniques on nucleosomes and chromatin microdomains, to verify if deep learning can restore stationary and dynamic data. It was noted that the supervised approach delivered better results. However there are instances where these algorithms produce deceiving results, despite 'looking good' to the naked eye. We anticipate broad application of this approach to critically evaluate artificial intelligence solutions for quantitative microscopy.

Basic Science ***Graduate Student***

POSTER #18

ONCOSTATIN M INFLUENCES CARDIAC ADAPTATION AND TUMOR PHENOTYPE IN PANCREATIC CANCER WITHOUT PROTECTING AGAINST CACHEXIA

Daenique H.A. Jengelley[1], Ashok Narasimhan[2], Meijing Wang[3], Joseph E. Rupert[3], Xiaoling Zhong[3], Andrew R. Young[3], Leonidas G. Koniaris[4], Teresa A. Zimmers[5]

[1] *Department Of Biochemistry And Molecular Biology, Indiana University School Of Medicine, Indianapolis, IN*

[2] *Indiana University School Of Medicine, Indianapolis, IN*

[3] *Department Of Surgery, Indiana University School Of Medicine, Indianapolis, IN*

[4] *Department Of Surgery, Indiana University School Of Medicine, Indianapolis, IN*

[5] *IU Melvin And Bren Simon Comprehensive Cancer Center, Indiana Center For Musculoskeletal Health, Richard Roudebush Veterans Administration Medical Center, Department Of Biochemistry And Molecular Biology, Department Of Anatomy, Cell Biology & Physiology, Department Of Surgery, Department Of Otolaryngology—Head & Neck Surgery, Indiana University School Of Medicine, Indianapolis, IN*

Email: *djengell@iu.edu*

In cancer cachexia, the Interleukin-6/GP130 family of cytokines activate signaling of the JAK/STAT pathway to mediate systemic inflammation and muscle and fat loss. Among family members, Interleukin-6 (IL-6) and Leukemia Inhibitory Factor (LIF) promote cachexia and pancreatic cancer. However, less is known of the related Oncostatin M, which has functions in inflammation, fibrosis, metabolism, and hematopoiesis. OSM is expressed by blood cells and is elevated in inflammation while OSMR is ubiquitously expressed. OSM can induce IL-6 secretion by binding OSM receptor (OSMR). Here we show that high expression of OSMR in tumor correlated with poor overall survival in patients with pancreatic cancer and that OSM was elevated in a mouse model of pancreatic cancer cachexia. Functionally, AAV-OSM administration to wildtype (WT) and *Il6* null mice caused local muscle atrophy and fibrosis, systemic bone loss, and cardiac dysfunction in non-cancerous mice, suggesting OSM could mediate cardiac and musculoskeletal effects in pancreatic cancer cachexia independent of IL-6. We hypothesized that inhibition of OSM and OSMR would be protective in cancer cachexia. 14-week-old WT, $Osm^{-/-}$, and $Osmr^{-/-}$ mice, were orthotopically implanted with the $Kras^{G12D}$;$Trp53^{R172H}$;Pdx1:Cre pancreatic tumor cell line, KPC32043, and followed for echocardiography, weight change, and body composition by EchoMRI. All groups were euthanized when one reached 5% fat mass. Cardiac dysfunction was detected earlier in $Osm^{-/-}$ KPC mice versus WT KPC mice, with increased ejection fraction and fractional shortening in the absence of cardiac wasting. At euthanasia, OSM plasma levels were elevated in WT and $Osmr^{-/-}$KPC mice compared to sham but minimally detected in $Osm^{-/-}$ mice. There were no differences in terminal cardiac, skeletal muscle or fat wasting among WT, $Osm^{-/-}$, $Osmr^{-/-}$ KPC mice. There were no differences in splenomegaly, organ wasting, or tumor size across genotypes. However, tumors from $Osm^{-/-}$ mice showed reduction in the stromal cell compartment and tumors from $Osm^{-/-}$and $Osmr^{-/-}$ mice had altered tumor proteomes as well as altered histological phenotypes in both tumor cells and cancer associated fibroblasts. These data indicate that OSM signaling via OSMR influences cardiac adaptation to cancer and modulates tumor aggressiveness in pancreatic cancer but is not necessary for cachexia.

Basic Science ***Graduate Student***

POSTER #19

INVESTIGATING THE ROLE OF THE KINESIN-14 TAIL DOMAIN IN CENTROSOME CLUSTERING

Emily Jones[1], Stephanie Ems-McClung[2], MacKenzie Emch[2], Stephanie Zhang[3], Claire Walczak[2]

[1] *IU School Of Medicine-Bloomington, Indiana University, Bloomington, IN*
[2] *IU School Of Medicine-Bloomington, Bloomington, IN*
[3] *Biology, Bloomington, IN*

Email: *jonesemj@iu.edu*

Centrosome amplification is a hallmark of cancer that correlates with poor outcomes. Cells with centrosome amplification form multipolar spindles, which lead to multipolar cell divisions and lethal levels of aneuploidy. However, many cancer cells have the ability to cluster centrosomes and form bipolar spindles, which enhances cell survival. Kinesin-14 proteins are important for centrosome clustering and are often amplified in many tumors. Inhibition of Kinesin-14 proteins in cancer cells with centrosome amplification leads to cell death; however, Kinesin-14s are not essential in normal cells. Therefore, targeting Kinesin-14s could provide a novel mechanism to selectively kill cancer cells without harming normal cells. Kinesin-14s are molecular motors that cross-link and slide both parallel and anti-parallel microtubules. Because microtubules near centrosomes are organized in a parallel manner, we hypothesize that the parallel microtubule cross-linking activity is critical for centrosome clustering. Previous work from our lab showed that Kinesin-14s can cross-link microtubules using their ATP-dependent kinesin-like motor domain and a second microtubule domain in the tail domain. How a single microtubule binding domain in the tail could be involved in cross-linking of microtubules of opposite polarity is not known. To address this question, we mapped the regions of the tail that are important for microtubule binding and found two independent microtubule binding domains, which we named MBD1 and MBD2. Biochemical analysis of these domains supports the idea that MBD1 mediates anti-parallel microtubule cross-linking and MBD2 mediates parallel microtubule cross-linking. We propose that MBD2 would thus be critical for centrosome clustering. To test this idea in cells, we are currently doing knockdown/rescue experiments using wild-type and mutant Kinesin-14s to ask if mutation of MBD2 impacts centrosome clustering. In addition, an analysis of cBioPortal revealed that while most patients with alterations in KifC1 (Kinesin-14 in humans) are amplifications of the gene, a small number of patients have mutations in highly conserved residues in MBD1. We postulate that mutations in MBD1 will reduce anti-parallel microtubule cross-linking and cause a commensurate increase in parallel microtubule cross-linking, leading to an increase in centrosome clustering, an idea that we are currently testing. Understanding the mechanisms by which Kinesin-14s cluster centrosomes will enable us to develop novel inhibitors that have the potential to treat cancers with centrosome amplification.

Basic Science ***Graduate Student***

POSTER #20

ROLE OF INTRINSICALLY DISORDERED RNA BINDING PROTEINS AS ONCOGENIC TRANSCRIPTIONAL CO-ACTIVATORS IN FUSION POSITIVE CANCERS

Renee Kinne[1]

[1] *Medical Sciences , Indiana University Bloomington, Bloomington, IN*

Email: *rekinne@iu.edu*

Chromosomal translocations that result in gene fusions are a common cause of cancer. In particular, gene fusions involving transcription factors are found in multiple cancers such as Ewing's sarcoma and renal-cell carcinoma. A commonality between these fusion proteins is that the transcription factors are fused to intrinsically disordered RNA binding proteins (ID-RBP). The intrinsically disordered regions of ID-RBPs allow them to bind together and form phase separated granules. In these fusion proteins, the ID-RBPs act as co-activators leading to oncogenic transcription but the mechanism for this activation is not fully known. Part of this activation may involve the paraspeckle, a nuclear phase-separated body that has an RNA scaffold. Many of the ID-RBPs found in these gene fusions are essential proteins for paraspeckle formation and function, making the paraspeckle an unexplored player in gene fusion oncogenic transcription. To investigate the mechanism, ID-RBPs will be cloned together with transcription factors they are not naturally found fused with and expressed in normal prostate cells. These fusions will be examined for oncogenic affects through functional assays such as migration. Paraspeckle presence and composition can then be examined by fluorescent imaging. This study will contribute to our understanding of gene targeting and activation based on protein ability to phase separate. This research could also further our understanding of the paraspeckles' potential role in cancer.

Basic Science ***Graduate Student***

POSTER #21

THE ROLE OF ENTEROENDOCRINE CELLS AND STAT3 IN COLON INFLAMMATION

Christopher Ladaika[1], Asdf Asdf[2,4], Heather O'Hagan[3]

[1] *Biology, Medical Science, Indiana University-Bloomington, Bloomington, IN*
[2] *Adsf, Dfgs, MA*
[3] *Medical Science, Indiana School Of Medicine-Bloomington, Indiana University-Bloomington, Bloomington, IN*
[4] *Sdfa*

Email: *cladaika@iu.edu*

As of 2015, it was estimated that three million people in the U.S. have been diagnosed with inflammatory bowel disease (IBD). IBD is characterized by the chronic inflammation of the gastrointestinal tract and includes both Crohn's disease and ulcerative colitis. IBD can be very debilitating and puts people at higher risk of developing other diseases such as colorectal cancer. Current therapeutics include broad-acting anti-inflammatory and immunosuppressive drugs. These therapeutics can induce remission, but many patients do not respond to therapy or experience relapses, indicating a need for new and more targeted therapies. Despite the prevalence, much is still unknown about IBD, including the role of epithelial cell types in colon inflammation.

One cell type of potential importance in colon inflammation and IBD are enteroendocrine cells (EECs). EECs are typically associated with secreting hormones to regulate absorption and motility, but other characteristics of EECs suggest they may play an important role in inflammation. For example, EECs express innate immune receptors and microbial metabolite receptors, suggesting EECs may be key in driving the inflammatory response to microbial pathogens, a common source of inflammation in the colon. Furthermore, EECs have been found to be increased in patients with IBD and positively correlated with markers of inflammation, which suggests that EECs are a cell type with clinical relevance in IBD. Despite noted features of EECs and correlative data in IBD, much is still unknown about EECs in inflammation including what inflammatory pathways are enriched in EECs and how inflammatory pathways may affect EEC differentiation.

The JAK/STAT pathway is typically considered for its role in immunity, but it also has established roles in cell differentiation. STAT3 for example has been implicated in the differentiation of spermatogonia and pancreatic endocrine cells. Interestingly, in both examples the role of STAT3 in cell differentiation centered around its ability to increase expression of the transcription factor *NEUROG3 (NGN3).* Interestingly, *NGN3* is also an essential transcription factor for EEC differentiation, but a connection between STAT3 and EEC differentiation in the colon has not yet been established.

Using a combination of physiologically relevant human colon organoids and the HT29 colon cancer cell line, both of which contain the major cell types found in the colon, I provide evidence that supports a role for EECs in promoting the transcriptional inflammatory response to the microbial irritant LPS, and present support for STAT3 in promoting the differentiation of EECs. Moving forward I plan to use scRNA-seq to further investigate the role of EECs in the transcriptional inflammatory response to LPS and the role of STAT3 in EEC differentiation and ATAC-seq to determine if the EEC mediated inflammatory response is due to regions of open chromatin that are unique to EECs.

Basic Science ***Graduate Student***

POSTER #22

TARGETING FGFR1 EXPRESSION THROUGH G-QUADRUPLEX STABILIZATION INHIBITS METASTATIC BREAST CANCER

Hang Lin[1]

[1] *Medicinal Chemistry And Molecular Pharmacology, Purdue University, West Lafayette, IN*

Email: *lin791@purdue.edu*

Metastatic breast cancer (MBC) is the most advanced stage of breast cancer. Our understanding of the molecular mechanisms which drive MBC remain incomplete. Epithelial-mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET) promote drug resistance and metastasis. It has been reported that fibroblast growth receptor 1 (FGFR1) plays a key role during the EMT: MET cycle. Furthermore, FGFR1 is amplified in 13% of breast cancer patients. Therefore, optimizing inhibition of FGFR1 is crucial for the therapeutic targeting of late-stage breast cancer. Herein, we examined the efficacies of FGFR kinase inhibitors in the murine 4T07 tumor model. Inhibition of FGFR kinase activity leads to tumor growth inhibition but fails to eradicate dormant breast cancer cells. Therefore, we explored broader approaches to inhibit FGFR1 expression in addition to blockade of its kinase activity. G-quadruplex (G4) is an emerging target for cancer therapies. Small molecules interact with and stabilize G4s formed in the promoters of oncogenes, effectively blocking the expression of critical cancer-related genes. Examination of the proximal FGFR1 promoter revealed several potential G4 forming sequences. Therefore, we evaluated G4 stabilizers in FGFR1 expressing, metastatic and drug-resistant cell lines. This approach resulted in dramatic downregulation of FGFR1 at the protein level after treatment. G4 stabilizing agents also interfere with ectopic FGFR1 expression and EMT-driven FGFR1 expression. Importantly, use of the clinical G4-targeting compound CX-5461 effectively blocked FGFR1 expression and inhibited FGFR1 downstream signaling, resulting in eradication of dormant breast cancer cells. Finally, *in vivo* application of CX-5461 reduced FGFR1 expression, blocked pulmonary tumor formation, and prolonged animal survival. In conclusion, consistent with clinical observations, our study demonstrates intrinsic resistance to FGFR kinase inhibitors in MBC. Our findings also indicate that targeting FGFR1 expression through G4 stabilization may represent an improved therapeutic strategy for MBC.

Basic Science ***Graduate Student***

POSTER #23

LONG NONCODING RNAS ARE ABUNDANT TARGETS OF ADAR3 IN GLIOBLASTOMA

Priyanka Mukherjee[1], Heather Hundley [2]

[1] *Cell, Molecular And Cancer Biology, Bloomington, IN*
[2] *Medical Sciences Program, Bloomington, IN*

Email: *primukh@iu.edu*

Adenosine-to-Inosine (A-to-I) editing occurs in almost 60% of human transcripts and is catalyzed by Adenosine Deaminases Acting on dsRNA (ADAR) family of RNA-binding proteins. In humans, A-to-I editing is primarily mediated by two ubiquitously expressed deaminases, ADAR1 and ADAR2. Both ADAR1 and ADAR2 are essential for normal neurological function and are associated with several pathologies including cancer, Alzheimer's, epilepsy and Aicardi-Goutières syndrome (AGS). Hence, extensive research has been directed towards understanding gene regulatory functions of the active deaminases. On the contrary, despite being discovered two decades ago, the physiological role of the brain-specific, deaminase-deficient ADAR family member, ADAR3, is largely unknown.

Loss of ADAR3 leads to altered gene expression profiles and is associated with learning and memory deficiencies in mice. Literature also demonstrates that ADAR3 is capable of binding both single and double stranded RNA molecules. However, molecular targets of ADAR3 and the mechanism underlying how ADAR3 binding to target transcripts influence gene expression profiles are still unknown. Additionally, the physiological significance behind why glioblastoma tissue samples have elevated ADAR3 protein expression when compared to adjacent normal tissues is still elusive. Our central hypothesis is that ADAR3 regulates glioblastoma progression through directly binding to target transcripts and modulating the tumor transcriptome and/or proteome by regulating transcript localization. In this regard, a high-throughput approach was employed in U373 glioblastoma cells to identify transcripts bound by endogenous ADAR3. We found that ADAR3 binds 1435 RNA targets of which almost 30% are long non-coding RNAs (lncRNAs). Since lncRNAs are known to play an important role in gene expression regulation in various cancers, our current efforts are directed towards delineating the global mechanism by which ADAR3 regulates cellular localization of bound lncRNA targets. Concurrently, we are also trying to understand the consequences ADAR3-mediated localization of target lncRNAs has on gene expression profiles in the context of glioblastoma progression. As very little is known about the molecular and cellular functions of ADAR3, insights gained from this study will be highly valuable in shedding light on the oncogenic potential of ADAR3.

Basic Science ***Graduate Student***

POSTER #24

PP2A ACTIVATION DRIVES EGFR SIGNALING IN KRAS MUTANT PDAC

Claire Pfeffer[1], Elizabeth Hoffman[2], Sydney Clifford[2], Gagan Mall[2], Garima Baral[2], Brittany Allen-Petersen[2,3]

[1] *Biological Sciences, Interdisciplinary Life Sciences , Purdue University, West Lafayette, IN*
[2] *Biological Sciences, Purdue University, West Lafayette, IN*
[3] *Purdue Center For Cancer Research*

Email: *cpfeffe@purdue.edu*

Pancreatic ductal adenocarcinoma (PDAC) has a low 5 year survival rate at just 10% and is set to become the 2nd leading cause of cancer related deaths by 2030. Over 90% of PDAC patients have oncogenic KRAS mutations. The majority of PDAC patients present with $KRAS^{G12D}$ mutations, which are currently considered undruggable. Therefore alternative therapeutic strategies to target KRAS-driven oncogenesis are needed. The first FDA-approved targeted therapeutic for PDAC was the EGFR inhibitor, Erlotinib. While EGFR signals downstream through KRAS, EGFR is still required for PDAC tumorigenesis, despite mutation of KRAS. While EGFR is critical for PDAC development, late stage PDAC patients may eventually develop a resistance to EGFR inhibitors, further promoting the critical need for new therapeutics in PDAC.

Protein Phosphatase 2A (PP2A) is a serine/threonine phosphatase that negatively regulates many downstream targets of both EGFR and KRAS, making the activation of PP2A a potential therapeutic target in PDAC. PP2A is a holoenzyme composed of three subunits: the scaffolding subunit (A subunit), the catalytic subunit (C subunit), and the regulatory subunit (B subunit). While the global role of PP2A is canonically a tumor suppressor, the role of PP2A in oncogenesis can vary based on the B subunit incorporated into the complex, influencing the targets of PP2A activity. PP2A is suppressed in PDAC by overexpression of PP2A endogenous inhibitors including cancerous inhibitor of PP2A (CIP2A) and SET. The knockdown of CIP2A with EGFR inhibitors as well as combinations of SET and EGFR inhibitors have shown synergism in lung cancer, suggesting that EGFR inhibition and PP2A activation is a promising combination. However, the application and exact mechanism of this synergistic relationship has yet to be elucidated.

Given the role of EGFR signaling in PDAC and the regulation of EGFR downstream signaling by PP2A, we sought to determine the role of PP2A in EGFR activation. We hypothesize that PP2A negatively regulated EGFR and that relationship could be therapeutically leveraged. We first assessed the impact of PP2A on EGFR signaling activation by activating PP2A activity through CIP2A knockdown or overexpression or knockdown of the B subunit, B56α. Using the Small Molecule Activator of PP2A (SMAP), DT-061, we assessed the impact of therapeutic activation of PP2A on EGFR signaling. Surprisingly, we found that PP2A activation lead to increased expression of EGFR ligands and EGFR activation. Finally, we combined DT-061 and EGFR inhibitors to identify if the combination shows synergy in KRAS mutant PDAC. Consistent with our hypothesis, this EGFR inhibition and PP2A activation combination is efficacious. Contradictory to our hypothesis, we have identified a novel role of PP2A activating EGFR and are continuing to explore the implications of this signaling.

Basic Science ***Graduate Student***

POSTER #25

IDENTIFYING CRITICAL TUMOR-SUPPRESSIVE MICRORNAS IN ANGIOSARCOMA

Annaleigh Powell-Benton[1], Emma Terwilliger[1], Hannah Maluvac[1], Anthony Murphy[1], Claire Pfeffer[1], Sagar Utturkar[2], Nadia Lanman[3], Jason Hanna[4]

[1] *Biological Sciences, Purdue University, West Lafayette, IN*

[2] *Purdue University Center For Cancer Research, Purdue University, West Lafayette, IN*

[3] *Purdue University Center For Cancer Research, Comparative Pathobiology, Purdue University, West Lafayette, IN*

[4] *Purdue University Center For Cancer Research, Biological Sciences, Purdue University, West Lafayette, IN*

Email: *powel172@purdue.edu*

Angiosarcoma is a rare, aggressive cancer that occurs in endothelial cells. The five-year survival rate of angiosarcoma is 30%. Due to the rarity of angiosarcoma, there is a lack of understanding of the drivers of the disease and novel therapeutic targets. MicroRNAs are short, non-coding RNAs that regulate messenger RNAs, and have many important cellular functions. Dysregulation of miRNAs has been shown to contribute to many diseases, including cancer. Although miRNAs have been explored extensively in other cancers, research into their role in angiosarcoma is limited. We are currently investigating the role of miR-497-5p in angiosarcoma. Of several microRNAs proposed to be dysregulated in angiosarcoma, we found that miR-497-5p most significantly and consistently suppressed cell viability in angiosarcoma cell lines. To determine the mechanisms and targets responsible for this phenotype we utilized a combinatorial approach with RNA-seq and target prediction algorithms. Based on this we have identified four candidate target genes that are downregulated in our cell lines as a result of miR-497 mimic transfection, predicted to be direct targets of miR-497-5p, and are upregulated in human angiosarcoma. miR-497 target gene validation and mechanistic studies on these target genes are ongoing. This work provides insight into the tumor-suppressing role of miR-497-5p in angiosarcoma and may lead to new therapeutic targets for this understudied and devastating cancer.

Basic Science ***Graduate Student***

POSTER #26

TRANSCRIPTOME-WIDE ANALYSIS OF ADAR3 BINDING AND REGULATION OF ADAR1 EDITING ACTIVITY IN GLIOBLASTOMA CELLS.

Reshma Raghava Kurup[1], Emilie Oakes[2], Aidan Manning[3], Pranathi Vadlamani[3], Heather Hundley[4]

[1] *Genome, Cell And Developmental Biology , Indiana University , Bloomington, IN*
[2] *Genome, Cell And Developmental Biology , Indiana University, Bloomington , IN*
[3] *Medical Sciences , Indiana University School Of Medicine, Bloomington, IN*
[4] *Medical Sciences, Indiana University School Of Medicine, Bloomington, IN*

Email: *rrkurup@indiana.edu*

Glioblastoma is one of the most lethal brain tumors and many RNA binding proteins (RBPs) are shown to contribute to glioma development and progression. Misregulation of RBPs contributes to pathogenic gene expression programs by influencing regulatory processes such as RNA modification, splicing, stability, localization, and translational efficiency. RBPs known as ADARs are required for normal neurological function, and altered ADAR function is associated with several pathologies such as cancer, autoimmune and neurodegenerative diseases. ADARs regulate one of the most abundant RNA modifications in humans, the conversion of adenosine to inosine, also referred to as A-to-I editing. In humans, there are three ADAR family members, ADAR1, ADAR2, and ADAR3. While ADAR3 is a member of the ADAR family of RNA editors due to a shared domain structure, ADAR3 lacks editing activity. However, ADAR3 is known to repress ADAR2-mediated editing of one essential neuronal transcript in glioblastoma cells. Recent studies also demonstrated increased expression of ADAR3 protein in glioblastoma tumors compared to adjacent normal tissue. However, the cellular targets and molecular function of ADAR3 in glioblastoma are largely unknown.

In this study, we performed a transcriptome-wide analysis to determine RNA targets of ADAR3 and the effects of ADAR3 on RNA editing in the U87 glioblastoma cell line. High-throughput sequencing analysis indicates that ADAR3 binds to over 3300 transcripts, and around 60% of ADAR3 targets were also previously found to be bound by ADAR1. ADAR3 expression resulted in differential editing of > 200 sites, with approximately 70% of sites exhibiting reduced editing. Around 58% of differentially edited sites were within transcripts bound by ADAR3, suggesting that most of the ADAR3 effects on editing might be caused by binding to specific transcripts. The majority of these differentially edited sites are located in 3' untranslated regions and were previously found to be catalyzed by ADAR1, indicating that ADAR3 acts as a negative regulator of ADAR1-mediated editing. Inhibition of ADAR1 editing by ADAR3 was investigated in one important, shared transcript encoding the essential innate immune response pathway gene, *MAVS*. Overexpression of ADAR3 and knockdown of ADAR1 results in reduced editing in the *MAVS* 3'UTR, leading to upregulation of MAVS protein without altering mRNA level. ADAR3 deaminase domain mutants with altered binding and editing activity were used to investigate the mechanism by which ADAR3 regulates editing and substrate selectivity. Together, this study provides insights into ADAR3-mediated regulation of ADAR1 editing activity and gene expression during normal development as well as in glioblastoma.

Basic Science ***Graduate Student***

POSTER #27

THE ROLE OF PP2A-B56α IN KRAS-MEDIATED PANCREATIC TUMORIGENESIS

Samantha Tinsley[1], Rebecca Shelley[2], Mary Thoma[3], Goutham Narla[4], Roalie Sears[3], Brittany Allen-Petersen[2]

[1] *Biological Sciences, Interdisciplinary Life Sciences, Purdue University, West Lafayette, IN*
[2] *Biological Sciences, Purdue University, West Lafayette, IN*
[3] *Molecular And Medical Genetics, Oregon Health And Sciences University, Portland, OR*
[4] *Internal Medicine, University Of Michigan, Ann Arbor, MI*

Email: *stinsley@purdue.edu*

Protein phosphatase 2A (PP2A) is a major serine/threonine phosphatase that regulates many cellular pathways including KRAS, whose oncogenic mutation is prevalent in 95% of patients with Pancreatic Ductal Adenocarcinoma (PDAC). Previous research has identified a decrease in global PP2A activity, as well as an increase in the expression of PP2A inhibitors, in PDAC cell lines. These studies suggest that suppression of PP2A activity may be important in PDAC maintenance.

The active PP2A holoenzyme consists of 3 subunits: the scaffolding subunit (A), the catalytic subunit (C), and the regulatory subunit (B). There are 20+ different B subunits that can be incorporated into the PP2A holoenzyme. The diversity of B subunits is responsible for substrate specificity. While global PP2A has tumor suppressive capabilities, the regulation of specific pathways by PP2A can dramatically change based on PP2A holoenzyme composition. Specifically, the B56α subunit of the heterotrimeric PP2A holoenzyme has been shown to negatively regulate cellular transformation and has decreased expression in PDAC, indicating that B56α suppression may aid in PDAC tumorgenicity. Therefore, there is a critical need to understand the mechanisms that alter PP2A function and substrate targeting.

Our research aims to investigate the impact of oncogenic KRAS on PP2A-B56α activity and how suppression of B56α impacts the initiation and progression of PDAC. Our preliminary studies suggest that induction of $KRAS^{G12D}$ increases the expression of cancerous inhibitor of PP2A (CIP2A), indicating that PP2A suppression may be an early event in PDAC initiation. Consistent with this hypothesis, our *in vivo* data show that the loss of B56α in the context mutant KRAS accelerates PDAC initiation, increasing the formation of precursor lesions. Additionally, loss of B56α in *ex vivo* acinar-to-ductal metaplasia assays promotes cellular plasticity in a cell-autonomous manner, thereby accelerating KRAS-dependent transdifferentiation. Future studies will investigate how mutant KRAS expression effects overall PP2A phosphatase activity, PP2A holoenzyme formation, and sequestration of B56α by endogenous inhibitors to further understand how suppression of PP2A-B56α contributes to development of PDAC. Together, these studies identify PP2A as a critical regulator of KRAS-induced cellular plasticity and suggest that the therapeutic reactivation of PP2A may be a novel therapeutic strategy in PDAC patients.

Basic Science ***Graduate Student***

POSTER #28

HOTAIR FUNCTIONS THROUGH NF-KB PATHWAY IN REGULATING OVARIAN CANCER STEM CELLS

Weini Wang[1], Fang Fang[1], Ali R. Ozes[1], Heather O'Hagan[1,2], Kenneth Nephew[1,2]

[1] *Medical Sciences Program, Indiana University, School Of Medicine, Bloomington, IN, Bloomington, IN*
[2] *Indiana University Melvin And Bren Simon Comprehensive Cancer Center, Indianapolis, IN*

Email: *wang654@iu.edu*

Ovarian cancer (OC) is the fifth leading cause of cancer-related death among U.S. women. Persistence of OC stem cells (OCSCs) is believed to contribute to resistance to platinum-based chemotherapy and disease relapse. Long non-coding RNA HOXC transcript antisense RNA (HOTAIR) has been shown to be associated with chemoresistance and overexpressed in many types of cancers, including high-grade serous OC (HGSOC). Previously published work has demonstrated that NF-κB was activated by HOTAIR through I-κB inhibition via trimethylation of histone H3 lysine K27, which contributes to chemoresistance in HGSOC. NF-κB-medicated signaling pathways involved in maintaining characteristics of CSCs, such as targeting stem cell markers, CD44, CD133, and ALDH1 has been demonstrated but not well defined in OC. The goals of this study are to understand the mechanism of HOTAIR-mediated EZH2 dependent signaling pathways in regulating OCSCs and develop novel strategies to target OCSCs and overcome OC recurrence and drug resistance. Quantitative RT-PCR analysis revealed that HOTAIR was overexpressed in OCSCs compared to non-OCSCs. In order to produce loss-of-function phenotypes of HOTAIR and investigate the function of this gene, we utilized the paired CRISPR guide RNA design to delete the functional sites of HOTAIR without affecting nearby protein-coding gene. Knockout of HOTAIR re-sensitized OC cells to platinum treatment and significantly decreased ($P<0.001$) OCSC population and stemness-related phenotypes, including spheroid formation and colony formation ability. ALDH1A1 and expression of other stemness-related genes, including Notch3, PROM1 were significantly decreased by HOTAIR knockout. Contrariwise, forced overexpression of HOTAIR in OC cells significantly increased ($P<0.05$) these stem-like characteristics. Integrated analysis of RNA-seq and ATAC-seq on control and HOTAIR knockout cells revealed that 76% overlapped signaling pathways, suggesting that HOTAIR mechanism of action included altering global chromatin dynamics to change downstream gene expression, in particular the NF-κB pathway. Furthermore, NF-κB nuclear accumulation and activation in OCSCs was increased compared to non-OCSCs and inhibiting NF-κB (pharmacologically or by NF-κB knockout) significantly decreased ($P< 0.01$) OCSC population and stemness-related phenotypes, including ALDH1 protein expression. However, in HOTAIR knockout cells, inhibition of NF-κB had no effect on OCSC characteristics, indicating that HOTAIR may function through constitutively activated NF-κB in regulating OCSCs. Collectively, these results strongly indicate that the HOTAIR- NF-κB axis plays an important role in regulating OCSCs and represents a potential therapeutic target to eliminate residual tumor cells after conventional chemotherapy and prevent OC recurrence and drug resistance.

Basic Science ***Graduate Student***

POSTER #29

INVESTIGATING MCAK AND KIF18B AS NEW CANDIDATES FOR ANTI-MICROTUBULE THERAPEUTIC DRUG TARGETS.

Jordon Winn[1], Sanjay Shrestha[2], Mark Hazelbaker[2], Stephanie Ems-McClung[2], Claire Walczak[2]

[1] *Biology, Indiana University - Bloomington, Bloomington, IN*
[2] *Medical Sciences, Bloomington, IN*

Email: *winnj@iu.edu*

Chromosome instability is a hallmark of many tumors and leads to aneuploid daughter cells. In normal cells, severe aneuploidy is lethal, but cancer cells are unique and can maintain some level of aneuploidy without lethality. A common therapeutic that may function by causing lethal levels of aneuploidy are the taxanes, such as paclitaxel. Taxanes bind microtubules (MTs) and modulate normal MT dynamics, which are critical for accurate chromosome segregation. However, taxanes and other anti-MT agents also affect the dynamics of MTs in neurons, causing severe neuropathy in patients. Finding new targets that alter MT dynamics and/or increase aneuploidy without affecting MT dynamics in neurons could provide new opportunities for therapeutic development. Mitotic Centromere-Associated Kinesin (MCAK), a Kinesin-13, is a major regulator of chromosome instability and aneuploidy. Functioning in normal cellular MT dynamics, MCAK binds directly to MT ends and induces their depolymerization. During mitosis, MCAK localizes to kinetochores where it uses its MT depolymerization activity to correct improper kinetochore attachments that cause lagging chromosomes leading to aneuploidy. A second kinesin motor, Kif18B (a Kinesin-8), is a plus-end directed MT motor protein that walks along MTs and destabilizes them. In cells, Kif18B localizes to the plus ends of astral microtubules and is required for chromosome alignment and proper spindle positioning. Both kinesins are also severely mis-regulated in various types of cancers, indicating that their function may be important to maintain proper ploidy and chromosome stability. Thus, inhibiting MCAK and/or Kif18B could lead to increased aneuploidy and cellular lethality in cancer cells. Interestingly, while MCAK can binds MT ends *in vitro*, it is proposed that Kif18B transports MCAK to the MT plus ends in cells. In addition, both kinesins bind end-binding protein (EB1), which is required for their accumulation on MT plus-ends. How the interaction between MCAK, Kif18B, and EB1 is regulated and how their activities coordinate cellular MT dynamics is unclear. To tease apart these relationships, we are characterizing their interactions to dissect their contributions in cellular MT dynamics. In addition, we are using super-resolution microscopy to define the spatial distribution of MCAK, Kif18B, and EB1 at MT ends. We have mapped the binding sites of EB1 on Kif18B as well as the importin α binding sites where the nuclear transport proteins, importin α/β, bind Kif18B. In addition, we found that EB1 and importin α/β are competitive binding partners for Kif18B. We are currently developing constructs to map the interaction domains in MCAK and Kif18B and are testing the contributions of EB1 and importin α/β binding to Kif18B in cells. Given that disruption of MT dynamics causes chromosome instability, looking more closely at MCAK/Kif18B interactions may allow us to better pinpoint targets for anti-microtubule agents for the treatment of cancer.

Basic Science ***Graduate Student***

POSTER #30

AN INVESTIGATION INTO CROSSTALK BETWEEN SENSORY NEURONS AND CANCER CELLS TO DRIVE THE GROWTH AND PROGRESSION OF PROSTATE CANCER

Hanyu Xia[1], Jill C. Fehrenbacher[1], Travis J. Jerde[1]

[1] *Department Of Pharmacology And Toxicology, Indiana University School Of Medicine, Indianapolis, IN*

Email: *xiah@iu.edu*

Prostate cancer (PCa) cells produce a variety of neurotrophic factors that promote cancer progression and the outgrowth of nerve fibers into the tumor microenvironment (TME). In addition, nerve fibers have been implicated as a conduit for metastasis, and perineural invasion has been correlated with increased risk of lethal PCa. Furthermore, recent publications have demonstrated that peripheral nerves can regulate cancer growth and progression. This has been demonstrated in PCa where denervation of sympathetic and parasympathetic nerves can inhibit PCa growth and migration, respectively. The prostate is densely innervated by sympathetic, parasympathetic, and sensory nerves, which are essential for normal prostate physiology.However, sensory innervation of the normal and malignant prostate is poorly characterized. It is well known that peptidergic sensory nerves (PSNs) are capable of anterograde release of neuropeptides that are essential for modulating inflammation and remodeling during tissue repair. Therefore, we hypothesize that sensory nerves and PCa are engaged in a feed-forward relationship, whereby the PCa TME drives sensory outgrowth and alters sensory function to increase the anterograde release of factors that contribute to the growth and metastasis of PCa.

Preliminary *in vitro* data demonstrate that conditioned media from an established PCa cell line, LNCaP, treated with IL-1α (10ng/ml) increases calcitonin gene-related peptide (CGRP) release from dorsal root ganglion (DRG) primary cultures of mouse sensory nerves beyond that elicited by vehicle, IL-1α (10ng/ml) alone, or LNCaP conditioned media alone. This suggests that PCa cells in the inflamed TME enhance the release of neuropeptides from sensory nerves. Interestingly, visual observations showed that LNCaP cells exhibited altered morphology, including dendritic projections and a mesenchymal appearance, with conditioned media from primary cultures of mouse DRG sensory nerves treated with IL-1α compared to vehicle alone. Additional experiments are being performed to validate these findings and identify the molecular mechanisms driving these observations.

To test whether PCa drives sensory nerve outgrowth, we will characterize sensory innervation of the prostate with and without an orthotopic tumor. 2D immunofluorescence studies have identified the presence of PSN tract-like structures in the murine prostate as previously published. However, state-of-the-art imaging will be required to interrogate whether PCa alters sensory innervation. Therefore, our group is currently optimizing whole-organ tissue clearing and immunofluorescent labeling techniques to image and render 3D visualizations of PSNs in the prostate. Furthermore, to assess the functional role of PSNs in PCa *in vivo*, we are developing a model of the selective PSN ablation in a novel syngeneic orthotopic mouse model of PCa. Together, this work will, for the first time, determine the spatial distribution and density of PSNs in the normal and malignant prostate, and determine whether PSNs play a role in PCa progression.

Basic Science ***Graduate Student***

POSTER #31

POTENTIAL ROLE OF CD14 IN LIVER REGENERATION INDUCED MUSCLE WASTING

Benjamin Johnson[1], Tewfik Hamidi[2], Leonidas Koniaris[2], Teresa Zimmers[2]

[1] *Indiana University School Of Medicine, Fort Wayne, IN*
[2] *Department Of Surgery, Indiana University School Of Medicine, Indianapolis, IN*

Email: *bejohn@iu.edu*

Background:

Organ cross-talk mechanisms are important drivers of cachexia, a systemic wasting disorder of a patient's muscle, fat, and bone. Our lab showed that muscle atrophy occurred in mice after a "regenerative" partial hepatectomy (PH) but not after a "non-regenerative" pneumonectomy, suggesting that injured regenerative organs such as the liver secrete factors that mediate muscle wasting. An important driver in liver regeneration is Yap1, a known transcription factor involved in organ size and regeneration. Interestingly, Yap1 overexpression in the liver induced hepatic growth and dramatic muscle atrophy. RNA-sequencing on Yap1-overexpressing livers identified Cd14 as a possible candidate involved in this cross-talk. This project will elucidate the role of Cd14 in liver regeneration and muscle atrophy in the mouse model and cancer patients.

Methods:

Western blot analysis was used to detect Cd14 levels in liver, muscle and serum from PH mice. Immunohistochemistry (IHC) and immunofluorescence (IF) were used to visualize Yap1 and Cd14 in PH and Yap1-overexpressing livers. IHC was used to localize CD14 expression in mouse liver metastases. Finally, human primary liver cancer and metastatic tissue microarrays (TMA) were used to detect CD14 expression by IHC.

Results:

Western blot and IHC showed a 3.3-fold increase in Cd14 levels in PH mouse serum compared to sham and 1.8-fold increase in livers from the PH group compared to sham, 24 hours after PH. IF showed higher Cd14 levels in Yap1-overexpressing hepatocytes. IHC staining revealed a higher Cd14 expression in hepatocytes surrounding metastasis. Finally, TMA analysis suggests that CD14 expression is increased in human liver cancer and liver metastasis.

Conclusion and potential impact:

Our data suggests that CD14 is a target of YAP1 that plays an important role in liver metastasis, inflammation, and regeneration. Future studies will investigate CD14 in liver-muscle crosstalk and the role of CD14 in cancer cachexia.

Basic Science ***Medical Student***

POSTER #32

SEMISYNTHESIS AND BIOLOGICAL EVALUATION OF CRUENTAREN A DERIVATIVES

Xiaozheng Dou[1], Chitra Subramanian[2], Hui Guo[3], Bhargav Patel[1], Terin D'Amico[1], John Rubinstein[3], Mark Cohen[2], Brian Blagg[1]

[1] *Department Of Chemistry & Biochemistry, University Of Notre Dame, Notre Dame, IN*
[2] *General Surgery Clinic, University Of Michigan, Ann Arbor, MI*
[3] *Department Of Medical Biophysics, University Of Toronto, Toronto,*

Email: *xdou@nd.edu*

The 90-kDa heat shock protein (Hsp90) is a molecular chaperone that plays essential roles for the folding, stabilization, activation, and degradation of over 400 client proteins, many of which are directly associated with cancer progression. Consequently, inhibition of the Hsp90 protein folding machinery has been an attractive anticancer target since its inhibitor results in a combinatorial attack on numerous oncogenic pathways. Unfortunately, 18 Hsp90 *pan*-inhibitors entered clinical trials and failed to reach FDA approval for use as a cancer monotherapy due to off- and on-target toxicities. The discovery and development of isoform-selective Hsp90 inhibitors is regarded as a promising approach to achieve the desired anticancer effect without the harmful side effects observed with Hsp90 *pan*-inhibitors. Cruentaren A, a potent cytotoxic natural product isolated from myxobaterium, selectively inhibits F1Fo ATP synthase, which disrupts its interactions with Hsp90α and induces Hsp90 client protein degradation without induction of the pro-survival heat shock response. Cruentaren A also exhibits sub-nanomolar activity against multiple human cancer cell lines, while manifesting >500 nM activity against normal cell lines, providing a large therapeutic window for drug development. However, its clinical development has been hampered by limited structure-activity relationship studies. Recently, we solved the first Cryo-EM structure of cruentaren A bound to F_1F_o ATP synthase, which allows for the rational design improved inhibitors. Herein we report the current progress towards the semisynthesis and biological evaluation of cruentaren A analogs.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #33

IGFBP1 MEDIATES MUSCULOSKELETAL DEFICITS IN COLORECTAL CANCER CACHEXIA

Joshua Huot[1], Fabrizio Pin[2], Alyson Essex[2], Andrea Bonetto[3]

[1] *Surgery, Anatomy, Cell Biology & Physiology, Indianapolis, IN*

[2] *Anatomy, Cell Biology & Physiology, Indianapolis, IN*

[3] *Surgery, Anatomy, Cell Biology & Physiology, Otolaryngology- Head & Neck Surgery, Indiana University School Of Medicine, Indianapolis, Indianapolis, IN*

Email: *jrhuot@iu.edu*

Background: Colorectal cancer (CRC) is often accompanied by cachexia, an uncured multi-organ wasting syndrome, debilitating musculoskeletal health and overall survival. In preclinical studies, we have shown that CRC is accompanied by metabolic perturbations of the liver, and further, that CRC liver metastases (LM) exacerbate musculoskeletal wasting. These observations, along with evidence that liver-derived factors (i.e., hepatokines) may poorly influence musculoskeletal health, suggest an endocrine role of the liver in mediating cancer-induced cachexia. Here, we identify the hepatokine, insulin-like growth factor binding protein 1 (IGFBP1) as a novel mediator of musculoskeletal wasting in CRC. **Methods:** Plasma from CRC patients and preclinical models of CRC (C26, MC38, HCT116, APC^{min}) were assessed for circulating IGFBP1 levels. AML12 hepatocytes were cultured with CRC cells (C26, MC38, HCT116) to assess tumor induced hepatic IGFBP1 production, while C2C12 myotubes and osteoclast precursor cells were cultured to examine effects of IGFBP1 on *in vitro* muscle atrophy and osteoclastogenesis. Lastly, 8-week-old male wild-type (WT) C57BL/6J and IGFBP1-KO mice were intrasplenically injected with MC38 tumor cells (mMC38) to mimic hepatic dissemination of cancer cells, while sham-operated animals received saline (n =5-7/group). Animals were assessed for muscle force 24-hours prior to euthanasia and skeletal muscles and bone were collected for mass and morphological analyses. **Results:** CRC patients and CRC tumor hosts (C26, MC38, HCT116, APC^{min}) consistently demonstrated markedly elevated circulating plasma IGFBP1 above controls ($p<0.05$), also supported by increased liver IGFBP1 mRNA expression in C26, MC38 and HCT116 hosts ($p<0.05$). Follow-up *in vitro* studies demonstrated that co-culturing C26, MC38, and HCT116 CRC cells with AML12 hepatocytes promotes increased IGFBP1 production ($p<0.01$). Of note, CRC cells lack IGFBP1 expression, thereby suggesting that IGFBP1 is purely host-derived. Treatment with recombinant IGFBP1 was sufficient to elevate osteoclastogenesis ($p<0.05$), while also promoting atrophy of C2C12 myotubes ($p<0.001$). Conversely, use of neutralizing antibodies against IGFBP1 reduced osteoclastogenesis and preserved C2C12 myotube size when exposed to serum from mice bearing CRC ($p<0.01$). WT mMC38 bearers displayed reductions in muscle mass (gastrocnemius: -16%; $p<0.0001$, quadriceps: -22%; $p<0.001$) and force (-24%; $p<0.01$), as well as in trabecular bone volume fraction (BV/TV: -53%; $p<0.01$) and trabecular number (Tb.N: -37%; $p<0.01$). Conversely, IGFBP1-KO tumor hosts exhibited preserved skeletal muscle mass (gastrocnemius: +9%; $p<0.01$, quadriceps: +18%; $p<0.01$), muscle force (+21%; $p<0.05$), and bone mass (BV/TV: +97%; $p<0.05$, Tb.N: +60%; $p<0.01$). **Conclusion**: Altogether, our data implicate IGFBP1, a uniquely host-derived factor, as a novel mediator of musculoskeletal deficits in cancer cachexia. As therapeutic interventions for cancer and cachexia often target tumor-associated factors, our work supports novel strategies to counteract host-derived factors in the treatment of cancer-associated muscle and bone deficits.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #34

SCAFFOLD-FREE BIOPRINTING OF BENIGN ENDOMETRIOTIC AND MALIGNANT OVARIAN TISSUES WITHIN ENDOMETRIOTIC MICROENVIRONMENT

Yuliya Klymenko[1,6], Lester Smith[2], Adrienne Sowers[3], Kenneth Nephew[4,6], Shannon Hawkins[1,5,6]

1 Obstetrics And Gynecology, Indiana University School Of Medicine, Indianapolis, IN

2 Radiology And Imaging Sciences, 3D Bioprinting Core, Indiana University, Indianapolis, IN

3 Indiana University School Of Medicine, Indianapolis, IN

4 Department Of Cellular And Integrative Physiology, Indiana University School Of Medicine, Bloomington, IN

5 Department Of Biochemist ry And Molecular Biology, Indiana University School Of Medicine, Indianapolis, IN

6 Melvin And Bren Simon Comprehensive Cancer Center

Email: yklymenk@iu.edu

Endometriosis, characterized by the growth of endometrial-like cell lesions outside the uterus, is a devastating, incurable disease affecting ~10% women worldwide, which results in reduced quality of life with chronic pelvic pain, infertility, and increased risk of ovarian cancer. A major obstacle impeding the progress in the disease research field is the lack of representative normal endometrial and endometriotic cell lines and model systems. The *objective* of the current work is to biofabricate scaffold-free three-dimensional (3D) self-supporting perfused endometriotic tissues for further assessment of endometriosis, the endometriotic microenvironment, and endometriosis-associated ovarian cancers. Fresh endometriotic lesions were dissociated to isolate primary stromal endometriotic cell cultures. Following expansion, primary human stromal cells were screened for their ability to generate solid, smooth, cohesive spheroids in ultra-low attachment U-bottom plates, which is a prerequisite for the subsequent 3D biofabrication with a Kenzan method. Upon spheroid parameters (diameter, density, smoothness, roundness) optimization, primary stromal endometriotic cells were employed for biofabrication on a Kenzan microneedle array using a Regenova Bio 3D Printer robotic system. After 24-96h incubation and removal from Kenzan needles, the multi-spheroid conglomerates retained as fused scaffold-free constructs, comprised from primary stromal endometriotic cells and self-released extracellular matrix. Importantly, co-culture of primary stromal cells with endometrioid ovarian cancer A2780 and SKOV3ip cells also rendered formation of more cohesive, rigid, and bioprintable heterotypic stromal/cancer spheroids in comparison with loosely connected, fragile monotypic A2780 and SKOV3ip aggregates, not competent for biofabrication. Additionally, primary endometriotic stromal cells, immortalized endometriotic epithelial cells (12z), and immortalized endometrioid ovarian cancer cell lines (A2780) were utilized to bioprint 9x9x1 monotypic endometriotic epithelial (12z only), 9x9x1 heterotypic endometriotic stromal/endometrial (stromal/12z), and 9x9x2 heterotypic endometriotic stromal/ovarian cancer (stromal/A2780) multi-spheroid constructs. These constructs adhered to type I collagen-precoated microchannel-containing perfusion platens, allowing the constructs to be removed from the Kenzan needles and placed into the separate FABRICA Bioreactor. An Ismatec digital peristaltic pump controlled continuous perfusion of bioprinted tissues with the appropriate cell medium through the tissue-platen microchannels for 24-72 hours at preselected flow rate (1-5 ml/min) under routine cell culture incubation conditions. Following perfusion, all tissues were imaged, removed from the platens, and saved for subsequent applications. Histological analysis and assessment of gene expression profiles of the perfused tissues are currently underway. Altogether, herein we report *in vitro* biofabricated and perfused scaffold-free 3D endometriotic and endometrioid ovarian cancer-like self-supporting perfused (SSuPer) tissues, comprised of multiple (stromal and epithelial) cell types. These novel complex and time-efficient models warrant further progress in research of endometriosis and associated cancers. Furthermore, employment of primary cells from human patient samples paves the way for advances in personalized medicine.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #35

PROCR+/ZEB1+/PDGFRα+ CELLS, ENRICHED IN THE BREAST TISSUES OF AFRICAN AMERICAN WOMEN, ALTER THE TISSUES MICROENVIRONMENT AND ARE PUTATIVE CELL-OF-ORIGIN OF ANAPLASTIC SARCOMAS

Brijesh Kumar[1], Poornima Bhat-Nakshatri[1], Harikrishna Nakshatri[2]

[1] *Departments Of Surgery, Indiana University School Of Medicine, Indianapolis, IN*

[2] *Departments Of Surgery, , Biochemistry And Molecular Biology, Indiana University School Of Medicine, Indianapolis, IN, VA Roudebush Medical Center, Indianapolis, IN*

Email: *kumarbr@iupui.edu*

Background: African American (AA) women suffer higher mortality from the aggressive breast cancer subtype, triple negative breast cancer (TNBC), than Caucasian (CA) women. Furthermore, basal-like and mesenchymal-like TNBCs with elevated intra-tumor heterogeneity are significantly more common in AA women. In addition, breast cancer recurrence after ductal carcinoma in situ (DCIS) diagnosis and eventual breast cancer related deaths are higher in AA women. Whether worse outcome in AA women is due to an increased incidence of TNBC or unique biological factors that promote aggressive biology is an important but unresolved challenge in cancer disparities research. We recently reported enrichment of a unique population of cells that express stemness-associated PROCR and ZEB1 in the normal breasts of AA women compared to CA women. These cells increased in the cancerous breasts of CA women suggesting their role in breast cancer progression.

Methods: Flow cytometry, adipogenic, osteogenic assay, co-culture and tumorigenesis were used to characterize population of cells that differ in the normal breasts of AA and CA women.

Results: Phenotypic characterization showed that PROCR+/ZEB1+ cells express PDGFRα, similar to adipogenic progenitors of the mouse mammary gland that transition into epithelial cells during lobulo-alveologenesis. These cells, renamed as PZP cells (PROCR+, ZEB1+, PDGFRα+), trans-differentiated into EpCAM+ epithelial cells upon transformation by $HRas^{G12V}$ ± SV40-T antigen suggesting that breast tumors in AA women can originate from two cell sources- PZP cells and luminal epithelial cells. Transformed PZP cells generated pleomorphic anaplastic sarcomas, which comprise of 0.5-1% of all breast neoplasms. Under adipogenic and osteogenic growth conditions, PZP cells transdifferentiated into adipocytes and osteocytes indicating their ability to alter the tumor microenvironment. To obtain potential insight into signaling pathway alterations in epithelial and PZP cells as a consequence of their cross-talk, we performed cytokine/chemokine profiling of factors secreted by an immortalized luminal epithelial cell line, a PZP cell line and both co-cultured together (50% of each cell line) for ~12 hours. While luminal epithelial cell line expressed several ligands such as PDGF-AA and osteopontin, which can affect trans-differentiation of PZP cells, PZP cells expressed factors such as EGF, HGF and SDF-1α, which can signal in luminal cells. Interestingly, IL-6 is produced only under co-culture condition suggesting that PZP-epithelial cell cross-talk can create pro-inflammatory, invasive, and metastatic conditions in the breast microenvironment.

Conclusions: Because the normal breasts of AA women naturally contain higher number of PZP cells, PZP-breast tumor cell interaction could occur early during tumor evolution in AA compared to CA women, which creates conditions conducive for early metastatic dissemination in AA women. These genetic ancestry-dependent differences in pro-inflammatory, invasive, and pro-metastatic status of the breast could explain for disparity in breast cancer outcomes.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #36

ASSISTANT SCIENTIST IN MEDICINE

Konstantina Kyritsi[1]

[1] *Gastroenterology, Indianapolis, IN*

Email: *kkyritsi@iu.edu*

Regulation of STING by Histamine Signaling in Human Intra- and Extra-hepatic Cholangiocarcinoma and 3D Spheroids

Background: Histamine (HA), synthesized by mast cells (MCs), exerts its effects through H1-H4 receptors (HRs). MC number increases in cholangiocarcinoma (CCA) and blocking MC-derived HA inhibits tumor growth. We have shown that the expression of STING (stimulator of interferon gene) expression increases in nude mice (nu/nu) treated with HA and decreases after treatment with cromolyn sodium, mepyramine (H1HR antagonist) or ranitidine (H2HR antagonist). **Aim:** To determine the role of HA-regulated STING in intra- and extra-hepatic CCA using 3D spheroid tumors. **Methods:** *In vitro*, we generated 3D spheroids with the CCA lines, Mz-ChA-1 (extrahepatic) and SG231 (intrahepatic), alone or in combination with human mast cells (hMCs), human hepatic stellate cells (hHSCs) or combination. After 48 hr of treatment with H-151 (STING inhibitor, 400 nM), HA (25 mM), cromolyn (25 mM), mepyramine (25 mM) or ranitidine (25 mM), we evaluated by: (i) *q*PCR the expression of STING, PCNA, VEGFA/C and the EMT markers S100A4 and vimentin; (ii) EIA kits HA and STING levels in culture media; (iii) immunofluorescence (IF) STING immunoreactivity in spheroid sections co-stained with chymase (MC marker), desmin (HSCs marker) and CK-19 (biliary marker).

The same parameters were measured in tumors from nu/nu mice injected with Mz-ChA-1 cells (3×10^6) and treated with HA, cromolyn or HR antagonists. In human CCA obtained from explants, STING expression in MCs, HSCs and cholangiocytes was evaluated by IF by co-staining for chymase, desmin or CK-19, respectively. **Results:** STING expression decreases in extrahepatic CCA, whereas expression increases in intrahepatic CCA shown by qPCR and western blot. 3D spheroids originated from Mz-ChA-1 or SG231 combined with hMCs, hHSCs or a combination and treated with HA had increased: (i) HA and STING levels; (ii) gene expression of STING, PCNA, angiogenesis and EMT markers. Inhibition of STING had no effect on Mz-ChA-1 CCA spheroid phenotypes, whereas blocking STING decreased SG231 CCA phenotypes. When HA/HR signaling was inhibited, both intra- and extra-hepatic 3D spheroid tumors had reduced (i) HA and STING levels; (ii) gene expression of STING, PCNA, angiogenesis and EMT markers. There was a colocalization of STING with MCs, cholangiocytes and HSCs in nu/nu tumors that increased after HA treatment, but decreased when the HA/HR axis was blocked. In human CCA explants, there was increased STING immunoreactivity in MCs, HSCs and CCA cells compared to controls. **Conclusion:** There is a dysregulation of STING signaling in CCA that is regulated by the HA/HR axis. 3D spheroids mimic human CCA tumors and demonstrate a novel technique to study CCA phenotypes.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #37

PROBING CANCER CELL BEHAVIORS USING VISCOELASTIC HYDROGELS

Fang-Yi Lin[1], Chien-Chi Lin[2]

[1] *Biomedical Engineering Department, Indiana University-Purdue University Indianapolis, Indianapolis, IN*
[2] *Biomedical Engineering, Indiana University Purdue University Indianapolis, Indianapolis, IN*

Email: *fli2@iu.edu*

Tumor progression is largely controlled by the tumor microenvironment, which involves different types of cells and the extracellular matrix (ECM). Mimicking ECM by synthetic hydrogels brings an opportunity to probe the impact of matrix mechanics induced mechanotransduction and phenotypic changes in cancer cells. Hydrogels crosslinked through dynamic covalent chemistry render viscoelastic properties that resembles the viscoelastic ECM. The fast equilibrium of bonding and debonding in dynamic covalent bonds allows cells to alter the microenvironment during proliferation and migration. To systematically tune hydrogel viscoelasticity, a synthetic hydrogel system was developed that incorporates thiol-norbornene and boronate ester-diol chemistries. The copolymers, termed PEHA, containing norbornene and boronic acid functional groups were synthesized via reversible addition-fragmentation chain transfer (RAFT) polymerization. The boronic acid content in PEHA copolymers were carefully regulated. By fabricating PEHA copolymers with thiolated gelatin, dopamine-conjugated poly(hydroxyethyl acrylate), and 4-arm thiol-functionalized poly(ethylene glycol), a hydrogel platform with tunable viscoelasticity was constructed for cell encapsulation. Human pancreatic ductal carcinoma cells (COLO-357) and human pancreatic cancer-associated fibroblast (CAF) were encapsulated alone or together in elastic and viscoelastic PEHA hydrogels. While COLO-357 cells proliferate better, CAF cells spread more in viscoelastic PEHA hydrogels. Moreover, in COLO-357 and CAF co-encapsulated PEHA hydrogels, COLO-357 cells exhibited epithelial-mesenchymal transition (EMT) in viscoelastic hydrogels but not in elastic hydrogels. This finding suggests that the viscoelasticity of matrix affects tumor progression and invasion into matrix. Future work will focus on developing the viscoelastic PEHA hydrogels for screening and testing anti-cancer drugs.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #38

IMPACT OF NOVEL Ku-DNA BINDING INHIBITORS ON THE DNA DSBs-INDUCED DNA DAMAGE RESPONSE.

Pamela L. Mendoza-Munoz[1], Navnath S. Gavande[2], Pamela S. VanderVere-Carozza[1], Katherine S. Pawelczak[3], Joseph R. Dynlacht[4], Joy Garrett [4], John J. Turchi[1,3,5]

[1] *Department Of Medicine, Indiana University, School Of Medicine, Indianapolis, IN*

[2] *Department Of Pharmaceutical Sciences, Wayne State University College Of Pharmacy And Health Sciences*

[3] *NERx Biosciences*

[4] *Department Of Radiation Oncology, Indiana University, School Of Medicine*

[5] *Department Of Biochemistry And Molecular Biology, Indiana University, School Of Medicine, Indianapolis, IN*

Email: *pmendoza@iu.edu*

The DNA-dependent protein kinase (DNA-PK) plays a critical role in the non-homologous end joining (NHEJ) double-strand break (DSB) repair pathway and the DNA damage response (DDR). Consequently, blocking DNA-PK kinase activity is a novel anti-cancer therapeutic strategy in combination with ionizing radiation (IR). Towards developing a new class of DNA-PK inhibitors, our laboratory has exploited the mechanism of DNA-PK activation which requires binding to DNA termini via the Ku 70/80 heterodimer. We have previously reported the development of Ku-DNA binding inhibitors (Ku-DBi's) that act via this novel mechanism of action to inhibit DNA-PK catalytic kinase activity. Ku-DBi's display nanomolar activity *in vitro*, possess cellular DNA-PK and NHEJ inhibitory activity, and sensitize non-small cell lung cancer (NSCLC) cells to DSB generating chemotherapeutics bleomycin and etoposide. In this study, we demonstrate that pre-incubation of our novel Ku-DBi's can potentiate the cellular effects of bleomycin and IR and also induce p53 phosphorylation through activation of the ATM pathway, which is concomitant by a decrease in DNA-PKcs autophosphorylation events at the S2056 (pS2056) cluster.

Using a combination of Western blot and immunofluorescence assays in the NSCLC NCI-H460 and A549 cell lines, Ku-DBi **245** treatment in combination with DNA DSBs-inducing agents such as bleomycin and IR showed a significant reduction of phosphorylation of DNA-PKcs at S2056 compared toDNA DSBs-inducing agent alone. In addition, analysis of phospho-ATM and phospho-p53 protein levels in these NSCLC cell lines, suggested activation of the ATM pathway as a function of **245** treatment followed by bleomycin, evidenced by an increase of phosphorylation of ATM at Ser1981, and p53 at Ser15.

These results demonstrate that Ku-DBi **245** blocks DNA-DSB dependent DNA-PKcs autophosphorylation, resulting in an increased bleomycin and radiation cellular sensitivity, along with inducing the activation of p53 through an ATM-dependent signaling pathway. These data are consistent with Ku-DBi's possessing a novel mechanism of action that abrogates autophosphorylation of DNA-PKcs and activates DDR signaling as part of an anticancer therapeutic strategy in combination with DNA DSBs-inducing agents.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #39

MECHANISMS TARGETING MUTANT P53 TO THE GENOME

Stephanie Metcalf[1], Peter Hollenhorst[2]

[1] *Medical Sciences, IU School Of Medicine, Bloomington, IN*
[2] *Medical Sciences, Bloomington, IN*

Email: *sametcal@indiana.edu*

Mutations in genes often result in tumor formation, especially when these mutations occur in tumor suppressors. TP53 is a tumor suppressor that is frequently mutated in cancer, with mutation in this gene occuring in nearly 50% of all cancer cases. Once mutated, p53 loses its tumor suppressive function while simultaneously gaining oncogenic function. One of the functions that mutant p53 loses is the ability to directly bind to chromatin, however, it has been reported that mutant p53 can still affect the transcriptome of cancer cells via interactions with other transcription factors. One of these interacting partners is ETS2. ETS2 belongs to the ETS transcription factor family, which has 28 family members. This family is characterized by their affinity for an ETS binding site (EBS). EBS's are present in 50% of all mutant p53 occupied promoters. Other ETS family members have also been linked to mutant p53 but these interactions have either been deemed as weak (ETS1) or have yet to be identified as direct (ERG).

To determine which ETS proteins interact with mutant p53 I conducted binding studies using purified ETS proteins and purified mutants of p53. My preliminary data shows that several ETS proteins interact with mutant p53 better than ETS2. I then sought to determine which residues are important for this interaction through truncation studies in which I used purified truncations of ETS proteins and purified mutants of p53. I found that ERG, one protein that strongly interacted with mutant p53, had two interaction interfaces. This may explain why the interaction is strong.

My next step is to determine which ETS proteins are responsible for the targeting of mutant p53 to the genome. To address the requirement for ETS to recruit mutant p53 to chromatin I performed chromatin immunoprecipitation sequencing studies in the presence or absence of different ETS factors to determine differences in mutant p53 binding. For these studies I knocked down ETS2 or ERG prior to performing p53 ChIP-Seq to determine differences in p53 binding to chromatin under these conditions. My preliminary analyses of these data indicate that each of the conditions resulted in different p53 binding patterns in the ChIP-Seq.

My future work will test phenotypes related to ETS/mutant p53 interactions. Ultimately, if I can identify ETS/mutant p53 interactions that are important for oncogenic function, these will be attractive targets for future drug development.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #40

DIFFERENTIAL SENSITIVITY OF MOUSE PDAC KRASG12D CELLS TO REF-1 REDOX-SIGNALING INHIBITORS: ROLE OF NF-KB AS A PRIMARY TARGET OF REF-1 IN KRAS DRIVEN PANCREATIC DUCTAL ADENOCARCINOMA

Mahmut Mijit[1], Randall Wireman[1], Lee Armstrong[1], Rachel Caston[1], Zonera Hassan[2], Christian Schneeweis[2], Melissa Fishel[3], Mark Kelley[1]

[1] *Department Of Pediatrics, Wells Center For Pediatric Research, Indiana University School Of Medicine, Indianapolis, IN*

[2] *Department Of Polyclinic For Internal Medicine, Technical University Of Munich, Munich, Germany,*

[3] *Department Of Pediatrics, Wells Center For Pediatric Research, Indiana University School Of Medicine, Indianapolis,*

Email: *mimaih@iu.edu*

Pancreatic Ductal Adenocarnicnoma (PDAC) is one of the deadliest cancers and has a poor response to current treatment regimens. Ref-1 redox signaling function activates several transcriptional factors (TFs) including NFkB, HIF-1a, STAT3 and others; all of which have been implicated in signaling in PDAC and are closely associated with cancer progression and development and therapy resistance. Using a panel of PDAC cell lines that were established from the genetically engineered mouse model of *Kras*^{G12D} mutation (KC), we screened these KC cell lines for sensitivity to Ref-1 redox signaling inhibitors, APX3330, APX2009, and APX2014 and ranked the lines based on inhibitor sensitivity. Using this data and bioinformatic tools, we identified key signaling pathways that may have a potential link with the differential sensitivity of PDAC cells to Ref-1 redox inhibitors. In particular, gene set enrichment analysis identified upregulation of IL-6-STAT and the NFkB-TNFa signaling pathways for APX inhibitor sensitive cell lines and mTOR and Myc pathways for resistant lines. Previous evidence suggests that NFkB, one of the targets of Ref-1, plays a crucial role in PDAC pathogenesis. Therefore, we hypothesized that targeting the Ref-1-NFkB axis through inhibition of the redox-signaling mechanism of Ref-1 could be an effective strategy for treating PDAC.

To further decipher the role of NFkB in response to Ref-1 inhibition in these lines, we used the KC3590 cell line that has a truncated, inactive NFkB (RelA) gene as well as KC3590 cells with the addition of full length active NFkB (clone 13; C13) added back. We demonstrated that NFkB deficient cells are more resistant to APX3330, APX2009, and APX2014, but their sensitivity is restored in the C13 cell line. Furthermore, we also investigated peroxiredoxin1 (PRDX1)-Ref-1 axis in APX inhibitor sensitivity. PRDX1 helps regulate the redox status of Ref-1.We demonstrated that upon PRDX1 knockdown, the cell line with NFkB add-back was more sensitive to APX inhibitors than the NFkB deficient cells. Additionally, using a gel-based protein thiol labeling assay, we measured the redox status of Ref-1, PRDX1, and NFkB in these cells as well as in cells challenged with APX compounds.

Finally, we interrogated potential involvement of STAT3 in KC cell response to APX inhibitors. Although knockdown of STAT3 did enhance the sensitivity of the NFkB deficient cells, no further enhancement was observed in the NFkB proficient line. This data suggests that either NFkB or STAT3 are critical in the response, but the loss of both does not further enhance cell death. Overall, PDAC *Kras*^{G12D} cells are more responsive to Ref-1 redox inhibition with a functional NFkB present. Our data further support a pivotal role of NFkB in mediating Ref-1 redox signaling in PDAC cells with the *Kras*^{G12D} genotype and provide novel therapeutic strategies to combat PDAC drug resistance.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #41

EVALUATION OF THE POTENTIAL INTERACTION BETWEEN ETOPOSIDE AND POLYETHERSULFONE FILTERS

Nick Miles[1], Andi Masters[2], Zeruesenay Desta [3], Jennifer Goldman[4], Sarah Suppes[5,7], Emma Tillman[6]

[1] *Division Of Clinical Pharmacology , Indiana University School Of Medicine, Indianapolis, IN*

[2] *Indiana University Comprehensive Cancer Center, Indiana University School Of Medicine, Indianapolis, IN*

[3] *Division Of Clinical Pharmacology , Indiana University School Of Medicine, Indianapolis , IN*

[4] *Department Of Pediatrics , Children's Mercy Hospital And The University Of Missouri-Kansas City, Kansas City , MO*

[5] *Division Of Clinical Pharmacology, Toxicology, And Therapeutic Innovation, Kansas City , MO*

[6] *Division Of Clinical Pharmacology, Indiana University School Of Medicine, Indianapolis, IN*

[7] *Children's Mercy Hospital*

Email: *milesn@iu.edu*

Etoposide, a topoisomerase II inhibitor, is used clinically to treat a wide range of cancer types, and has been associated with anaphylactic infusion reactions. Reported incidence of infusion reactions with etoposide is around 2%, while some studies have seen rates as high as 27%. Recent studies suggest that there may be a link between the use of an in-line IV filter and increased incidence of infusion reactions in pediatric patients. In a previous study, we identified a hydrophilic polyethersulfone filter implemented at Children's Mercy Hospital (CMH) of Kansas City from 2017-2020 as a possible cause of increased rates of pediatric etoposide infusion reactions. In this analytical study we aimed to assess if there was any measurable change to the chemical structure of etoposide or its degradation products after passing through a polyethersulfone membrane filter of the same brand used at CMH. Etoposide 0.4 mg/mL infusion was prepared under standard aseptic conditions, and then set up using a standard IV infusion set with an in-line filter in place. Samples were taken in triplicate from three locations: 1) the injection port of the IV bag, 2) pre-filter IV tubing, and 3) post filter IV tubing. Samples 2 and 3 were collected using a needless access port. Samples were diluted and analyzed using HPLC-MS/MS. The selected reaction monitoring (SRM) Q1/Q3 (m/z) transition for etoposide was 606.2/228.8 and the DAD wavelengths were monitored at 210, 220, 254, and 280nm and run for 30 minutes. Etoposide peak was seen at each of the observed wavelengths occurring around 10 minutes into the analysis. Additional peaks were also observed which were not seen in the etoposide SRM scan, but each of these additional peaks were consistent between each of the sampled locations and occurred at the same time points in each sample. Based these results, a chemical change in etoposide is unlikely to be the primary cause of increased rates of infusion reactions related to in-line filtration of etoposide.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #42

CD166 AS NOVEL THERAPEUTIC TARGET TO OVERCOME PLATINUM RESISTANCE IN OVARIAN CANCER

Mayuri Prasad[1], Qipeng Fan[1], George Sandusky[2], Salvatore Condello[1]

[1] IUSM, Indianapolis, IN
[2] Hematology/ Oncology, Indianapolis, IN

Email: *msprasad@iupui.edu*

CD166 as novel therapeutic target to overcome platinum resistance in ovarian cancer

Mayuri Prasad[1,2], Qipeng Fan[1,2], George E Sandusky[3], and Salvatore Condello[1,2]

[1]Indiana University School of Medicine, Department of Obstetrics & Gynecology

[2]Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, Indiana.

[3]Division of Hematology/Oncology, Indiana University Melvin and Bren Simon Comprehensive Cancer Center.

Abstract

Background: Ovarian cancer (OC) is the most lethal gynecological cancer, characterized by chemoresistance and fatal tumor recurrence after primary treatment. The development of intra-peritoneal metastases is correlated with the formation of multicellular spheroids by OC cells disseminated in the peritoneal fluid. Within a spheroid, OC cells undergo an epithelial-to-mesenchymal transition (EMT), which involves a change in cell adhesion molecules (CAMs) expression and acquisition of cancer stem cell (CSC) characteristics, protecting OC cells during chemotherapy. Freshly isolated OC primary cells obtained from patients' ascites showed a significant expression of CD166 (cluster of differentiation 166). This protein is one of the CAMs involved in tumor progression and its modulation impacts cell-cell adhesion, leading to impaired ability of tumor cells to metastasize. Despite a functional role in the tumorigenic CSC phenotype, how CD166 is regulated during OC progression remains poorly understood.

Hypothesis: Our preliminary data functionally linking CD166 to the tumorigenic CSC phenotype and its overexpression in chemoresistant OC cells provide a strong rationale to investigate this molecule as a potential new therapeutic target in OC.

Methods: We compared CD166 expression in primary OC cells grown as spheroids and monolayers and in chemoresistant versus sensitive OC cell lines by realtime-PCR and Western Blot (WB). CD166 blockade by shRNA knockdown (KD) or anti-CD166 inhibitory antibody (clone AZN-L50) was evaluated on spheroid assay. ChIP assay detected the interaction between the transcriptional complex β-catenin/TCF/LEF1 and the CD166 promoter. Combinatorial carboplatin and AZN-L50 treatment was assessed on spheroid assays and by WB of pro-apoptotic proteins.

Results: OC cells grown as spheroids showed a significant increase in CD166 expression compared to the same cells grown as monolayers (n=3; $P<0.0001$). A comparison of normal adjacent ovarian epithelium, localized, and metastatic OC tissues on a multitissue array (TMA) revealed that CD166 expression coincided with an advanced promigratory phenotype (n= 88, $P<0.001$). In addition, survival analysis associated high CD166 levels with poor overall survival in patients with HGSOC in the TCGA OC database (P=0.005). CD166 blockade by either shRNA mediated KD or AZN-L50 treatment (10 mM) led to a significant decreased in spheroid formation (n=3; $P<0.0001$). Mechanistically, b-catenin KD decreased CD166expression levels (n=3; $P<0.001$) and b-catenin coimmunoprecipitated with the CD166promoter, suggesting that CD166 is a direct b-catenin target. Combination treatment of carboplatin and AZN-L50 showed synergistic effects in decreasing OC spheroids compared to single agents alone (n=3; $P<0.0001$). Finally, a combination of carboplatin and

CD166 blockade increased cleaved-PARP and -caspase-3 compared to single agent alone, indicating sustained DNA damage.

Conclusions: CD166 is strongly linked to chemoresistance and is regulated by the oncogenic Wnt/b-catenin pathway. Our data suggest that inhibition of CD166 along with carboplatin treatment could be a potential combination therapy in OC.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #43

EXTRACELLULAR DEK TREATMENT MIMICS HYPOXIC BLOCKADE OF EXTRA PHYSIOLOGIC OXYGEN STRESS IN HUMAN AND MOUSE HEMATOPOIETIC CELLS

James Ropa[1], Scott Cooper[1], Lindsay Beasley[1], David Markovitz[2], Hal Broxmeyer[1], Maegan Capitano[1]

[1] *Microbiology And Immunology, School Of Medicine, Indiana University, Indianapolis, IN*
[2] *Internal Medicine, Medical School, University Of Michigan, Ann Arbor, MI*

Email: *jropa@iu.edu*

Hematopoietic cell transplantation (HCT) is a life-saving treatment for malignant and non-malignant hematologic diseases. Cord blood (CB) derived hematopoietic stem (HSC) and progenitor cells (HPC) are an important source for HCT; however, CB HSC numbers are limiting. Thus there is a strong need to enhance collection, expansion, and/or engraftment of CB derived HSC. HSC/HPC self-renewal, proliferation, survival, and function are regulated by extracellular signals from cytokines and chemokines. DEK, a nuclear regulator of chromatin availability, also functions as a cytokine that regulates HSC and HPC by enhancing pools of long-term stem cells while decreasing pools of functional HPC. To explore the effects of extracellular DEK (ecDEK) treatment on human HSC/HPC, we treated human CB CD34+ cells with human ecDEK protein and isolated HSC, multipotent progenitors (MPP), common myeloid progenitors (CMP), and granulocyte-macrophage progenitors (GMP) and performed mRNA-sequencing to profile ecDEK-dependent transcriptomes of these separate populations of HSC/HPC. These analyses revealed unique and overlapping pathways affected by ecDEK treatment in the different cell populations. Fast gene set enrichment analysis (FGSEA) revealed that after ecDEK treatment GMP upregulate gene programs associated with leukocyte migration, CMP upregulate gene programs associated with myeloid cell development, and MPP and HSC both upregulate cytokine signaling responses, though HSC also exhibited a unique downregulation of cell cycle genes. Interestingly, ecDEK treatment induced upregulation of genes associated with hypoxic responses in cells and a concordant downregulation of genes that are downregulated by hypoxic exposure in all four cell populations, suggesting that DEK may stimulate pathways related to hypoxia responses and/or response to exposure to extra physiologic oxygen. In fact, the effects on HSC and HPC induced by ecDEK is similar with previous work demonstrating that isolating HSC/HPC at 3% O_2compared to ambient air conditions (~21% O_2) leads to a preserved pool of functional HSC but a lower number of functional HPC, due to a phenomenon termed extracellular physiologic shock/stress (EPHOSS). To explore whether DEK regulates pathways associated with EPHOSS, we assessed the effects of *in vivo*ecDEK treatment on HSC/HPC isolated and kept at 3% O_2 or HSC/HPC exposed to 21% O_2. In contrast to untreated HSC/HPC isolated at 3% O_2, ecDEK treated cells isolated at 3% O_2 did not exhibit further increases in LT-HSC numbers or decreases in pools of functional HPC over ecDEK treatment alone. Further, ecDEK prevents *ex vivo*apoptosis of CB HSC/HPC populations. Thus, ecDEK may neutralize the effects of EPHOSS by counteracting gene programs induced by ambient air exposure, preventing cellular stress and resulting in HSC preservation. This has important implications for the role of ecDEK in normal and diseased hematopoiesis, as well as clinical implications wherein ecDEK may be used to mimic the HSC preserving effects of isolation at 3% O_2.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #44

ST2 AS CHECKPOINT TARGET FOR COLORECTAL CANCER IMMUNOTHERAPY

Kevin Van der Jeught[1], Yifan Sun[1], Yuanzhang Fang[1], Zhuolong Zhou[1], Hua Jiang[2], Tao Yu[1], Jinfeng Yang[2], Malgorzata Kamocka[3], Ka Man So[4], Yujing Li[1], Haniyeh Eyvani[1], George Sandusky[5], Michael Frieden[1], Harald Braun[6], Rudi Beyaert[6], Xiaoming He[7], Xinna Zhang[1], Chi Zhang[1], Sophie Paczesny[2], Xiongbin Lu[1]

[1] *Department Of Medical And Molecular Genetics, Indianapolis, IN*
[2] *Department Of Pediatrics, Indianapolis, IN*
[3] *Department Of Medicine, Indianapolis, IN*
[4] *Indiana University Center For Computational Biology And Bioinformatics, Indianapolis, IN*
[5] *Department Of Pathology And Laboratory Medicine, Indianapolis, IN*
[6] *Ghent University*
[7] *University Of Maryland, , MD*

Email: *kvanderj@iu.edu*

Immune checkpoint blockade immunotherapy delivers promising clinical results in colorectal cancer (CRC). However, only a fraction of cancer patients develop durable responses. The tumor microenvironment (TME) negatively impacts tumor immunity and subsequently clinical outcomes. Therefore, there is a need to identify other checkpoint targets associated with the TME. Early-onset factors secreted by stromal cells as well as tumor cells often help recruit immune cells to the TME, among which are alarmins such as IL-33. The only known receptor for IL-33 is stimulation 2 (ST2). Here we demonstrated that high ST2 expression is associated with poor survival and is correlated with low CD8+ T cell cytotoxicity in CRC patients. ST2 is particularly expressed in tumor-associated macrophages (TAMs). In preclinical models of CRC, we demonstrated that ST2-expressing TAMs (ST2+ TAMs) were recruited into the tumor via CXCR3 expression and exacerbated the immunosuppressive TME; and that combination of ST2 depletion using *ST2*-KO mice with anti–programmed death 1 treatment resulted in profound growth inhibition of CRC. Finally, using the IL-33trap fusion protein, we suppressed CRC tumor growth and decreased tumor-infiltrating ST2+ TAMs. Together, our findings suggest that ST2 could serve as a potential checkpoint target for CRC immunotherapy.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #45

DEFINING THE MAF-DEPENDENT TRANSCRIPTIONAL REGULATORY NETWORK IN T(14;16) MULTIPLE MYELOMA

Sabine Wenzel[1], Enze Liu[1], Valeriy Lyzogubov[2], Brian Walker[1]

[1] *Medicine, Indianapolis, IN*

[2] *University Of Arkansas Medical Sciences, Little Rock, AR*

Email: *sabiwenz@iu.edu*

Despite recent advances in treatment options for multiple myeloma patients, the clinical outcome for those carrying the t(14;16) translocation is still considered poor. This patient subgroup, which overexpresses the transcription factor c-MAF due a chromosomal translocation bringing *MAF* under the control of the IGH enhancer, does not benefit from proteasome inhibitor or immunomodulatory drugs compared to other high-risk groups. In addition, overall higher mutational load and enrichment for APOBEC cytidine deaminase signature mutations, which have a strong prevalence in cancer and implications in treatment resistance, have been associated with the t(14;16) subgroup. We hypothesize that elevated *APOBEC3B* expression levels in t(14;16) patients links *MAF* over-expression to drug-resistance.

Human myeloma cell lines with high c-MAF levels were used for shRNA-based knock-down of either *MAF* or *APOBEC3B* to investigate whether *APOBEC3B* expression is linked to *MAF* overexpression. As verified by RT-qPCR and western blotting, knock-down of *MAF* in either JJN3 t(14;16) or RPMI8226 t(16;22) results in reduced *APOBEC3B* expression (fold-change: 1.8 (JJN3), 2 (RPMI8226)) and protein (fold-change: 1.7 and 1.9) levels. Knock-down of *APOBEC3B* results in reduction of expression (fold-change: 2.2 and 2.8) and protein levels (fold-change: 2.2 and 2.6) in JJN3 and RPMI8226, respectively. *MAF* levels were unaffected supporting a co-dependence of *APOBEC3B* expression on cellular c-MAF. To further study the effects of overexpression of MAF and its downstream targets, we generated CRISPR/Cas9 engineered JJN3 t(14;16) cells with a DNA-binding-deficient c-MAF. Two isolated clones with either 51 aa (clone 1) or 48 aa (clone 2) c-terminal deletions showed significant reduction of known c-MAF target genes like *ITGB7* and were used in further global expression studies (RNAseq) to identify c-MAF controlled genes relevant to multiple myeloma. There were 387 (clone 1) or 388 (clone 2) downregulated genes, of which there were 203 in common between the knockout clones. Significant decrease of *APOBEC3B* RNA expression ($p<0.05$) was observed in clone 1 (fold-change: 2.26). In clone 2 they were reduced but did not reach significance. Reduced APOBEC3B protein levels were confirmed by western blotting and deaminase assays. Moreover, we integrated our experimental data with RNA-seq patient data to identify clinically relevant c-MAF target genes. 84 (clone 1) and 100 (clone 2) genes identified as differentially expressed were reversely dysregulated in the t(14;16) patient subgroup (n=26) compared to the non-translocated patient group (n=352). Representative validated c-MAF target genes, *NUAK1* and *ITGB7,* were present in the patient dataset. With the initial results strongly supporting a regulatory role of c-MAF on APOBEC3B, our JJN3-MAF-KO clones will allow us to further dissect this regulatory network and the implications on treatment resistance.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #46

HISTAMINE PROMOTES TUMORIGENESIS, INFLAMMATION, DUCTULAR REACTION AND INFLAMMATION IN AGED MDR2-/- MICE, A MODEL OF PRIMARY SCLEROSING CHOLANGITIS

Tianhao Zhou[1], Lindsey Kennedy[1], Vik Meadows[1], Debjyoti Kundu[1], Heather Francis[1]

[1] *Department Of Medicine, Indiana University, Indianapolis, IN*

Email: *tnh.zhou@gmail.com*

Background: Primary sclerosing cholangitis (PSC) is characterized by biliary damage and fibrosis. Multidrug resistance-2 gene knockout (*Mdr2*$^{-/-}$)mice: (i) develop cholestatic liver disease; (ii) mimic some characteristics of human PSC; and (iii) develop hepatocellular carcinoma (HCC) within one year. We have demonstrated that depletion of the l-histidine decarboxylase/histamine (HDC/HA) axis using 12wk old *HDC/ Mdr2*$^{-/-}$ double knockout (DKO) mice results in decreased: (i) liver damage; (ii) mast cell (MC) activation; (iii) ductular reaction and (iv) hepatic fibrosis/inflammation compared to controls. The **aim** of this study was to determine the effects of depletion of the HDC/HA axis on tumor formation and progression in aged *Mdr2*$^{-/-}$and DKO mice. **Methods:** Male and female 52 week old homozygous DKO mice and age-matched *Mdr2*$^{-/-}$ mice were used. Livers were evaluated for tumor formation and lobular damage by H&E staining. In total liver we evaluated: (i) HDC/histamine receptor (HR) expression by *q*PCR, (ii) MC activation by *q*PCR for chymase and tryptase expression; and (iii) ductular reaction by immunohistochemistry for CK-19. Changes in liver fibrosis were evaluated by Sirius red staining in liver sections, and *q*PCR for αSMA and Col1a1. Hepatic stellate cell (HSC) activation was studied by immunofluorescence for SYP9. Inflammation was evaluated by expression of IL-6 and TNFα and by immunohistochemistry for F4/80. HA serum levels were measured by EIA in all groups. In livers and tumors (when present), we evaluated angiogenesis by VEGF and vWF mRNA expression and epithelial mesenchymal transition (EMT) by immunohistochemistry for vimentin and E-cadherin. **Results:** Depletion of the HDC/HA axis in 52 wk old DKO mice resulted in little to no tumor formation compared to age-matched *Mdr2*$^{-/-}$ mice, which displayed large HCC tumors. The HDC/HA/HR axis and MC activation were reduced in DKO mice compared to controls. Ductular reaction and hepatic fibrosis were increased in *Mdr2*$^{-/-}$ mice, which were reduced in DKO mice. Furthermore, angiogenesis, EMT, and inflammation were all reduced in DKO compared to age-matched Mdr2$^{-/-}$ mice. **Conclusion:** Our data demonstrates that depletion of the HDC/HA axis blunts tumor growth and progression in *Mdr2*$^{-/-}$mice.Therefore, the HDC/HA axis plays a critical role in promoting hepatic damage and tumor formation, and drugs targeting this axis may result in amelioration of cancer progression.

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #47

DEVELOPMENT OF PATIENT-DERIVED XENOGRAFTS OF PRIMARY AND METASTASIS OF BREAST CANCERS WITH UNIQUE PROPERTIES

Katie Batic[1], Henry Mang, Brijesh Kumar, Poornima Bhat-Nakshatri, Hong Shao, Sarat Chandarlapaty, Aaron A Cohen-Gadol, George Sandusky, Kathy D Miller, Harikrishna Nakshatri

[1] *Surgical Oncology, IU School Of Medicine, Indianapolis, IN*

Email: *kotyson@iu.edu*

Background: Several patient-derived breast cancer xenografts (PDXs) have been described in the literature. However, the majority of them are from triple negative breast cancer (TNBC). There is an urgent need to develop more PDXs from estrogen receptor (ER+) positive breast cancers as well as breast tumors from metastatic sites because ER+ breast cancers are more common and breast cancer metastasis may contain distinct genome/epigenome than primary tumors and respond differently than primary tumors to therapy.

Methods: We transplanted primary tumors, cells from pleural effusions, and metastasis collected from chest wall, lymph node, or brain of breast cancer patients into the mammary fat pad of NSG mice. Mice were also implanted with 60-days slow release estradiol implants (72 mg). Once tumors reached 1000 mm^3, a fragment of tumor was analyzed by H&E, and for levels of ER, Progesterone receptor (PR), FOXA1, GATA3, CK14, and CK19. Lungs and liver of mice at the time of sacrifice were analyzed by H&E for metastasis. All PDXs were tested for mycoplasma contamination and *Corynebacterium bovis* infection, which is common among PDXs. Genomic DNA from blood of the donor as well as patient-derived xenografts were sequenced to determine cancer-specific genomic aberrations in select few cases. In select cases, we established cell lines from primary tumors using our recently described method.

Results: We have successfully established PDXs from eight breast cancer patients including an ER+/PR+ primary tumor of a BRCA2 mutation carrier, ER+/PR- tumor from an anti-estrogen resistant pleural effusion, ER+/PR+ tumor from a lymph node metastasis, a brain metastasis of a TNBC, and a chemoresistant inflammatory TNBC from a BRCA1 mutation carrier. Tumors were from self-reported White, Black and Hispanic women. Several of PDXs showed lung metastasis including ER+/PR- PDX from pleural effusion. Multiple aliquots of PDXs have been cryopreserved and cryopreserved PDXs have been successfully reimplanted to obtain tumors. Mutations in homologous recombination pathway genes were common across three PDXs that have been sequenced. Two PDXs showed missense mutation of PIK3CD, a less studied member of the PI3 kinase family.

Conclusions: We have created a series of PDXs that are less common in the literature. These PDXs are useful for evaluating drugs that are effective against metastatic breast cancers as well as inhibitors against specific kinases such as PIK3CD. These PDXs as well as primary tumor-derived cell lines are available for researchers.

Basic Science ***Research Technician***

POSTER #48

OXIDATIVE DNA DAMAGE AND CISPLATIN NEUROTOXICITY IS EXACERBATED BY REDUCING THE EXPRESSION OF 8-OXYGUANOSINE GLYCOSYLASE AND AP ENDONUCLEASE 1 IN SENSORY NEURONS

Adib Behrouzi[1], Hanyu Xia[1], Eric Thompson[1], Mark Kelley[2], Jill Fehrenbacher[1]

[1] *Pharmacology And Toxicology, School Of Medicine, Indiana University, Indianapolis, IN*
[2] *Pediatrics, School Of Medicine, Indiana University, Indianapolis, IN*

Email: *abehrouz@iu.edu*

Chemotherapy-induced peripheral neuropathy is a debilitating and dose-limiting adverse effect of anticancer treatment affecting cancer patients and survivors. Cisplatin, a common chemotherapeutic, is one of the most toxic chemotherapeutics and causes numbness, hypersensitivity to cold, and burning pain. Cisplatin generates DNA adducts and oxidative stress, the latter of which can result in oxidative DNA damage. The base excision repair (BER) pathway is the primary way sensory neurons repair cisplatin-induced oxidative lesions, such as 8-oxyguanosine (8-oxoG); however, the role of the different BER enzymes has not been assessed in sensory neurons. Understanding how oxidative DNA damage alters neuronal function is critical to understanding the mechanisms for cisplatin-induced neurotoxicity. Here, we utilized an in vitro model of cultured sensory neurons and siRNA technology to decrease the expression of the first two enzymes of the BER pathway, 8-oxoG glycosylase (OGG1) and AP endonuclease 1 (APE1) and measured sensory neuronal function and morphology. The content and stimulated release of the neuropeptide, calcitonin gene-related peptide (CGRP), was assessed as a surrogate for neuronal function in combination with immunohistochemical identification of cisplatin-induced DNA adducts and 8-oxoG. We found that knockdown of either BER enzyme alone did not affect CGRP release in the absence of cisplatin treatment, but increased the sensitizing effects of cisplatin on CGRP release. Surprisingly, combination knockdown had an additive effect in the presence of cisplatin, even though the enzymes are thought to participate in a linear pathway. Decreasing OGG1 had a dramatic effect on neuronal morphology even in the absence of cisplatin, suggesting a novel role for the BER enzyme in regulating neurite homeostasis. Collectively, these data suggest that OGG1 and APE1 are neuroprotective in the presence of oxidative stress, such as that induced by cisplatin. Further research will examine how each of these BER enzymes contributes to neuronal homeostasis in conditions of high oxidative stress.

Basic Science ***Research Technician***

POSTER #49

THE BREAST TISSUE MICROBIOME IN CANCER SUSCEPTIBILITY

Rana German[1], Pam Rockey[1], Hongyu Gao[2], Xiaoling Xuei[2], Harikrishna Nakshatri[2], Anna Maria Storniolo[2], Natascia Marino[2]

[1] *Susan G. Komen Tissue Bank At IUSCCC*
[2] *IU School Of Medicine*

Email: *rgerman@iu.edu*

Introduction: Breast cancer is a complex disease that involves numerous genetic alterations as well as changes in the microenvironment surrounding the cancer cells. In the past decades, microbial composition of human body (microbiota) raised significant attention in cancer biology. Breast tissue, once believed to be sterile, contains a specific microbial population. Moreover, the comparison of breast tissue samples shows differences in the composition and abundance of some specific bacterial taxa (known as dysbiosis) between breast cancer (BC) patients and healthy individuals. We hypothesize that dysbiosis occurs in early phase of BC development, promotes BC and is linked to BC risk factors.

Methods: We employed 16S rRNA paired end sequencing (MiSeq, Illumina) to define the microbiome of normal breast tissues (N=106) donated by healthy women, and examine the link of the microbiota profile with age, BMI, menopausal status, parity of the donors. BC tissues (N=11), normal breast adjacent to tumor (NAT, N-42), metastatic BC tissue (N-6), and various negative controls (N=41) were also included into the analysis. Data analysis of the FASTQ files was performed using the online tool SHAMAN (https://shaman.pasteur.fr/) applying the RDP database for taxonomy.

Results: The bacterial abundance in the samples was heterogeneous with an average alpha diversity of 38. *Proteobacteria*, *Firmicutes*, and *Actinobacteria* were the most abundant phyla in the tissues. Among the more abundant genera, we detected *Ralstonia* abundant in NAT (p=0.02) and tumor (although not significant) and *Enterococcus* abundant in NAT (p=0.03) as compared with normal breast, while *Methylobacterium* more abundant in normal breasts as compared with the other analyzed tissues. No significant correlation with age, BMI, racial background, menopausal status and parity was identified.

Conclusion: Our preliminary analysis identified bacterial genera abundant in either the normal or the NAT breast, suggesting a potential role in carcinogenesis. To address the interindividual heterogeneity we are increasing the sample size. This is the first study providing a comprehensive microbiota profiling of the normal breast tissue with the goal to define links between dysbiosis and BC susceptibility.

Basic Science ***Research Technician***

POSTER #50

REGULATION OF ENTEROENDOCRINE PROGENITOR FORMATION BY CoREST IN *BRAF* MUTANT COLORECTAL CANCER

Thomas Huntington[1], Samuel Miller[2], Heather O'Hagan[3]

[1] *Medical Sciences Program, Indiana University School Of Medicine, Bloomington, Indiana., Bloomington, IN*

[2] *Genome, Cell, And Developmental Biology, Department Of Biology, Indiana University Bloomington, Bloomington, Indiana., Medical Sciences Program, Indiana University School Of Medicine, Bloomington, Indiana., Bloomington, IN*

[3] *Medical Sciences Program, Indiana University School Of Medicine, Bloomington, Indiana., Cell, Molecular And Cancer Biology Graduate Program, Indiana University School Of Medicine, Bloomington, Indiana., Indiana University Melvin And Bren Simon Comprehensive Cancer Center, Indianapolis, Indiana., Department Of Medical And Molecular Genetics, Indiana University School Of Medicine, Indianapolis, Indiana., Bloomington, IN*

Email: *thunting@iu.edu*

Colorectal Cancer (CRC) has a lifetime prevalence of 4.1-4.4%, and of individuals who develop CRC, 5-15% develop an especially severe form, *BRAF* mutant CRC. In the normal colon, stem cells at the bases of crypts of Lieberkuhn give rise to pluripotent progenitor cells that migrate upwards and differentiate into nutrient-absorbing enterocytes or secretory cells. We have shown that in *BRAF* mutant CRC, differentiation of hormone-secreting enteroendocrine cells (EEC's) deviates from that in the normal colon. DNA hypermethylation of the *NEUROD1* promoter in *BRAF* mutant CRC blocks the full differentiation of EEC progenitors and contributes to enrichment of EEC progenitors in these cancers. Additionally, LSD1, a lysine demethylase that is overexpressed in CRC, is necessary for formation of EEC progenitors, and its loss decreases tumor growth and metastasis. LSD1 acts in concert with the histone deacetylase HDAC1 and a scaffolding protein, RCOR1, as part of a CoREST complex, repressing transcription of tumor suppressor genes by demethylating histone H3K4. We hypothesized that LSD1-dependent regulation of EEC progenitor formation occurs through CoREST complex activity, in conjunction with HDAC1 and RCOR1. To assess this hypothesis, we treated HT29 cells with three different classes of LSD1 inhibitors. We found that expression of early EEC marker genes was significantly decreased only by Corin, a dual HDAC1 and LSD1 inhibitor, and not by inhibitors targeting LSD1's catalytic activity or interactions with associated transcription factors. We next examined whether other isoforms of HDAC1 and RCOR1 play roles in co-regulating (with LSD1) formation of EEC progenitors in *BRAF* mutant CRC. Previous studies have demonstrated redundancy between HDAC1 and HDAC2 in some cell types and suggested that both may be therapeutic targets in CRC. Our data suggest that HDAC1, but not HDAC2, is necessary for EEC progenitor formation: In HT29 cells, we found that knockdown (KD) of HDAC1 significantly decreased early EEC marker gene expression, while HDAC2 KD either increased or had no effect on this gene expression. Previous research in non-cancerous hematopoietic cells indicates that RCOR2 can compensate for loss of RCOR1 in helping to drive erythro-megakaryocytic differentiation, while RCOR3 counteracts this process by competitively inhibiting RCOR1. Our data suggest similar activity in *BRAF* mutant CRC: Following KD of RCOR1 or RCOR2, early EEC marker gene expression significantly decreased, as in LSD1 or HDAC1 KD. However, following RCOR3 KD, early EEC marker gene expression significantly increased. This finding suggests that RCOR3 may counterbalance activity of RCOR1 and RCOR2, antagonizing early EEC formation. Altogether, these findings suggest that CoREST complexes, HDAC1, and the

different RCOR proteins affect formation of EEC progenitors. As we previously showed that EEC progenitors promote cell survival and colony formation in HT29 cells, these findings suggest each is a potential therapeutic target in *BRAF* mutant CRC.

Basic Science ***Undergraduate Student***

POSTER #51

HSF1-MYC OVARIAN CANCER CELL TARGETING USING BI6727

Bobby Walker[1], Imade Williams, PhD[1], Richard Carpenter, PhD[1]

[1] *Medical Sciences, Indiana University, Bloomington, IN*

Email: *Bowalker@iu.edu*

Ovarian cancer is the fifth leading cause of cancer related deaths among women, with an estimated 21,410 new cases in 2021. Heat shock factor 1 (HSF1) transcription factor and protein is the master regulator of the heat shock response and has been studied extensively, more recently, for its role in cancer metastasis and in promoting the early stages of cancer cell malignancy. Using publicly available data from the Cancer Genome Atlas (TCGA), we have found that ovarian cancer patients have lower recurrence free survival (RFS) when HSF1 activity is high and coupled with MYC amplification, suggesting these two proteins may cooperate to support ovarian cancer growth. In the present study, we have targeted polo-like kinase 1 (PLK1), which sits upstream of both HSF1 and MYC, in HSF1/MYC (dual) –amplified ovarian cancer cells. We hypothesize that MYC and HSF1 are working in conjunction with one another to promote ovarian cancer cell growth. BI6727 (Volasertib), is an ATP-competitive PLK1 inhibitor. We performed viability assays in ovarian cancer cell lines with MYC and HSF1 gene amplification (OVCAR8, SNU119, OVCAR4) compared to non-amplified ovarian cancer cell lines (OVSAHO, CAOV3, PEO1) and assessed the cellular response to 48h of PLK1 inhibition. We obtained half maximal inhibitory concentrations (IC_{50}) for each cell line and found that the dual-amplified ovarian cancer cell lines were 150-fold more sensitive to BI6727 (IC_{50}=29.5nM) on average compared to the non-amplified ovarian cancer cell lines (IC_{50}=4.59μM). These results have important implications for dual-targeting of MYC and HSF1 in ovarian cancer and can be applied for testing combinatorial treatment strategies with current standard of care chemotherapeutic carboplatin to combat chemoresistance. Furthermore, these results suggest that MYC and HSF1 co-amplification may sensitize ovarian cancer cells to PLK1 targeted therapy.

Basic Science ***Undergraduate Student***

POSTER #52

IT IS ROUGHER ON ME THAN IT IS ON HIM: FAMILY CAREGIVER-GENERATED AND PRIORITIZED ILLNESS CONCERNS WHILE PATIENTS UNDERGO CANCER TREATMENTS

Kristin Levoy PhD, MSN, RN, OCN[1,7], Jesse Wool MBE, BSN, RN, CCRN[2,8], Rebecca Ashare PhD[3], William Rosa PhD, MBE, ACHPN, FAANP, FAAN[4,9], Frances Barg PhD[5], Salimah Meghani PhD, MBE, RN, FAAN[6]

[1] *School Of Nursing, Indiana University, Indianapolis, IN*

[2] *Department Of Nursing Operations, Philadelphia, PA*

[3] *Department Of Psychiatry , University Of Buffalo, Buffalo, NY*

[4] *Department Of Psychiatry And Behavioral Sciences, New York, NY*

[5] *Department Of Family And Community Health, Perelman School Of Medicine, University Of Pennsylvania, Philadelphia, PA*

[6] *Department Of Biobehavioral Health Sciences, School Of Nursing, University Of Pennsylvania, Philadelphia , PA*

[7] *Center For Aging Research, Regenstrief Institute*

[8] *Children's Hospital Of Philadelphia*

[9] *Memorial Sloan Kettering Cancer Center*

Email: *klevoy@iu.edu*

Introduction: Research eliciting patients' illness concerns has typically focused later in the cancer continuum, rather than during cancer treatments. Family caregiver concerns are overlooked during this time. Less is known about how patients and caregivers prioritize concerns during cancer treatments, which holds potential for improving supportive oncology care (i.e., primary palliative care).

Aim: Elicit and compare which domains of supportive oncology are of highest importance to patients and caregivers during cancer treatments.

Methods: Freelisting, a cognitive anthropology method, was used to elicit concerns in order of importance.Freelist data were analyzed using Smith's salience index. Qualitative interviews were conducted with a caregiver subsample to add explanatory insights.

Results: In descending order, pain, death, fear, family, and awful were salient Freelist items for patients *(n*=65), while sadness, time-consuming, support, anger, tired, death, and frustration were salient for caregivers *(n*=24). When integrated with supportive oncology domains, patients' concerns reflected a prioritization of the physical (pain) and emotional (death, fear, awful) domains, with less emphasis on social (family) aspects. Caregivers' prioritized the emotional (sadness, anger, death, frustration) and social (time-consuming, support) domains, with less emphasis on the physical (tired) aspects.

Conclusion: Our findings suggest that enhancing primary palliative care delivery by oncology teams requires systems thinking to support *both* the patient and caregiver as the primary unit of care. Primary palliative care may be improved by prioritizing interventions that address physical concerns among patients as well as key social concerns among caregivers to support the complex caregiving role while patients undergo cancer treatments.

Behavioral ***Faculty***

POSTER #53

CHARACTERISTICS OF HOME-BASED SELF-SAMPLING FOR HUMAN PAPILLOMAVIRUS (HPV) AMONG LOW-INCOME WOMEN USING CONJOINT ANALYSIS

Erika Biederman[1], Victoria Champion[2], Gregory Zimet,

[1] *Of Nursing, INDIANAPOLIS, IN*
[2] *Nursing*

Email: *emcconne@iu.edu*

Introduction

Home-based self-sampling for HPV testing may help to increase overall cervical cancer screening rates among low-income women by overcoming barriers associated with provider-based screening. The purpose of this study was to assess preferred characteristics for TYPE of HPV self-sampling kit (cervicovaginal or urine collection), DELIVERY of the kit (mail, pharmacy pick-up, or clinic pick-up), RETURN of the kit (mail, pharmacy drop-off, or clinic drop-off), and HPV RESULTS delivery (mail, phone call, or text message).

Methods

Data were gathered via an online survey from a sample of low-income women (household income<$50,000) provided by Dynata (n=940). They evaluated scenarios that varied along 4 dimensions: TYPE, DELIVERY, RETURN, and RESULTS. A fractional factorial design generated 9 representative scenarios with varying characteristics along each dimension. Each scenario was rated on a 0-100 scale. Ratings-based conjoint analysis (RBCA) created importance scores (IS) that showed how much each dimension contributed to the ratings of the scenarios. Part-worth utilities (PWU) generated by RBCA indicated the relative preference for a characteristic within each dimension.

Results

The women ranged in age from 30-65 (M=51). The most important dimension (IS=32.97) was DELIVERY, with a preference for mail (PWU=1.94) or pharmacy pick-up (PWU=1.49) over clinic pick-up (PWU=-3.43). The next most important decisional factor (IS=25.09) was RETURN, with a preference for clinic drop-off (PWU=1.5) and mailed return (PWU=.5) over pharmacy drop-off (PWU=-2.31). Then test TYPE had an IS of 22.59 with a preference for urine collection (PWU=1.84) over cervicovaginal collection (PWU=-1.84). The least important decisional factor was RESULTS (IS=19.35), with participants preferring a phone call (PWU=147) over mailed delivery of results (PWU=0.21) or text messages (PWU=-1.68).

Conclusions

Overall, the most preferred test was a urine test delivered by mail, dropped off at a clinic, with results communicated by phone. Researchers could use these findings to better understand facilitators of, and barriers to, self-testing protocols.

Behavioral ***Graduate Student***

POSTER #54

PERCEIVED ILLNESS SEVERITY AND TERMINALITY IN ADVANCED LUNG AND PROSTATE CANCER PATIENTS

Ellen Krueger[1], Ekin Secinti[1], Catherine Mosher[1]

[1] *Department Of Psychology, Indiana University-Purdue University Indianapolis, Indianapolis, IN*

Email: *efkruege@iu.edu*

Among advanced cancer patients, reporting a terminally ill health status has shown mixed associations with symptoms and illness acceptance. We extended this literature by examining potential clinical correlates of both perceived terminal illness and serious illness in advanced lung and prostate cancer patients.

Advanced cancer patients (inoperable lung cancer: *n*=102; prostate cancer: *n*=99; 72% male; 85% White; mean age=67 years; mean time since diagnosis=3 years) were recruited from medical centers in the midwestern U.S. Patients completed standard measures of demographics, medical comorbidities, functional status, distress (anger about cancer, anxiety, depression), physical symptoms (pain, fatigue, sleep problems), and illness acceptance. Patients also reported whether they were "relatively healthy," "seriously but not terminally ill," or "seriously and terminally ill." Cancer data were collected via chart review. Logistic regressions examined associations between each patient characteristic and perceived health status.

Of the 198 patients with complete data, the majority (66%) identified as relatively healthy, whereas others identified as seriously but not terminally ill (22%) or seriously and terminally ill (12%). Patients with lung cancer and a worse functional status were more likely to identify as terminally ill than those with prostate cancer and better functional status (*p*s<.05). Other study variables were unrelated to this outcome.

Both demographic (indices of lower socioeconomic status) and medical factors (lung cancer, worse functional status, greater medical comorbidities) were related to a higher likelihood of reporting serious illness, irrespective of perceived terminality (*p*s<.05). Controlling for demographic and medical factors, worse pain and anxiety were also related to a higher likelihood of reporting serious illness (*p*s<.05). Other study variables were unrelated to this outcome.

Findings suggest that many advanced lung and prostate cancer patients are unaware of the severity of their illness. The association between pain and perceived serious illness may be related to interpreting pain as a sign of disease progression and its functional impact. Not surprisingly, greater anxiety was also related to reports of serious illness. On average, anger about cancer was low and illness acceptance was high regardless of perceived health status. Next steps include examining predictors of perceived health status throughout the illness trajectory.

Behavioral ***Graduate Student***

POSTER #55

FACILITATORS AND BARRIERS TO IMPLEMENTATION OF TOBACCO CESSATION SERVICES IN COMMUNITY PHARMACIES

Katy Ellis Hilts [1], Alexander V. Prokhorov[2,5], Robin L. Corelli[3], Karen S Hudmon[4]

[1] *School Of Nursing, Indiana University , Indianapolis, IN*
[2] *The University Of Texas , Houston, TX*
[3] *Department Of Clinical Pharmacy, , California San Francisco , San Francisco , CA*
[4] *Department Of Pharmacy Practice, School Of Pharmacy , Purdue University , Indianapolis, IN*
[5] *MD Anderson Cancer Center*

Email: *kaaellis@iupui.edu*

Background:

Tobacco use and smoking account for approximately 80% of head, neck, and lung cancer deaths in the U.S. Continued use of tobacco after a cancer diagnosis adversely impacts treatment effectiveness, overall survival, risk of subsequent primary malignancies, and decreased quality of life. As travel is often a barrier to accessing care, community pharmacies, which are highly accessible, can be an important referral site for cancer patients to receive assistance with quitting in their local communities. While evidence suggests pharmacy-delivered cessation services are clinically and cost-effective, few studies have examined contextual factors influencing implementation of these services in community pharmacies. This study aimed to characterize pharmacists' and pharmacy technicians' perceptions of facilitators and barriers to implementing brief interventions (Ask-Advise-Refer; AAR) for tobacco cessation in pharmacies.

Methods:

A total of 124 pharmacists and 127 technicians, representing 64 community pharmacies, participated in a 6-month randomized trial comparing two approaches to enhance implementation of brief tobacco cessation interventions. Guided by Rogers' Diffusion of Innovation Theory, data from self-administered questionnaires [baseline, 3 months, 6 months], were used to characterize perceptions of the AAR framework and factors influencing its adoption within the pharmacy setting. Standard summary statistics, reported as means, were computed for pharmacy-specific facilitators and barriers, and these results were then compared between pharmacists and pharmacy technicians.

Findings:

Regarding facilitators, pharmacy personnel rated (1=not at all to 4=highly) the AAR approach as moderately compatible with daily workflow (3.02), advantageous compared to other cessation counseling approaches (3.13), acceptable for implementing into routine practice (3.11), and appropriate for use within their pharmacy (3.27). Clarity of the three steps comprising AAR was rated as moderate to high, with a mean of 3.51. Compared to technicians, pharmacists provided higher mean ratings for advantage ($p<0.05$) and acceptability ($p=0.05$). The highest-rated barrier was lack of available time (3.47), followed by discomfort in asking patients about tobacco use (2.89), lack oftraining (2.67), perceived importance of cessation counseling as part of their job (2.56), and lack of confidence for counseling patients about quitting (2.51). When considering these barriers to implementing AAR, technicians had lower ratings of perceived importance ($p<0.05$) and expressed less confidence for discussing quitting with patients ($p<0.01$).

Conclusion:

The delivery of tobacco cessation services in community pharmacies has the potential to fill gaps in access to these services, which can lead to reductions in tobacco use and improved outcomes for cancer patients. Our results suggest that contextual factors might impact implementation of AAR interventions for tobacco cessation in pharmacy settings. Future research should further explore system-level barriers and facilitators

while also identifying and exploring unique experiences of pharmacy team members when integrating tobacco cessation interventions into routine community pharmacy practice.

Behavioral ***Post-Doctoral/Medical Fellow***

POSTER #56

IDENTIFYING BARRIERS TO HPV VACCINATION FOR PATIENTS WITH SICKLE CELL DISEASE AND CHILDHOOD CANCER SURVIVORS

Mahvish Rahim[1], Seethal Jacob[1], Mary Ott[2], Meagan Miller[3], Carolyn Meagher[2], Sara Hardman[4], Gabi Lozano[4], Gregory Zimet[2], Scott Coven[1]

[1] *Pediatric Hematology/Oncology, Riley Hospital For Children*
[2] *Department Of Adolescent Medicine, Riley Hospital For Children*
[3] *Department Of Hematology/Oncology*
[4] *IUPUI*

Email: *Mqrahim@iu.edu*

Background:

Human papillomavirus (HPV) vaccination prevents the development of HPV-associated malignancies. A retrospective chart review of childhood cancer survivors and patients with sickle cell disease (SCD) at Riley Hospital for Children found suboptimal HPV vaccination rates in this population. There was a notable difference between the rate of HPV vaccination and other age-appropriate vaccinations, indicating substantial missed opportunities for HPV vaccination administration. Additionally, childhood cancer survivors are at increased risk for secondary malignancies, demonstrating the need to better understand barriers to vaccination against a cancer-causing organism for our adolescent and young adult patients. In the general pediatric population, HPV vaccine hesitancy has been shown to be multi-factorial. Less is known about vaccine hesitancy in hematology and oncology populations.

Objective: Our objective was to explore patient/parent vaccine hesitancy, beliefs regarding HPV vaccination, and system-level barriers.

Design/Method: We used qualitative methods because less is known about vaccine hesitancy in this population. We recruited HPV vaccine decision-makers in Sickle Cell Disease and Oncology Survivor clinics, including caregivers of patients aged 9-18 years, and both caregivers of and patients themselves aged 18-21years. Data were collected via audio recorded qualitative interviews conducted either in person or virtually. Questions assessed trust in the medical system, HPV vaccine baseline knowledge, and misconceptions about the vaccine. Interviews were transcribed and analyzed with thematic content analysis, with coding based on over-arching themes such as vaccine misconceptions, side effects, disease interactions with the HPV vaccine. These themes were organized into a model of caregivers' and patients' experiences, attitudes, and barriers with regards to HPV vaccination.

Results: The most common themes identified were misconceptions and lack of baseline knowledge. Many did not know what the HPV vaccine was, as they had not previously been counseled on it or they could not remember the indication for the HPV vaccine. Misconceptions included the perception that vaccination was a gateway to sexual activity, that HPV vaccine was new, or exclusively for females. There was concern regarding potential for increased risk of side effects with the HPV vaccine in the setting of a chronic disease. We found that patients and caregivers stated that they would be reassured about the vaccine by a strong HPV vaccination recommendation from a provider, and many preferred counseling about the safety of the vaccine in the context of their child's or their own illness, indicating a trust between the provider and the patient.

Conclusion:

The findings from this study demonstrate the need for improved HPV vaccination counseling and promotion in sickle cell disease and oncology survivor patient populations. Improved counseling from pediatric hematology-oncology subspecialists with regards to HPV vaccination can potentially have a strong impact on a family's understanding of the vaccine in the context of their adolescent's chronic illness.

Behavioral ***Post-Doctoral/Medical Fellow***

POSTER #57

PALPITATIONS AND CO-OCCURRING MENOPAUSAL SYMPTOMS IN WOMEN PRIOR TO BREAST CANCER SURGERY

Ying Sheng[1], Janet Carpenter[1], Steven Paul[2], Christine Miaskowski[2]

[1] *IU School Of Nursing, Indianapolis, IN*
[2] *University Of California, San Francisco, CA*

Email: *shengyin@iu.edu*

Background: Palpitations are described as racing, pounding, or skipped heart beats that are associated with greater sleep disturbance, depression, anxiety, and stress. Palpitations in women with breast cancer may be related to the cancer and/or its treatment. Unlike other menopausal symptoms (e.g., hot flashes, night sweats) associated with breast cancer and its treatments, palpitations are understudied. Therefore, a need exists to evaluate the occurrence of palpitations, associated risk factors, and the co-occurrence of other menopausal symptoms in these patients.

Purpose: Evaluate for differences in demographics and clinical characteristics, as well as the occurrence of as well as the severity and distress associated with 45 menopausal symptoms between women with breast cancer who did and did not report palpitations prior to surgery.

Methods: This analysis utilized data from a longitudinal study of neuropathic pain and lymphedema in women who underwent breast cancer surgery. Prior to surgery, 393 women completed questionnaires on demographic and clinical characteristics, functional status using the Karnofsky Performance Status scale, common medical conditions using the Self-Administrated Comorbidity Questionnaire, palpitations (yes/no), and menopausal symptoms using the Menopausal Symptoms Scale. Patients were categorized into two groups (i.e., those with and those without palpitations). Data were analyzed using descriptive statistics, t-tests, Fisher Exact tests, Mann-Whitney U tests, and Chi-square tests.

Results: Of the 398 patients, 15.1% (n=60) had and 84.9% (n=338) did not have palpitations prior to breast cancer surgery. Patients with palpitations had lower annual household income (p=.001), lower functional status (89.7±11.1 versus 93.9±10, p=.004), higher comorbidity burden (5.3±3.2 versus 4.1±2.7, p=.004), were more likely to report back pain (43.3% versus 25.4%, p=.008), and higher total number of menopausal symptoms (excluding palpitations) (21.5±7.8 versus 10.4±7.8, p<.001), compared to those without palpitations. Patients with palpitations reported higher severity scores for difficulty concentrating (4.3±2.3 versus 3.2±1.9, p=.001), dizziness (4.8±2.6 versus 2.6±1.4, p=.004), swollen hands/feet (5.3±2.4 versus 3.1±1.7, p=.001), and nighttime waking (5.5±2.8 versus 4.2±2.4, p=.001). In addition, they reported higher distress scores for anxiety (5.7±2.7 versus 4.2±2.7, p=.001), hot flashes (5.0±3.3 versus 3.3±2.7, p=.009), swollen hands/feet (5.7±2.7 versus 3.1±2.3, p=.002), and nighttime waking (5.4±3.1 versus 3.7±3.0, p=.001).

Conclusions: While the etiology of these palpitations warrant investigation, these findings suggest that 15% of women prior to surgery experience this symptom. Palpitations appear to co-occur with a number of menopausal symptoms. Future research is warranted on the impact of palpitations on other aspects of women's lives including quality of life and coping. In addition, the mechanisms that underlie palpitations warrant investigation.

Behavioral ***Post-Doctoral/Medical Fellow***

POSTER #58

COMMUNICATION ABOUT COST OF CANCER CARE: ATTITUDES AND PRACTICES OF ONCOLOGISTS

Ahmed Bilal Khalid[1], Samuel Urrutia[2], Nasser Hanna[1]

[1] *Indiana University School Of Medicine, Indianapolis, IN*
[2] *The University Of Texas MD Anderson Cancer Center, Houston, TX*

Email: *ahkhalid@iu.edu*

Introduction: With the cost of cancer care rising, it is becoming ever so important for physicians to be mindful of financial toxicity to their patients. Patient-provider cost discussions are associated with lower out of pocket costs (Hong el al, Value in Health 2020), yet few patients report having this discussion with their providers. Little is known about the oncologist's perspective on cost discussion including their preparedness, current practice, and barriers in not being able to have this discussion.

Methods: An online survey was administered to Hematology/Oncology attending physicians and fellows at Indiana University. The objectives were to assess attitudes on communication about cost of cancer care and awareness around financial toxicity of cancer therapy. The questions included physician perception on patient's attitudes towards cost discussion, current state of cost discussion in their clinic and their preparedness on having this discussion in the future.

Results: 27/48 (56%) fellows and faculty members completed the survey. All respondents believed cost communication is associated with improved patient outcomes and 22/27 (81%) believed cost communication is desired by most patients. 22/27 (81%) indicated cost of care were discussed in less than half of patient encounters. 22/27 (81%) of respondents also believed clear communication about cost should be a part of a patient's clinic visit. When asked to rate their preparedness level on a scale of 0-100 in discussing cost of cancer care in their clinics, the median preparedness level was 20. Median awareness level about patient's insurance plan was also 20 and median awareness level about patient out of pocket cost was 21. 26/27 (96%) believed knowing cost of therapy and out of pocket cost would change the way they practice oncology. Not knowing the cost of cancer care themselves was rated as the greatest barrier behind not being able to discuss cost by 20/27 physicians (74%).

Conclusion: Despite physicians believing cost of cancer care should be discussed in the clinic, most fail to do so. Physician education on cost of cancer care and training on how to have this discussion in the clinic is needed.

Behavioral ***Resident physician***

POSTER #59

USING RISK FACTORS TO ESTIMATE RISK FOR EARLY ONSET SPORADIC COLORECTAL CANCER (EOCRC): WHETHER TO SCREEN AND HOW

Joanne Daggy[1], Timothy Stump[2], Barry Barker[3,5], Thomas Imperiale[4,5,6]

[1] *Biostatistics, Medicine, Indianapolis, IN*
[2] *Bostatistics, Medicine, Indianapolis, IN*
[3] *Health Services Research And Development, Indianapolis, IN*
[4] *Medicine, Health Services Research And Development, Medicine, Indianapolis, IN*
[5] *Roudebush VA Medical Center*
[6] *Regenstrief Institute, Inc.*

Email: *jdaggy2@iu.edu*

Using Risk Factors to Estimate Risk for Early Onset Sporadic Colorectal Cancer (EOCRC): Whethe and How

BACKGROUND: Knowing risk for EOCRC would help decide whether to screen persons < age 50. factors for EOCRC have been identified, but how to use them for decision making is unclear. We process of going from risk factors to risk estimation and whether and how to screen.

METHODS: We used data from 450 cases of EOCRC among 35-49 year-old male Veteran controls matched for site and year of case diagnosis. The top 15 risk factors based univariable p-values were selected for a multivariable logistic regression model. Mode with 10-fold cross-validation determined final model (Table 1). To make the model decision-making, we calculated the relative risk (RR) estimate for 5 risk scenarios: scenario (no factors present), 3 intermediate risk (some risk factors present), and 1 high-ri factors present), and applied these RR estimates to each of 3 SEER age groups (35-39, 40 49 years). For each scenario, we multiplied the baseline SEER CRC incidence rate x (1- A Risk) x RR estimate to calculate a revised CRC incidence rate ("colon age"). When confidence limit of the revised estimate was ≥ current SEER CRC incidence for 50-54 y recommendation for screening was made; when it was ≥ that for 70-74 year olds, scre colonoscopy was recommended.

RESULTS: Among 2250 subjects (mean [SD] age 44.3 [4.0]; 65.6% Caucasian), a 12 model demonstrated good fit (mean squared error = 0.14) and discrimination (c-statist Table 2 shows 5 risk scenarios for each age group, revised incidence rates, comparison to 50-54 age group, and whether / how to screen. Among 35-to-39-year olds, only the h group (all factors present) would be screened (with colonoscopy). Among 40-44-year intermediate risk persons would be screened non-invasively, and high-risk persons with co Only low-risk 45-49 year olds could remain unscreened, with non-invasive screening intermediate risk, and colonoscopy for the remaining 3 intermediate / high risk groups. (Ta

CONCLUSION: By using risk factors for EOCRC among male Veterans and SEER CRC rates to estimate “colon age”, this analysis provides a framework for using these factors fo about whether and how to screen for EOCRC. For persons < age 50 whose colon age > screening may be recommended. Further study of this model and framework are required.

Table 1. Risk Factors for Early Onset Colorectal Cancer (EOCRC) in Male Vetera

Risk Factor	Value associated with increased risk	Odds Rati CI)*
Age	Older age (per year)	1.08 (1.05
Current vs former / no alcohol use	Current use	1.74 (1.40
FDR or SDR with CRC	Present	2.31 (1.68
FDR or SDR with any visceral cancer	Present	1.67 (1.27
Charlson comorbidity score	Higher score (per unit)	1.14 (1.05
BMI ≤ 20kg/m^2 ≥ 6mo. before diagnosis	≤ 20kg/m^2 vs. ≥ 30kg/m^2	3.56 (1.43
Reported regular exercise	None	2.08 (1.39
Multivitamin use	None	1.80 (1.18

bootstrap estimates.

Table 2. Revised SEER CRC Incidence Rates ("Colon Age") and Screening Decisions for 15 Scenari
Risk Factor Profiles for 37, 42, and 47 year olds

Age	Baseline SEER CRC incidence rate / 100K	Risk factors present	Risk profile	Revised SEER CRC incidence based on risk: rate and 95% CI		Screening (test) > SEER incidenc year old (67.9/1 colonoscopy if > old (179.9/100K
				Rate	95% CI*	
37	10.4	None	Low	0.25	0.10-0.47	No
		Some	Intermediate	6.41	3.44-9.99	No
				17.3	5.3-58.5	No
				19.7	(6.1-64.8	No
		All	High	330.3	102.2-874.4	Yes (colon
42	19.4	None	Low	0.67	0.30, 1.24	No
		Some	Intermediate	17.5	10.4, 27.7	No
				47.2	14.9, 157.0	Yes (non-i
				53.7	17.1, 177.3	Yes (non-i
		All	High	904.1	290.2, 2331	Yes (colon
47	34.9	None	Low	1.75	0.81, 3.26	No
		Some	Intermediate	46.0	28.5, 72.6	Yes (non-i
				124.9	38.4, 421	Yes (colon
				141.8	43.3, 477	Yes (colon
		All	High	2400	828.2, 6343	Yes (colon

* CIs estimated from based on quantiles of the bootstrap estimates.

** 2012-2016 SEER CRC incidence rates per 100K: **50-54 years = 67.9**; 55-59 years = 79.7; 60-64 =
69 = 143.7; **70-74 = 179.9**; 75-79 years = 226.6.

POSTER #60

SARS COV-2 PILOT PROJECT, NATIONAL HISTORY OF SARS-COV-2 IN CANCER PATIENTS A NATIONAL STUDY,

Nicholas Pettit[1],

[1] *Emergency Medicine, Zionsville, IN*

Email: *nrpettit@iu.edu*

There is a need to characterize patients with cancer with suspected acute respiratory syndrome coronavirus 2 (SARs-CoV-2).

Methods This is a planned secondary analysis of patients with suspected SARS-CoV-2 with (n= 2,871) and without (n= 2,871) a diagnosis of cancer. This data is from a multicenter registry of 116 emergency departments (ED) in 27 US states. In a nested case-control design we compared mortality, hospitalization, ventilatory support, presence of new blood clots, circulatory support, and mortality for patients with and without cancer, based on COVID-19-positivity.

Results Patients with a positive COVID-19 test, irrespective of cancer-status were more likely to be male (53.7%, 52.9%, cancer positive and cancer free, respectively). Black or African American race were seen at higher rates based on COVID-19-positivty, with 33.8% of the cancer positive and 36.8% of the cancer-free, COVID-19-positive cohort, compared to 13.6% and 24.7% respectively. Similarly, Hispanic/Latino ethnicity were more commonly seen in the COVID-19-positive cohort. Among cancer subjects, most patients were found to have a localized solid tumor (72.0% vs 80.9%, COVID-19 negative vs -positive, respectively). The most common types of cancers observed based on COVID-19-positivity were breast (25.9%), prostate (15.8%), and colorectal cancer (12.3%). Patients with cancer and positive COVID-19 test had the highest rate of mortality among all cohorts (25.5%), more likely to be hospitalized, more likely to be admitted to the ICU, more likely to require ventilatory support, and more likely to require circulatory support. Lastly, 35.8% of the COVID-19 positive cancer cohort and 31.8% of COVID-19 positive cancer free cohort were black race, compared to 14.3% of COVID-19 negative cancer cohort and 19.2% COVID-19 negative cancer free cohort.

Conclusion Among ED patients with cancer, clinical outcomes associated with SARS-CoV-2 infection were worse compared to patients without cancer, with racial disparities in both the presence of COVID-19 positivity and mortality.

Population Science/Epidemiology ***Faculty***

POSTER #61

DISPARITIES IN OUTCOMES AMONG PATIENTS DIAGNOSED WITH CANCER IN PROXIMITY TO AN EMERGENCY DEPARTMENT VISIT; A STATEWIDE EPIDEMIOLOGIC ASSESSMENT OF NEW CANCER DIAGNOSES

Nicholas Pettit[1]

[1] *Emergency Medicine, Zionsville, IN*

Email: *nrpettit@iu.edu*

Asuspected diagnosis of cancer in the emergency department (ED) may be associated with poor outcomes however data are limited.This is case-control analysis of the Indiana State Department of Health Cancer Registry, and the Indiana Network for Patient Care. First time cancer diagnoses appearing registries from 2013-2017 were included. Cases were patients who had an ED visit in the 6 months before their cancer diagnosis; controls had no recent ED visits. 134,761 first-time cancer patients were identified, including 15,432 (11.5%) cases. The mean age was same at 65, more of the cases were Black than the controls (12.4% vs 7.4%, P<.0001) and more were low income (36.4%. vs 29.3%). The most common ED-associated cancer diagnosis was lung cancer at 18.4% of the cases. Cases observed an over three-fold higher mortality, with cumulative death rate of 32.9% for cases vs 9.0% for controls (P<.0001). Adjusted regression analysis predicting mortality produced an odds ratio of 4.12 (95% CI 3.72-4.56) for ED associated cancers.These data suggest that additional work is needed to reduce disparities among ED-associated cancer diagnoses.

Population Science/Epidemiology ***Faculty***

POSTER #62

SITES OF CANCER DIAGNOSES ACROSS IU HEALTH: A METHODOLOGY REPORT (2007-2017)

Eric Vachon[1,4], Oindrila Bhattacharyya [2,4], David Haggstrom[3,4,5]

[1] *IU School Of Nursing, Indianapolis , IN*
[2] *IU Department Of Economics , Indianapolis, IN*
[3] *IU School Of Medicine, Indianapolis, IN*
[4] *Regenstrief Institute*
[5] *Indianapolis VA*

Email: *evachon@iu.edu*

Background: While cancer death rates across the US and Indiana have continued to decrease, incidence rates have maintained or even increased with some cancer sites. Overall incidence rates are higher in Indiana than the national average. With Indiana University (IU) Health being the largest healthcare system and cancer treatment provider in Indiana, it is vital to understand vast cancer population cared for by IU Health.

Purpose: The purpose of this work is to describe the process of utilizing the IU Health Cancer Registry to descriptively analyze population of cancer patients diagnosed within IU Health from 2007-2017. This work will describe the process of narrowing down raw electronic health record data into cleaned cancer sites that can be easily reported and utilized for data analysis.

Methods: Data were obtained from the IU Health Cancer Registry via the Regenstrief Institute. All individuals diagnosed with cancer in the IU Health system during 2007-2017 that were reported to the registry and had available data were included. Variables pulled from the cancer registry included various demographic and cancer variables, including International Classification of Diseases (ICD) site codes, and ICD histology codes. All unique ICD site and histology codes across the sample were gathered to be narrowed down to create a final cancer site variable. ICD-9 and ICD-10 codes were both included, depending on the year of diagnosis. A team consisting of a nurse/cancer health services researcher, physician/cancer health services researcher, and economics and health services pre-doctoral fellow utilized clinical expertise, the NCI Surveillance, Epidemiology, and End Results (SEER) database, and American Cancer Society Facts and Figures to collapse the ICD codes into cancer sites. Once site codes were narrowed, a new variable was created in STATA 16.1, with a numeric value assigned to each cancer site.

Results: The sample consisted of 65,879 cancer patients across Indiana. The data set included 305 unique ICD primary site diagnosis codes and 576 unique ICD histology codes (each participant had one site and one histology code). This work resulted in a final cancer site variable consisting of 25 categories, two of which were “unknown,” if the primary site could not be determined, and “other,” if the site could not categorized into one of the 23 sites. Other and unknown made up <5% of the sample. The three most prevalent cancer sites were breast, lung, and prostate – all with more than 11% of the sample, which is fairly consistent with national incidence rates.

Conclusions: This process allows us to report on the incidence rates of cancer sites across IU Health from 2007-2017 and is a primary step in the data management phase for a larger secondary analysis to examine trends of healthcare utilization of cancer patients across Indiana.

Population Science/Epidemiology ***Faculty***

POSTER #63

USING ELECTRONIC HEALTH RECORDS TO ANALYZE PATIENT HEALTHCARE UTILIZATION DATA: A METHODOLOGIC APPROACH

Oindrila Bhattacharyya[1,4], Eric Vachon[2,4], David Haggstrom[3,5,4]

[1] *IUPUI School Of Liberal Arts, Department Of Economics, Indianapolis, IN*

[2] *IU School Of Nursing, Indianapolis, IN*

[3] *Division Of General Internal Medicine & Geriatrics, Indiana University School Of Medicine, Indianapolis, IN*

[4] *Center For Health Services Research, Regenstrief Institute Inc*

[5] *Indianapolis VA HSR&D Center For Health Information And Communication, Roudebush VA*

Email: *oinbhatt@iu.edu*

Background: Utilizing electronic health record (EHR) data has the potential to impact patient care and clinical research on a much larger scale than prospective data collection. However, as technology advances and plays an increasingly important role in managing health information, data quality concerns are becoming more prevalent in health informatics and information management. Raw EHR data is rarely, if ever, in a manageable state for data analysis, with concerns of inaccuracy, duplications, missing data, and other issues. With greater access to EHR data and the potential impact, there is a need to develop methodologic approach to cleaning raw EHR in order to be utilized for data analysis.

Purpose: To describe a methodologic approach for cleaning raw EHR data for hospital admissions and emergency room (ER) visits for cancer (cases) and non-cancer (controls) patients across Indiana.

Methods: Data were obtained from the Indiana Network for Patient Care (INPC) database via the Regenstrief Institute. A modified data quality framework developed by Kahn et al. (2012) was utilized to guide this approach. Interactions were first split into care setting – hospitalizations and ER visits. The current, previous and following admission and discharge dates are denoted as A_n and D_n, A_{n-1} and D_{n-1} and A_{n+1} and D_{n+1} respectively, where n is the number of interactions corresponding to each id. Admission dates were dropped if greater than the corresponding discharge dates as it wasn't reasonable, followed by identifying duplicate admission and discharge dates. Next, few special cases of duplicates were dealt with within each id and their care settings; firstly, two consecutive admission dates were counted as one if the corresponding discharge dates were missing, otherwise counted as two separate admission dates if they had corresponding discharge dates. Secondly, A_n that fell within the range of A_{n-1} and D_{n-1} with either missing corresponding D_n or with D_n being $\leq D_{n-1}$ were eliminated. Thirdly, A_n being same with D_n and A_{n+1}, with $D_n < D_{n+1}$ were eliminated. Fourthly, interactions where D_n were same as A_n and D_{n-1}, with $A_n > A_{n-1}$ were eliminated. Lastly, overlapping interactions where A_n fell within the range of A_{n-1} and D_{n-1} and $D_n > D_{n-1}$, the minimum of the two admission dates, A_n and A_{n-1} and the maximum of the two discharge dates, D_n and D_{n-1} were included. The analyses were performed using Stata 16.1. Stata codes and date examples will be provided during presentation.

Conclusion: By developing and following a step-by-step framework for cleaning EHR data, we can hope to ensure the highest quality data to utilize for analysis in order to enhance patient care and outcomes and clinical research moving forward.

Population Science/Epidemiology ***Graduate Student***

POSTER #64

CHARACTERIZATION OF CUTANEOUS METASTASIS OF COLON CANCER REMAINS CHALLENGING

Joseph Nenow[1]

[1] *Transitional Year, Indianapolis, IN*

Email: *josephnenow@gmail.com*

Intro: Colon cancer is the 3rd highest prevalence of all cancer subtypes in the US. Despite their potential for aid in diagnosis, the cutaneous metastasis of colon cancer has been poorly described.

Methods: A Pubmed search was conducted using search terms “cutaneous metastasis colon” with “cancer” or “adenocarcinoma”, which yield 145 and 95 results respectively. Once reviewed for redundancy and relevance, 52 articles remained. Inclusion criteria: skin biopsy to confirm metastasis and cutaneous findings, English language. Gender, age, morphology, and distribution of lesions were included in the study. The study included 60 patients total with average age of 65 and ranging from 35-92.Males accounted for 55% of patients, females 43%, unspecified 2%.

Results: Relative to available data per category, lesions tended to be non-tender (63% vs. 38% non-tender), flesh-colored (38% vs. 24% pink-red/hyperpigmented, 14% white) and nodules (38%) or masses (37%). Relative to the total number of individual skin lesions, lesions tended to be, in descending order, nodular 36%, masses 34%, firm consistency 33%, erythema 19%, multiple skin lesions 19%, previous trauma 18%, induration 11%, flesh-colored 11%, skin degradation 11%, plaques 11%, irregular margins 10%, papule 8%, well-defined 8%, multi-coccoid 8%, non-tender 7%, pink-red 7%, hyper pigmented 7%, ovoidal 5%, exophytic 5%, tender 4%, white 4%, and cyst-like 4%. By distribution, lesions were disproportionately reported to occur on head (27%), anterior torso (31%), and groin (11%) relative to body surface area. Lesions were proportionately unlikely in to be found on the extremities relative to body surface area: thighs 6%, axilla 5%, and hand 2%.

Discussion: Extrapolation was limited be the dearth of standardized data available for analysis. Herein, we propose criteria for reporting of physical exam findings of cutaneous metastases. Accurate characterization of cutaneous metastasis of morphology may assist in diagnosis of cancer type. Analysis of the distribution of cutaneous lesions is useful to direct physical exam findings in patients with histories of cancer.

Population Science/Epidemiology ***Graduate Student***

POSTER #65

A NEGATIVE BINOMIAL REGRESSION MODEL FOR ESTIMATION RISK OF NON-MELANOMATOUS AND MELANOMATOUS SKIN CANCER.

Joseph Nenow[1], Kenai Rhodes

[1] *Transitional Year, Indianapolis, IN*

Email: *josephnenow@gmail.com*

Intro

Seasonality is a well-documented phenomenon in the diagnosis of many pathologies (e.g. melanomatous and non-melanomatous skin cancers, benign skin lesions, and internal malignancies). Explanations for this phenomenon revolve around discussions of access to care, exposure to UV radiation, or the role of vitamin D in cancer pathogenesis. Recent research on seasonal diagnosis in skin cancer has utilized a quantitative approach to predict the risk of cancer diagnosis based on summed recent UV exposure. We aim to expand this technique to analyze the risk of a diagnosis of basal cell carcinomas, squamous cell carcinomas, and melanomas for patients in Greenville, NC from 1994-2017.

Methods

Historical data regarding loco-regional UV intensity was gathered from the National Oceanic and Atmospheric Administration website for Raleigh, NC from 1994-2017. Dates for biopsy confirmation of skin cancer were collected from ECU physicians over a similar period. Five periods of time were chosen over which to sum UV Data (month of diagnosis, the first, second-, and third-month preceding diagnosis, as well as the sum of UV exposure over months one and two. Diagnosis data were grouped by type of cancer diagnosis (i.e. basal cell carcinoma, squamous cell carcinoma, or melanoma). RStudio was used to generate negative binomial models for predicted risk of diagnosis.

Results

For both melanoma and squamous cell carcinoma, no failed achieved statistical significance. The strongest relationship between melanoma and cumulative UV dose existed during the concurrent month of diagnosis ($p = 0.056$). For squamous cell carcinoma, the strongest relationship existed between the date of squamous cell diagnosis and cumulative UV exposure three months preceding diagnosis ($p = 0.0715$). For basal cell carcinoma, cumulative UV exposure in the two months preceding diagnosis was associated with statistical significance ($p = 0.0008$).

Conclusion

Previous research has identified cumulative UV exposure in the two months preceding diagnosis as an effective method for addressing the role of UV exposure in the seasonal diagnosis of melanoma. Basal cell carcinoma has been traditionally expected to develop over the course of several months.

Here the risk of diagnosis with basal cell carcinoma has been most strongly associated with the sum of UV data in the two months preceding diagnosis. UV exposure has been associated with the development of skin cancer in sun-exposed areas. However, limitations in access to patient data include gender, age, and location of skin cancer challenge further discussion at this time. Further research should address the utility of classifier models for predicting risk in basal cell carcinoma.

Population Science/Epidemiology ***Graduate Student***

POSTER #66

ASSOCIATION OF OMEGA-3 FATTY ACID INTAKE WITH TELOMERE LENGTH IN US MEN

Bojung Seo[*1], Keming Yang[*2], Ka Kahe[3,4], Abrar A. Qureshi[5], Andrew T. Chan[2], Immaculata De Vivo[6,7], Edward L. Giovannucci[6,7,8], Eunyoung Cho[5], Hongmei Nan[1,9]

[1] *Department Of Epidemiology, Richard M. Fairbanks School Of Public Health, Indiana University, Indianapolis, IN, USA*

[2] *Clinical And Translational Epidemiology Unit And Division Of Gastroenterology, Massachusetts General Hospital And Harvard Medical School, Boston, MA, USA*

[3] *Department Of Obstetrics And Gynecology, Vagelos College Of Physician And Surgeons, Columbia University, New York, USA*

[4] *Department Of Epidemiology, Mailman School Of Public Health, Columbia University, New York, USA*

[5] *Department Of Dermatology, Warren Alpert Medical School Of Brown University, Providence, RI, USA*

[6] *Channing Division Of Network Medicine, Department Of Medicine, Brigham And Women's Hospital And Harvard Medical School, Boston, MA, USA*

[7] *Department Of Epidemiology, Harvard T.H. Chan School Of Public Health, Boston, MA, USA*

[8] *Department Of Nutrition, Harvard T.H. Chan School Of Public Health, Boston, MA, USA*

[9] *Department Of Global Health, Richard M. Fairbanks School Of Public Health, Indiana University, Indianapolis, IN, USA*

Email: *boseo@iu.edu*

**Contributed equally as co-first author*

Correspondence to Dr. Hongmei Nan:
Department of Global Health, Richard M. Fairbanks School of Public Health, Indiana University, 1050 Wishard Blvd, RG 6086, IN 46202, USA (Phone: 317-274-3120; Email: hnan@iu.edu)

Our knowledge of the relationship between omega-3 fatty acid intake and leukocyte telomere length (LTL) in men is limited. In this study, we aimed at cross-sectionally exploring the association of omega-3 fatty acid intake with LTL in US men. We included 2,494 participants with LTL measurement from 4 nested case-control studies of Health Professionals Follow-up Study (HPFS). Individuals with previous histories of cancers, diabetes and cardiovascular diseases at or prior to blood collection were excluded. Blood collection was performed between 1993 and 1995 and relevant information including omega-3 intakes was collected in 1994 by questionnaire. The LTL was long-transformed and Z scores of the LTL were calculated for statistical analyses by standardizing the LTL in comparison with the mean within each selected nested case-control study. We found that consumption of docosahexaenoic acid (DHA) was positively associated with LTL. In the multivariable-adjusted model, compared to the individuals who had least intake of DHA (i.e., first quartile group), the percentage differences [95% confidence intervals (CIs)] of LTL were -3.5% (-13.6~7.7%), 7.4% (-3.9%~20.1%), and 8.9% (-2.8%~22.1%) for those individuals who were in the second, third, and fourth quartile group of the consumption, respectively (P for trend = 0.04). Additionally, we found that men who have higher consumption (>=median) of canned tuna had relatively longer LTL, compared to those with less consumption (<median); in the multivariable-adjusted model, the percentage difference (95% CI) of LTL was 10.6% (1.4%~20.6%) (P value = 0.02). Our results suggest that higher intakes of DHA and canned tuna are associated with longer LTL.

Population Science/Epidemiology ***Graduate Student***

POSTER #67

A QUALITATIVE FRAMEWORK-BASED EVALUATION OF APPLYING THE EXTENSION FOR COMMUNITY HEALTH CARE OUTCOMES (ECHO) MODEL FOR CANCER PREVENTION AND SURVIVORSHIP CARE: KEY FACTORS TO PARTICIPATION IN IMPLEMENTATION

Zheng Milgrom[1,3], Tyler Severence[2,4], Caitlin Scanlon[4], Anyé Carson[1], Andrea Janota[1], Terry Vik[2,4], Joan Duwve[1], Brian Dixon[1,3], Eneida Mendonca[2,3]

[1] *Richard M. Fairbanks School Of Public Health, Indiana University, Indianapolis, IN*
[2] *Department Of Pediatrics, Indiana University School Of Medicine, Indianapolis, IN*
[3] *Center For Biomedical Informatics, Regenstrief Institute*
[4] *Riley Hospital For Children*

Email: *omilgrom@iu.edu*

Background: Cancer is the second leading cause of death in Indiana. To address the increasing cancer burden, a tele-mentoring program using the Extension for Community Health Care Outcomes (ECHO) model focusing on cancer prevention, screening, and survivorship care (Cancer ECHO program), was deployed in September 2019 to provide continuing medical education and guide best-practice care to primary care providers (PCP). The purpose of this study was to conduct a formative evaluation of the program and to identify and explore complex factors that influence the adoption and implementation of the program.

Methods: We utilized quantitative program administrative data, qualitative data from semi-structured interviews (N=22) with the participants (hub and spoke members) of Cancer ECHO and the participants of other ECHO programs at Indiana University (IU). The qualitative data were analyzed thematically, using a conceptual model of Consolidated Framework for Implementation Research (CFIR).

Results: During the pilot year, 147 unique individuals have participated the program at least once with an average of 14.5 per session. The CFIR constructs of external policy and incentives and available time resources were the most prominent among the six barriers to participation. Relative advantage, design quality, tension for change, learning climiate, access to knowledge and information, reflecting and evaluation arose as facilitators, while knowledge and beliefs received mixed results.

Conclusions: This study identified a relative lower PCP participation in the Cancer ECHO program and multiple interacting CFIR constructs factors influencing the program adoption and implementation. The CFIR approach guided us to understand the barriers and facilitators that have broader applicability to other ECHO replications.

Statement of Significance:

The pandemic-catalyzed rapid expansion of telehealth usage imposed the importance of virtual collaboration in clinical care and education. Tele-mentoring using the ECHO model is well-suited to democratize complex cancer control knowledge among health professionals in medically isolated communities. However, the success of new ECHO adoption relies on understanding key contextual factors and little is known in the context of cancer prevention and survivorship.

The significance of this work is that by examining a pilot program using an implementation science framework, we informed the future intervention about the potential acceptance issue and the reasons through the audience's lens. The results allowed us to understand the complexity of applying the ECHO model on cancer prevention and survivorship care and determined actionable facets for guiding ongoing program adaptations.

Research Stage: Complete

Propose for: Poster

Keywords: Telehealth, implementation science, continuing medical education, cancer control

Population Science/Epidemiology ***Post-Doctoral/Medical Fellow***

POSTER #68

COVID-19 DIAGNOSIS AND THE RISK OF ALL-CAUSE MORTALITY AMONG ADULTS NEWLY DIAGNOSED WITH CANCER: USING A LARGE ELECTRONIC HEALTH RECORDS DATABASE.

Nimish Valvi[4], Hetvee Patel[1], Giorgos Bakoyannis[1], David Haggstrom[2,5], Sanjay Mohanty[2], Brian Dixon[3,4]

[1] *Biostatistics, Richard M. Fairbanks School Of Public Health, Indiana University, Indianapolis, IN*
[2] *School Of Medicine, Indiana University, Indianapolis, IN*
[3] *Epidemiology, Richard M. Fairbanks School Of Public Health, Indiana University, Indianapolis, IN*
[4] *Regenstrief Institute*
[5] *Regenstrief Insitute*

Email: *nvalvi@regenstrief.org*

Introduction: Individuals with cancer are reported to have poorer COVID-19 outcomes in single hospital studies early on in the pandemic. However, few studies have examined large, multi-center cancer populations. Our aim was to examine the association of COVID-19 diagnosis and all-cause mortality in a large cancer population using a statewide electronic health record (EHR) database.

Methods: A retrospective cohort study of individuals 18 years and older with newly diagnosed cancer in the state of Indiana between January 1, 2020 and December 31, 2020. Data were utilized from the Indiana Network for Patient Care (INPC), which contains electronic health records for individuals across 38 distinct health systems including the IU Simon Cancer Center. New cancer diagnosis was assessed using International Classification of Disease (ICD) codes documented in patients' EHR. The exposure variable was COVID-19 diagnosis based on a confirmatory RT-PCR assay test from a throat or nose swab positive for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The primary outcome of the study was all-cause mortality after diagnosis of COVID-19. We used Kaplan-Meier analysis to estimate the unadjusted survival over time. We examined the association of COVID-19 diagnosis and all-cause mortality using unadjusted and adjusted Cox-proportional hazard regression, controlling for age, race, sex, number of chronic diseases, and cancer type to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs).

Results: A total of 24,306 individuals received a new diagnosis of cancer during the study period. The mean (SD) age was 65 (±13.9) years and those diagnosed with COVID-19 were slightly younger [64.3 (±14.7)]. The majority of the population was White (82.2%) with males (51.4%) and females (48.6%). The highest incidence of malignancies was for breast (13.9%) and prostate (13.3%) cancer. In the adjusted model, there was a 37% increased (HR: 1.37, 95% CI: 1.15-1.63) risk of death among those with a COVID-19 diagnosis compared to those without. The risk of death increased 36% (HR: 1.36, 95% CI: 1.30-1.42) per 10-year increase in age with males having a 20% (HR: 1.20, 95% CI: 1.08-1.34) greater risk of death compared to females. Individuals with two or more chronic diseases had 2-fold (HR: 2.10, 95% CI: 1.84-1.34) risk of death compared to those without a chronic disease. Among cancers, the highest mortality was observed in those with other digestive cancers (HR: 1.68, 95% CI: 1.43-1.97) and lung cancer (HR: 1.54, 95% CI: 1.33-1.78) compared to the other cancer group.

Conclusion: COVID-19 infection increases risk of death among individuals newly diagnosed with cancer. Mortality risk further increases based on demographic factors, certain cancer subtypes, and comorbidities. Our results highlight the need for cancer populations to take extra precautions against COVID-19 infection, including vaccination and social distancing.

Population Science/Epidemiology ***Post-Doctoral/Medical Fellow***

POSTER #69

A SINGLE CELL ATLAS OF THE HEALTHY BREAST TISSUES REVEALS CLINICALLY RELEVANT CLUSTERS OF BREAST EPITHELIAL CELLS.

Poornima Nakshatri[1]

[1] *Surgery, Indianapolis, IN*

Email: *pnakshat@iupui.edu*

Poornima Bhat-Nakshatri[1], Hongyu Gao[2,3], Liu Sheng[2,3],Patrick C. McGuire[3], Xiaoling Xuei[3], Jun Wan[2,3], Yunlong Liu[2,3], Sandra K. Althouse[4], Austyn Colter[5], George Sandusky[5], Anna Maria Storniolo[6], and Harikrishna Nakshatri[1,2,7,8*]

Single cell RNA sequencing (ScRNA-seq) is an evolving technology used to elucidate the cellular architecture of adult organs. Previous ScRNA-seq on breast utilized reduction mammoplasty samples, which are often histologically abnormal. We report a rapid tissue collection/processing protocol to perform ScRNA-seq of breast biopsies of healthy women and identify 23 breast epithelial cell clusters. Putative cell-of-origin signatures derived from these clusters are applied to analyze transcriptomes of ~3000 breast cancers. Gene signatures derived from mature luminal cell clusters are enriched in ~68% of breast cancers, whereas a signature from a luminal progenitor cluster is enriched in ~20% of breast cancers. Overexpression of luminal progenitor cluster-derived signatures in HER2+ but not in other subtypes is associated with unfavorable outcome. We identify TBX3 and PDK4 as genes co-expressed with estrogen receptor (ER) in the normal breasts, and their expression analyses in >550 breast cancers enable prognostically relevant subclassification of ER+ breast cancers.

Keywords: Normal breasts, single cell analyses, epithelial cell clusters, cell-of-origin, breast cancer

Translational/Clinical Research ***Faculty***

POSTER #70

HORMONALLY REGULATED MYOGENIC MIR-486 INFLUENCES SEX-SPECIFIC DIFFERENCES IN CANCER-INDUCED SKELETAL MUSCLE DEFECTS

Ruizhong Wang[1], Poornima Bhat-Nakshatri [1], Xiaoling Zhong[1], Teresa Zimmers[1], Harikrishna Nakshatri

[1] *Surgery, Indianapolis, IN*

Email: *rewang@iupui.edu*

Abstract

Cancer-induced skeletal muscle defects show sex-specific differences in severity with men performing poorly compared to women. Hormones and sex chromosomal differences are suggested to mediate these differences, but the functional skeletal muscle markers to document these differences are unknown. We show that the myogenic microRNA miR-486 is a marker of sex-specific differences in cancer-induced skeletal muscle defects. Cancer-induced loss of circulating miR-486 was more severe in men with bladder, lung, and pancreatic cancers compared to women with the same cancer types. In a syngeneic model of pancreatic cancer, circulating and skeletal muscle loss of miR-486 was more severe in male mice compared to female mice. Estradiol (E2) and the clinically used selective estrogen receptor modulator toremifene increased miR-486 in undifferentiated and differentiated myoblast cell line C2C12 and E2-inducible expression correlated with direct binding of estrogen receptor alpha (ERα) to the regulatory region of the miR-486 gene. E2 and toremifene reduced the actions of cytokines such as myostatin, transforming growth factor β, and tumor necrosis factor α, which mediate cancer-induced skeletal muscle wasting. E2- and toremifene-treated C2C12 myoblast/myotube cells contained elevated levels of active protein kinase B (AKT) with a corresponding decrease in the levels of its negative regulator PTEN, which is a target of miR-486. We propose an ERα:E2-miR-486-AKT signaling axis, which reduces the deleterious effects of cancer-induced cytokines/chemokines on skeletal muscle mass and/or function.

Keywords: breast cancer; estradiol; miR-486; skeletal muscle; systemic effects.

Translational/Clinical Research ***Faculty***

POSTER #71

THE ALTERNATIVE SPLICING LANDSCAPE IN MULTIPLE MYELOMA IS DETERMINED BY IGH TRANSLOCATIONS AND MUTATIONS OF RNA PROCESSING GENES

Enze Liu[1], Sabine Wenzel[1], Brian Walker[1]

[1] *Division Of Hematology And Oncology, School Of Medicine, Indiana University, Indianapolis, IN*

Email: *enzeliu@iu.edu*

Background:

Alternative Splicing (AS) plays a key role in regulating numerous cellular processes in both normal and malignant cells. Previous studies have revealed mutations in the spliceosome complex, such as *SF3B1,* can cause increased AS frequencies in multiple myeloma (MM) patients, and patients with increased levels of AS are associated with a poor prognosis. Other frequently mutated genes involved in RNA processing include *DIS3* and *FAM46C*, thus, systematically investigating other causes of AS abnormalities and pathologies in MM patients is highly necessary.

Method:

RNA-seq data from 598 newly diagnosed MM patients from the MMRF CoMMpass study were utilized to generate AS comparisons. They were previously annotated for cytogenetic, copy number, and mutation data (version IA16). RNA-seq data were aligned to HG38 using STAR and Salmon. SUPPA2 was used for calling AS differences. For each identified AS event, the splicing level was defined by Percentage of Spliced-In (PSI) while the mean difference of splicing levels between two groups was measured by ΔPSI (dPSI) and by the P-value from independent T-tests against PSIs in the two groups. Filtering thresholds were determined to find high-quality differentially spliced events and were filtered for those also present in normal PCs (GSE110486). Geneset enrichment analysis (GSEA) was performed to identify dysregulated pathways caused by differential splicing and differential expression. Survival analysis was performed on clinical annotations of 598 NDMM patients while the Logrank test and Cox regression were used to evaluate the risk of AS and other genomic factors. Kaplan-Meier curves were plotted for various subgroups.

Results:

We compared 16 major cytogenetic subgroups, including Ig translocations (t(4;14), t(14;16), t(11;14)), hyperdiploidy, mutations in KRAS, NRAS, BRAF, FAM46C, SF3B1, DIS3 and TP53, combined events (t(4;14) plus DIS3 mutation), as well as those with biallelic abnormalities (DIS3, FAM46C, and TP53). Samples with SF3B1 hotspot mutations identified the greatest number of AS events (n=862), and samples with any SF3B1 mutation had approximately half as many. IGH translocations had an equivalent number of AS events to those with SF3B1 mutations, with t(14;16) having the most (n=587) followed by t(11;14) (n=366), and t(4;14) (n=256). We observed an increased number of significant AS events in bi-allelic DIS3 and FAM46C groups (n=404 and 171) compared to their mono-allelic abnormalities (n=114 and 35). As DIS3 mutations are enriched in the t(4;14) subgroup we also examined that interaction and found significantly more AS events (n=481; $p<0.01$) in the combination compared to either event alone. As expected, KRAS, NRAS and BRAF mutations did not have enrichment for AS events (n=2, 15, 23, respectively).

Most AS events were unique to each subgroup, exemplifying the AS heterogeneity in these subgroups. Among overlapped events, an alternative first (AF)

exon in ACACA was consistently more spliced in t(14;16), t(11;14) and t(4;14) groups (dPSI=0.18, 0.10, 0.12, P=2x10-5, 2x10-9, 5x10-5). ACACA encodes an enzyme that significantly affects MM cell growth and viability, suggesting that similar regulations exist in the three translocation groups. Unique events were also detected including an AF event in MIB2 (E3 Ubiquitin Protein Ligase 2) in the t(11;14) group (dPSI=0.17, P=7x10-14), and a skipped exon in UBXN4 (related to ER stress) in t(14;16) group (dPSI=0.1, P=3x10-4).

AS heterogeneity also leads to functional heterogeneity in the three groups. Besides commonly downregulated RNA catabolic processes, cell adhesion, migration and mobility related pathways are enriched pathways in t(14;16); cell growth related pathways in t(11;14); and ERK related pathways in t(4;14).

High-risk events were identified through survival analysis, including an AF event of MBNL1 (Muscleblind Like Splicing Regulator 1) in the t(14;16) (Hazard Ratio (HR)=4.61, p=0.02). Similarly, high risk was associated with an AF event in TFG (Trafficking from ER to Golgi Regulator) in t(11;14) (HR=6.69, p=2.7e-5) and an Alternative 5' site (A5) event in SNHG5 (Small Nucleolar RNA Host Gene 5) in t(4;14) (HR=8.90, p=0.03).

Translational/Clinical Research Faculty

POSTER #72

MITIGATION OF CDK4/6 INHIBITOR RESISTANCE IN RELAPSED PEDIATRIC AND AYA OSTEOSARCOMA BY PI3K/MTOR INHIBITION

Farinaz Barghi[1], Pankita H. Pandya[2], M. Reza Saadatzadeh[3], Khadijeh Bijangi-Vishehsarai[2], Barbara J. Bailey[4], Erika A. Dobrota[4], Courtney Young[4], Melissa A. Trowbridge[5], Kathryn L. Coy[5], Henry Elmer Mang[5], Anthony L. Sinn[5], Harlan E. Shannon[4], Jamie L. Renbarger[2], Karen E. Pollok[6]

[1] *Department Of Medical And Molecular Genetics, Indiana University School Of Medicine, Department Of Pediatrics, Herman B Wells Center For Pediatric Research, Indiana University School Of Medicine, Indianapolis, IN*

[2] *Department Of Pediatrics, Hematology/Oncology, Indiana University School Of Medicine, Department Of Pediatrics, Herman B Wells Center For Pediatric Research, Indiana University School Of Medicine, Indianapolis, IN*

[3] *Department Of Pediatrics, Herman B Wells Center For Pediatric Research, Indiana University School Of Medicine, Department Of Pediatrics, Hematology/Oncology, Indiana University School Of Medicine, Indianapolis, IN*

[4] *Department Of Pediatrics, Herman B Wells Center For Pediatric Research, Indiana University School Of Medicine, Indianapolis, IN*

[5] *In Vivo Therapeutics Core, Indiana University Melvin And Bren Simon Comprehensive Cancer Center, Indiana University School Of Medicine, Indianapolis, IN*

[6] *Department Of Pediatrics, Hematology/Oncology, Indiana University School Of Medicine, Department Of Pediatrics, Herman B Wells Center For Pediatric Research, Indiana University School Of Medicine, In Vivo Therapeutics Core, Indiana University Melvin And Bren Simon Comprehensive Cancer Center, Indiana University School Of Medicine, Department Of Medical And Molecular Genetics, Indiana University School Of Medicine, Indianapolis, IN*

Email: *fbarghi@iu.edu*

Osteosarcoma (OS) is the most common bone malignancy in children, adolescents, and young adults (AYA). Despite treatment with chemotherapies, approximately 35% of OS patients develop metastases and relapse. Alteration of cyclin-dependent kinases 4 and 6 (CDK4/6) is one of the top actionable signatures, based on genomic data from pediatric and AYA patients enrolled in the Precision Genomics program at Riley Hospital for Children, Indiana University Health. Interaction of retinoblastoma protein (RB)-E2F transcription factors is regulated by the cyclin D-CDK4/6 complex. Upon RB phosphorylation mediated by cyclin D-CDK4/6 complex, the RB-E2F complex dissociates, and cell cycle progression ensues. FDA-approved CDK4/6 inhibitors (CDK4/6i) are typically classified as cytostatic resulting in cell cycle arrest in contrast to cell death. In addition, chronic administration of CDK4/6i can lead to acquired resistance in RB1-proficient (RB+) tumors through activation of compensatory pathways such as PI3K and MAPK. Aberrant PI3K activation has also been demonstrated in OS patients. We hypothesized that dual inhibition of CDK4/6 and PI3K pathways will be efficient and well-tolerated in RB+ OS models with hyperactivation of the cyclin D-CDK4/6 complex. RB+ OS cell lines and an RB+ patient-derived xenograft (PDX)-derived xenoline (TT2-77) were studied. Whole-genome sequencing demonstrated that the original OS biopsy and the TT2-77 PDX generated from harbor signatures associated with CDK4/6 pathway up-regulation. Cell growth response to CDK4/6i (Palbociclib or Abemaciclib) and PI3K/mTOR inhibitor (PI3K/mTORi, Voxtalisib, or LY3023414) resulted in additive-to-synergistic inhibition of growth in RB+ OS cell lines at clinically relevant concentrations indicated by the combination index and Bliss independence analyses. A pharmacodynamic (PD) study of Palbociclib (CDK4/6i) on the TT2-77 PDX tumors from mice treated with vehicle vs. Palbociclib for five days was conducted, and both global/phospho-proteomics and kinome profiling analyses were performed to evaluate down-stream target modulation and provide insight into potential CDK4/6i-induced compensatory pathways. Down-regulation of RB1 and MKI67 phosphopeptides and total protein levels of CDK1, as well as DNA replication proteins, were observed, confirming that Palbociclib targets the cell cycle-related proteins. Kinome

profiling analysis indicated AXL receptor tyrosine kinase upregulation following CDK4/6i therapy. AXL activity can promote cell proliferation through downstream effector molecules, including PI3K pathway proteins. Thus, this may be an alternative approach to target CDK4/6i resistance in OS. Furthermore, TT2-77 PDX mice were treated with Palbociclib (50 mg/kg) and Voxtalisib (50 mg/kg) once a day for four weeks. Tumor growth was significantly decreased in single-agent groups compared to vehicle ($p<0.01$) and was well tolerated. Despite the slower tumor growth kinetics, tumor growth still progressed under single-agent therapy. These data highlight the **need for combination therapy, and experiments are in progress to evaluate anti-tumor response and safety of combination Palbociclib and Voxtalisib in a panel of OS PDX.**

Translational/Clinical Research ***Graduate Student***

POSTER #73

CANCER ASSOCIATED FIBROBLASTS PROVIDE A CANCER STEM CELL NICHE THAT LEADS TO DISEASE RELAPSE IN OVARIAN CANCER

Yiming Fang[1,2], Xue Xiao[1,2], Ji Wang[1,2], Subramanyam Dasari[1,2], Kenneth Nephew[1,2], Dmitriy Zamarin[3], Anirban Mitra[1,2]

[1] *Indiana University School Of Medicine, Bloomington, IN*
[2] *IUSCCC*
[3] *Memorial Sloan Kettering Cancer Center*

Email: *yimfang@iu.edu*

Epithelial ovarian cancer is the deadliest gynecologic malignancy with a 5-year survival rate of only 48%. While most ovarian cancer patients respond to chemotherapy initially, frequent relapse and development of chemoresistance result in poor patient outcomes. Cancer stem cells (CSCs) consist of a small subpopulation in the tumor that are typically resistant to cytotoxic chemotherapy and are a potential cause of relapse and chemoresistance. Using patient specimens and in vitro models, we determined that CSCs are enriched in a niche adjacent to cancer associated fibroblasts(CAFs) after chemotherapy. Cancer associated fibroblasts (CAFs) are a major constituent of the ovarian cancer tumor microenvironment and are highly enriched in the residual tumors following chemotherapy. Therefore, we studied the mechanism by which CAFs promote ovarian cancer chemoresistance and disease relapse by providing an optimal microenvironment for CSCs. CAFs isolated from ovarian cancer patient tumors were used in heterotypic 2D or 3D coculture systems with high-grade serous ovarian cancer cell lines or with patient-derived ovarian cancer cells to study their effect on CSCs and chemoresistance. Matched pre-and post-chemotherapy patient tumors were used to confirm our findings. CAFs significantly increased resistance to carboplatin and enriched CSCs by increasing their symmetric division as well as dedifferentiation of bulk ovarian cancer cells. An in vivo limiting dilution assay demonstrated that pre-coculture with CAFs increased in vivo tumor initiation capacity of the ovarian cancer cells by 10-fold. The CSC-CAF crosstalk responsible for CSC induction was found to be mediated by Wnt5a signaling. CRISPR knockdown of Wnt5a in CAFs or treatment with a specific Wnt5a inhibitor abrogated the induction of CSCs by CAFs. Only cancer cells with ROR2, a Wnt coreceptor, respond to Wnt5a signaling triggered by CAFs and developed into CSCs. Responders were found to signal through a non-canonical Wnt pathway involving the coreceptor ROR2, protein kinase C (PKC), and cAMP Responsive Element Binding Protein 1 (CREB1). Inhibition of each of them prevented CSC induction and functional rescue experiments confirmed the sequence of the Wnt5a-ROR2-PKC-CREB1 axis. Treatment of mouse xenografts, established by co-injection of CAFs and ovarian cancer cells, with the Wnt5a inhibitor sensitized them to carboplatin, and eliminated the CSCs in the residual tumors. Our results indicate that CAF-derived Wnt5a is instrumental in ovarian cancer CSC growth and maintenance. Since this mechanism can replenish CSC by increasing their symmetric division as well as stimulating dedifferentiation of subpopulations of bulk cancer cells, targeting it effectively prevents tumor relapse after cytotoxic chemotherapy. In the long term, our studies will broaden the understanding of the mechanism of CSC maintenance by the tumor microenvironment and contribute towards the development of novel therapeutic approaches to prevent ovarian cancer chemoresistance and relapse.

Translational/Clinical Research ***Graduate Student***

POSTER #74

DETERMINING THE ROLE OF AKT-EZH2 IN MEDIATING THE TRANSCRIPTIONAL RESPONSE TO ROS IN COLON CANCER

Ahmed Ghobashi[1]

[1] *Indiana University-Bloomington, Bloomington, IN*

Email: *aghobash@iu.edu*

Determining the role of AKT-EZH2 in mediating the transcriptional response to ROS in colon cancer

Ahmed Ghobashi

PhD advisor: Heather O'Hagan

Colorectal cancer (CRC) is the third leading cause of cancer related mortalities in the US. Inflammation, one of the major risk factors of CRC development, increases the production of reactive oxygen species (ROS), which play a role in tumor initiation and progression through activation of many cellular survival signaling pathways such as PI3K/AKT pathway. Activation of PI3K/AKT pathway mediates phosphorylation of many protein substrates including EZH2. EZH2 is a methyltransferase that catalyzes trimethylation of H3K27 (H3K27me3) as a catalytic subunit of polycomb repressive complex 2 (PRC2). Pharmacological inhibition of EZH2 ameliorates intestinal inflammation and delays colitis-associated CRC. EZH2 is proposed to act as an oncogene that plays a significant role in proliferation of many tumors including CRC. However, the molecular mechanism by which EZH2 drives CRC development and progression is not fully understood. *My overall goal is to study the role of EZH2 in promoting inflammation-induced CRC tumorigenesis.* I demonstrated that in response to the ROS, H_2O_2, EZH2 interacts with AKT in SW480 colon cancer cells which led me to hypothesize that AKT interacts with EZH2 to regulate the transcriptional response to ROS. Aberrant activation of WNT/β-catenin pathway has been demonstrated to be the initiating and promoting event in the majority of CRC development. My preliminary data demonstrates that in response to ROS, EZH2 interacts with and methylates β-catenin. Interestingly, inhibiting AKT activity abolishes both EZH2-β-catenin interaction and EZH2-induced β-catenin methylation. Altogether, these findings lead me to *hypothesize that ROS-mediated AKT activation induces AKT-EZH2 interaction which promotes EZH2-induced β-catenin methylation to regulate β-catenin transcriptional activity in response to* H_2O_2. This work will further elucidate non-histone protein targets of EZH2 in CRC. Current EZH2 inhibitor studies predominantly focus on H3K27me3 levels as the pharmacodynamic readout. However, the level of H3K27me3 is not the only way for measuring the oncogenic EZH2 activity due to its other non-histone targets. Therefore, identifying and understanding non-histone targets for EZH2 may provide promising cancer therapeutic interventions.

Translational/Clinical Research ***Graduate Student***

POSTER #75

DEFINING THE MECHANISTIC ROLE OF HEAT SHOCK FACTOR 1 (HSF1) IN METASTATIC BREAST CANCER COLONIZATION.

Natasha Hockaden[1]

[1] *Medical Sciences, Bloomington, IN*

Email: *nhockade@iu.edu*

Breast cancer is the most commonly diagnosed cancer in women and is the second leading cause of cancer-related deaths in women. Approximately 20-30% of patients will develop metastases and metastasis is responsible for greater than 90% of breast cancer deaths. Metastasis is a complex process in which the cells combat many forces to survive and spread to different areas of the body. Metastatic colonization is the rate-limiting step of metastasis and is an inefficient process in which most cells die and only a small fraction of those that survive can form metastases. Our lab has previously shown that heat shock factor 1 (HSF1) is involved in the early steps of breast cancer metastasis, particularly invasion, by promoting epithelial-to-mesenchymal transition (EMT). Utilizing an HSF1 gene expression signature that assesses HSF1 transcriptional activity, we further found that patients with high HSF1 activity have significantly worse metastasis-free survival, further suggesting HSF1 may play a role in metastasis. The physiological function of HSF1 is to regulate the cellular stress response to aid in cell survival in response to external stressors. We have also published that HSF1 can regulate the breast cancer stem cell populations. Because of these functions and the fact that the process of metastatic colonization is known to involve the stem cell population and incur external stressors, we hypothesized that HSF1 may function in metastatic colonization in addition to EMT and metastatic dissemination. To test this, we injected human breast cancer MDA-MB-231 cells with or without HSF1 knockdown into the left ventricle of nude mice. This model injects cells directly into the circulation allowing for assessment of metastatic tumor formation in which the major barrier will be metastatic colonization. We observed that cells without HSF1 had a significantly reduced metastatic burden, indicating HSF1 is necessary for the completion of metastasis and colonization. Consistent with these findings, bone metastatic tumor specimens from patients show increased HSF1 activation compared to their matched primary breast tumors. The mechanism by which HSF1 enables metastatic colonization is unknown. Metastatic colonization likely requires at least two stages that include tumor initiation (or early colonization) characterized by the seeding of a tumor followed by tumor expansion (or late colonization) characterized by rapid proliferation and an increase in tumor size. We aim to identify in which of these stages is HSF1 activated and what mechanism initiates HSF1 activity. We hypothesize that HSF1 may be activated by protein aggregation induced by the stressors during colonization. We will also test the contribution of HSF1-driven stemness in this process. Because we see HSF1 hyperactivation in metastatic tumors and HSF1 is required for completion of metastasis, we also will test whether HSF1 is a viable therapeutic target in preclinical models of metastatic breast cancer.

Translational/Clinical Research ***Graduate Student***

POSTER #76

HSF1 DOWNREGULATION OF CCL5 REDUCES CD8 + T CELL TRAFFICKING IN BREAST CANCER

Curteisha Jacobs[1]

[1] *Biology , Indiana University- Bloomington , Bloomington , IN*

Email: *cujacobs@iu.edu*

1 and 8 women will be diagnosed with breast cancer in their lifetime. Breast cancer is the second leading cause of cancer related death in women. Cytotoxic CD8+ T cell infiltration in breast tumors improve patient outcome and reduces cancer progression. Our previous work has linked the transcription factor HSF1 to breast cancer progression and metastasis. We also recently observed that when HSF1 activity is high there are less genes enriched for cytotoxic immune cells in breast tumors. We observed this negative relationship between HSF1 and CD8+ T cell presence in several computational datasets as well as a cohort of primary breast cancer patient specimens. To functionally test this relationship between HSF1 and CD8+ T cells, HSF1 was knocked down using shRNA in 4T1 mouse breast cancer cells and injected into the mammary fat pad of Balb/c mice. Tumors with HSF1 knockdown had lower tumor volumes and increased CD8+ T cell infiltration. To test the functional role of the relationship between HSF1 and CD8+ T cell presence, 4T1 cells with HSF1 knockdown were injected into Balb/ca mice with or without CD8+ T cell depletion. With depletion the HSF1 knockdown groups had larger tumors, suggesting a functional role for HSF1 to inhibit CD8+ T cell infiltration and protect the tumor from immune-mediated killing. CD8+ T cells are recruited by cytokines such as CCL5 and CXCL16. To investigate cytokine secretion, surrounding medium from 4T1 cells with control or HSF1 shRNA were subjected to a cytokine array wherein we observed that CCL5 secretion was significantly increased with the loss of HSF1. Upon further investigation, we found that HSF1 suppresses the expression of CCL5 in breast cancer cells, both at the mRNA level and protein secretion. To ascertain the importance of CCL5 in HSF1-regulated suppression of CD8+ T cell recruitment, we observed that conditioned medium from 4T1 cells with HSF1 knockdown increases migration of CD8+ T cells from Balb/c mice in a transwell migration assay. Furthermore, knockdown of CCL5 reversed this CD8+ T cell migration indicating that CCL5 is a primary mechanism for CD8+ T cell recruitment with HSF1 knockdown. HSF1 ChIP-Seq indicates HSF1 does not directly target CCL5 gene promoter but NF-κB is a canonical regulator of CCL5 gene expression. We, and others, have observed that HSF1 can suppress the activity of NF-κB. Therefore, we hypothesize that HSF1 indirectly regulates CCL5 levels via suppression of the the NF-κB pathway. Therefore, our study points to HSF1 as a novel regulator of tumor interaction with the immune system and may indicate therapeutically targeting HSF1 may enhance immune checkpoint therapy by increasing immune cells within tumors.

Translational/Clinical Research ***Graduate Student***

POSTER #77

TONSL IS AN IMMORTALIZING ONCOGENE OF THE CHROMOSOME 8q24.3 AMPLICON

Aditi Khatpe[1], Rebecca Dirks[2], Poornima Nakshatri[2], Henry Mang, Katie Batic, Sarah Sweizy[2], Jacob Olson[3], Xi Rao[4], Yue Wang[4], Hiromi Tanaka[4], Sheng Liu[4], Jun Wan[4], Duojiao Chen, Yunlong Liu[4], Fang Fang[4], George Sandusky[5], Kenneth Nephew[6], Kathy Miller[7], Harikrishna Nakshatri[2]

[1] *IU School Of Medicine, Department Of Biochemistry And Molecular Biology, Indianapolis, IN*
[2] *IU School Of Medicine, Department Of Surgery*
[3] *IUPUI Purdue School Of Science*
[4] *IU School Of Medicine, Department Of Medical And Molecular Genetics*
[5] *Department Of Pathology And Laboratory Medicine*
[6] *IU School Of Medicine, Bloomington, Medical Sciences Program*
[7] *IU Simon Cancer Center*

Email: *askhatpe@iu.edu*

Introduction: Immortalization is defined as a process by which cells acquire the ability to divide and reproduce indefinitely. Immortalization is the first event during cancer initiation. Our understanding of genomic events during cancer initiation including immortalization is still very limited. The goal of this study is to identify genomic events during cancer initiation using an isogenic breast cancer progression model starting with primary breast epithelial cells from healthy women.

Methods:RNA sequencing was performed with primary breast epithelial cells isolated from core biopsies of seven healthy women of diverse genetic ancestry and their immortalized counterparts. Various filters were applied to identify functionally important genes transcriptionally deregulated during immortalization. Publicly available cancer transcriptome databases and CRISPR screens were used to determine cancer relevance and gene essentiality, respectively, of differentially expressed genes. Chromosome 8q24.3 localized gene TONSL (Tonsoku like, DNA Repair Protein) was identified as a top hit and was further verified for its role during immortalization by overexpressing in primary breast epithelial cells. TONSL mediated changes in epigenome, transcriptome, and immortalization were studied with ATAC seq, RNA seq, and TRAP (Telomeric Repeat Amplification Protocol) assay, respectively. Therapeutic effect of targeting TONSL was studied *in vivo* and *in vitro* using two breast cancer cell lines with (MD-436, HCC1937), and without (MD-231, MD-468) chr8q24.3/TONSL amplification with CBL0137, an anticancer drug which targets TONSL-FACT complex and shown clinical efficacy in a Phase I trial.

Results: Over 1,500 unique genes were differentially expressed between primary and immortalized cells; ~700 of which were upregulated in the latter. Differentially expressed genes were integral part of cancer-related pathways such as growth, cell cycle, death, and survival. TONSL, which has previously been co-implicated during UV-induced DNA damage, homologous recombination during replication-associated DNA damage, demonstrated elevated expression in immortalized cells compared to primary cells and further upregulated in RAS transformed cells. TONSL-induced transcriptome/epigenome changes in primary cells revealed its role in altering expression of genes in the DNA repair pathways and cell cycle control of chromosome replication. TONSL overexpressing cells exhibited higher telomerase activity compared to primary cells and their transformation with defined oncogenes generated ER+ invasive ductal carcinoma in NSG mice. Breast cancer cell lines with chr8q24.3 amplification were sensitive to CBL0137 *in vivo* and *in vitro*.

Conclusion: This study identified TONSL as an immortalizing oncogene as its upregulation during tumor initiation, potentially due to chromosome amplification, increases telomerase activity required for immortalization. CBL0137 could be developed as a therapy for ~20% of breast cancers that have TONSL amplification.

Translational/Clinical Research ***Graduate Student***

POSTER #78

TARGETING ALDH1A1 AND REGULATORY NETWORKS THAT SUPPORT STEMNESS IN OVARIAN CANCER CELLS

Vaishnavi Muralikrishnan[1], Fang Fang[2], Ram Podicheti[3], Thomas Hurley[4], Kenneth Nephew[5,6]

[1] *Cell Molecular And Cancer Biology, School Of Medicine, Indiana University, Bloomington, IN*
[2] *Center For Medical Genomis, School Of Medicine, Indiana University, Indianapolis, IN*
[3] *Center For Genomics And Bioinformatics, Indiana University, Bloomington, IN*
[4] *Biochemistry And Molecular Biology, School Of Medicine, Indiana Univeristy, Indianapolis, IN*
[5] *School Of Medicine, Indiana Univeristy, Bloomington, IN*
[6] *Indiana University Melvin And Bren Simon Comprehensive Cancer Center*

Email: *vmuralik@iu.edu*

High grade serous ovarian cancer (HGSOC) is the leading cause of death from gynecologic malignancies. Ovarian cancer stem cells (OCSCs) are hypothesized to be largely responsible for the emergence of chemoresistant tumors, and we have previously shown that OCSCs contribute to recurrent, drug resistant HGSOC using aldehyde dehydrogenase 1A1 (ALDH1A1) activity as a robust functional marker to identify OCSCs. ALDH1A1 is an intracellular enzyme that oxidizes toxic aldehydes to carboxylic acids and plays a role in controlling cell differentiation pathways. However, the mechanism by which ALDH1A1 maintains stemness phenotype remains poorly understood. To examine the effect of ALDH1A1 upregulation on cellular survival signals in OCSCs, we used a novel ALDH1A1-specific small molecule inhibitor named compound 974. Treatment of HGSOC cell lines with compound 974 reduced ($p<0.01$) ALDH enzyme activity and inhibited stem-like properties including spheroid formation ($p<0.01$) and clonogenic survival ($p<0.05$). Stemness genes PROM1, BMI1, OCT4 and Nanog were inhibited ($p<0.01$) by compound 974. ALDH1A1 inhibition also reduced ($p<0.05$) cisplatin IC50 and synergized with cisplatin treatment. To further examine the effect of compound 974 to inhibit ALDH1A1 and consequently tumor initiation, mice were injected with 10^6, 10^5 and 10^4 OVCAR3 cells treated *in vitro* with compound 974 (5μM for 48h). In a parallel study, mice were injected with 10^6, 10^5 and 10^4 of ALDH1A1 knockdown or scrambled control cells. In both cases, ALDH1A1 inhibition led to a significant delay in tumor initiation compared to the control. Extreme limiting dilution analysis revealed that ALDH1A1 inhibition reduced ($p<0.05$) CSC frequencycompared to control. To understand the mechanism by which ALDH1A1 maintains OC stemness,OVCAR3 cells treated with vehicle or compound 974 were analyzed by RNA-sequencing and bioinformatics. Transcriptomic analysis revealed that compound 974 significantly (FDR < 0.05, fold change >2) downregulated expression of markers strongly associated with CSC phenotypes (NF-κB, KLF4, SOX9, FZD7) and chemoresistance (ABCB1). Ingenuity Pathway Analysis for downstream regulators of differentially expressed genes revealed senescence pathway as one of significantly altered pathways. Analysis by qRT-PCR revealed that compound 974 inhibited ($p<0.01$) chemotherapy-induced expression of senescence marker p21 and senescence associated secretory phenotype (SASP) such as IL6, IL8 and IL1α. Compound 974 also inhibited ($p<0.05$) the chemotherapy induced beta-galactosidase activity. By using a specific inhibitor, we conclude that ALDH1A1 plays a functional role in OCSC biology in general and for the first time describe a role for a specific ALDH isoform in chemotherapy-induced senescence. Targeting ALDH1A1 in OCSCs could be part of a therapeutic strategy to inhibit tumor relapse and overcome chemoresistance.

Translational/Clinical Research ***Graduate Student***

POSTER #79

THE HDAC INHIBITOR ROMIDEPSIN IS A PROMISING THERAPEUTIC FOR METASTATIC OSTEOSARCOMA

Emily Seiden[1], Patrick Getty[2], Christopher Collier[1], Ed Greenfield[1]

[1] *School Of Medicine, Indiana University, Indianapolis, IN*
[2] *Case Western Reserve University, Cleveland, OH*

Email: *eseiden@iu.edu*

Introduction: Osteosarcoma is the most common primary malignant bone tumor and predominately affects children, adolescents, and young adults. The five-year survival rate of those with detectable pulmonary metastases is only 30%, indicating that standard therapy (MAP: methotrexate, doxorubicin, cisplatin) is not effective in this patient group. It is therefore necessary to identify novel therapies for osteosarcoma that target the progression of pulmonary metastases. Previously, our lab screened 114 FDA-approved anti-cancer drugs to identify agents that decrease the growth of 3D spheroids (sarcospheres) generated from three human highly metastatic osteosarcoma cell lines. Sarcospheres more closely mimic pulmonary metastases compared with a cell monolayer. The top hits from the initial screen included both of the histone deacetylase inhibitors (HDACi's) that were tested. In follow-up experiments with and without MAP, romidepsin was the most effective of the five FDA-approved HDACi's and the seven that are in clinical trials. Our goal was therefore to further evaluate romidepsin as a potential therapy for metastatic osteosarcoma.

Methods: Highly uniform sarcospheres (~400μm in diameter) were generated from highly metastatic human cell lines (143B, MG63.3) and primary cells from a canine osteosarcoma by our previously validated centrifugation-based method. Sarcospheres were matured for 24 hours, then incubated with or without romidepsin treatment for 48 hours. Viability was measured using resazurin reduction. Size was assessed by measuring sarcosphere area from brightfield images obtained using the Incucyte® S3 Live-Cell Analysis System. The Incucyte® Cytotox Green Dye was used to stain for cell death.

Results: Romidepsin decreased viability of sarcospheres generated from the three different highly metastatic osteosarcoma cell lines LM7, MG63.3, and 143B with IC50s of 3 nM, 5nM, and 28 nM respectively. Following 48 hours, high concentrations of romidepsin caused a regression of viability below levels prior to drug treatment. However, the size plateaued, likely due to persistence of dead cells. Consistent with that possibility, Incucyte® Cytotox Green Dye staining showed that 1 μM romidepsin causes cell death throughout the sarcosphere after 48 hours. Over the course of the 48 hour treatment period, we showed that romidepsin dose dependently decreased sarcosphere size beginning at 22 hours. Using cells derived from a primary canine osteosarcoma to generate sarcospheres, we showed that romidepsin treatment decreased viability below levels prior to drug treatment with an IC50 of 77 nM.

Discussion: This study identified romidepsin as a promising therapeutic to target the progression of micrometastases in osteosarcoma. These *in vitro* sarcosphere results show that romidepsin decreases sarcosphere viability and size. These results provide strong justification to pursue future *in vivo* studies to determine whether romidepsin blocks progression of metastases, and thereby improves survival, in murine and canine osteosarcoma models.

Translational/Clinical Research ***Graduate Student***

POSTER #80

MCAK AS A THERPEUTIC TARGET IN TRIPLE-NEGATIVE BREAST CANCER

John Smith[1], Stefan Husted[1], Jay Pilrose[1], Disha Kuchangi[1], Richard Carpenter[1], Claire Walczak[1]

[1] *Indiana University - Bloomington, Bloomington, IN*

Email: *jcs12@iu.edu*

Triple negative breast cancer (TNBC) is the most lethal breast cancer subtype with no targeted therapies available. The standard of care for TNBC is chemotherapy using a combination of microtubule poisons and DNA damaging agents. Microtubule poisons, like paclitaxel, were initially thought to function by inducing cell cycle arrest during mitosis, and ultimately, apoptosis. Recent studies, however, suggest that paclitaxel induces lethal levels of aneuploidy in tumor cells. While these drugs are initially effective in treating cancer, patients often relapse with drug resistant tumors. Identifying proteins that limit aneuploidy may therefore be valuable targets for therapeutic development. One potential target is the microtubule depolymerizing kinesin, MCAK, which prevents chromosome mis-segregation by correcting mal-attached kinetochores during mitosis. To evaluate MCAK as a therapeutic target in cancer, we first probed its expression levels in the TCGA and the GSE47651 breast tumor databases and found MCAK to be highly upregulated in TNBC. High MCAK expression was associated with elevated tumor stage, increased metastasis, and reduced metastasis-free survival. Knockdown of MCAK in multiple tumor-derived cell lines caused an approximate 5-fold reduction in the IC_{50} for paclitaxel, but there was no change in a normal diploid line, suggesting MCAK loss has a cancer-specific effect. Treatment of cells with paclitaxel or knockdown of MCAK each caused an increase in aneuploidy, but combination treatments did not have an additive effect, suggesting that another mechanism is likely responsible for the increase in taxane sensitivity. One possibility is MCAK's recently uncovered role in DNA repair, which would allow MCAK inhibition to modulate DNA damage in combination with other drugs. Consistent with this hypothesis, bioinformatics studies suggest that MCAK expression levels are correlated with a decreased sensitivity to the topoisomerase inhibitor topotecan, an interaction we are currently testing in the lab. To identify potential MCAK therapeutics, we developed a screening pipeline for putative MCAK inhibitors and have established three candidate inhibitors that all caused increased aneuploidy in breast cancer cells. At least one of these inhibitors caused a potent reduction in colony formation assays. In addition, preliminary data show that MCAK loss increases aneuploidy in a taxane-resistant cell line, opening the possible utility of MCAK inhibitors in patients with taxane-resistant cancers. Collectively our work will expand the field of precision medicine to include aneugenic drugs, while giving treatment options to breast cancer patients with relapsed or drug-resistant disease.

Translational/Clinical Research ***Graduate Student***

POSTER #81

PARACRINE INTERACTIONS WITH ADJACENT CANCER ASSOCIATED FIBROBLASTS IMPART CHEMORESISTANCE TO OVARIAN CANCER CELLS

Xue Xiao[1,2], Yiming Fang[1,2], Anirban Mitra[1,2]

[1] *Indiana University School Of Medicine – Bloomington, Bloomington, , IN*
[2] *Indiana University Simon Comprehensive Cancer Center, Indianapolis*

Email: *xuexiao@iu.edu*

Ovarian cancer is the most lethal gynecologic cancer and the fifth leading cause of cancer-related deaths among women in the US. This high death rate is mainly due to extensive metastasis and a high recurrence rate. The standard of care involves debulking surgery and Carbo-Taxol chemotherapy. While most patients respond well initially, some cancer stem cells survive and cause ovarian cancer relapse. Cancer-associated fibroblasts (CAFs) are key components of the tumor microenvironment (TME) that have been suggested to play important roles in regulating tumorigenesis. Moreover, the residual tumors following neoadjuvant chemotherapy are typically fibrotic, indicating the potential role of CAFs in providing a niche for surviving cancer cells.

Like cancer cells, CAFs are heterogenous and our research has shown a subpopulation of CAFs secrete Wnt5a to regulate cancer cells and enrich the cancer stem cell population. My analysis of multiple publicly available datasets revealed that CAFs have significantly higher Wnt5a expression than cancer cells, further confirming the role of CAF-derived Wnt5a in clinical samples. Deconvolution of TCGA ovarian cancer data further revealed that the percentage of CAFs in the TME increases with the cancer stage. Deconvolution analysis of the Australian Ovarian Cancer Study dataset containing transcriptomic information of chemo-resistant and chemo-sensitive patient tumors showed significant CAF enrichment in chemoresistant tumors. TUNEL and immunofluorescent staining were done in frozen sections of chemo-naïve ovarian cancer patient tumors and compared to tumors from patients who had received prior chemotherapy. While chemo naive tumors had minimal apoptotic cells, chemotherapy-induced apoptosis in cancer cells was further away from CAFs. Interestingly, the cancer cells adjacent to CAFs were spared.

Our previous research had shown that Wnt5a triggers cancer stem cell enrichment in two ways. It stimulates ovarian cancer stem cell self-renewal by promoting symmetric division and increases the dedifferentiation of subpopulations of bulk ovarian cancer cells. To identify and characterize the subpopulations of CAFs that are capable of inducing stemness and chemoresistance as well as the subpopulation of ovarian cancer cells that are responsive to these signals, we applied a single-cell RNA sequencing (scRNA-seq) approach. Heterotypic 3D cocultures of patient-derived ovarian cancer cells and CAFs were thus analyzed. We characterized the pathways activated in CAF subpopulations with high Wnt5a expression. We also found cancer cell subpopulations, that respond to CAF signals and become cancer stem cells, using trajectory analysis. Our research uncovered the heterogeneity of CAFs in ovarian cancer TME and demonstrated the role of certain subpopulations that can serve as a cancer stem cell niche, giving rise to disease relapse. My long-term goal is to determine the molecular mechanism of the CAF-cancer stem cell crosstalk and specifically target this communication to prevent ovarian cancer recurrence and improve patient outcomes.

Translational/Clinical Research ***Graduate Student***

POSTER #82

CHARACTERISTICS OF SIDE EFFECTS EXPERIENCED IN PEDIATRIC ONCOLOGY PATIENTS RECEIVING THE COVID-19 VACCINE

Whitney Carroll[1], Dr. Seethal Jacob [2], Dr. Jennifer Belsky[3]

[1] *Marian University College Of Osteopathic Medicine, Indianapolis, IN*

[2] *Division Of Hematology/Oncology, Riley Hospital For Children, Indianapolis, IN, Center For Pediatric And Adolescent Comparative Effectiveness Research, Indianapolis, IN, Indianpolis, IN*

[3] *Division Of Hematology/Oncology, Riley Hospital For Children, Indianapolis, IN,, Department Of Pediatrics, Indiana University School Of Medicine, Indianapolis, IN, Indianapolis, IN*

Email: *wcarroll248@marian.edu*

Characteristics of side effects experienced in pediatric oncology patients receiving the COVID-19 vaccine

Abstract:

Purpose: In patients with cancer, lymphadenopathy is a concerning feature while receiving chemotherapy or radiation, and for survivors, lymphadenopathy is often a sign of disease recurrence. Lymphadenopathy following the Coronavirus Disease 2019 (COVID-19) vaccination that may mimic metastasis or cancer recurrence will result in multiple laboratory tests, imaging, and surgical biopsy. It is imperative to start understanding the distinguishing features of reactive lymphadenopathy following COVID-19 vaccination compared to the well-known characteristics of malignancy. No study to date has investigated the incidence and characteristics of lymphadenopathy on imaging in children and adolescent young adults (AYA) with cancer or sickle cell disease (SCD) receiving the vaccine. The primary objective of our study was to investigate secondary effects of the COVID-19 vaccine in children with cancer or SCD with regards to physical exam findings, imaging, and outcomes.

Methods: We retrospectively analyzed data from Riley Hospital for Children, including patients aged 0-35 with an oncologic or sickle cell diagnosis from January 2017 – December 2020. We investigated patients 1) aged 0 < 28 years of age, 2) received any COVID-19 vaccination, 3) have an active or previous oncology or SCD diagnosis and/or 4) a new patient referral for lymphadenopathy 8 weeks post-vaccination.

Results: We identified 154 unique patients with cancer or SCD who received the COVID-19 vaccination. Among these patients the most common oncologic diagnoses were leukemia (n=25, 16.2%), central nervous system tumors (n=25, 16.2%), lymphoma (n=23, 14.9%), sarcoma (n=15, 9.7%), other solid tumors (n=42, 27.3%). Of our patients with SCD (n=32, 20.8%), the most common genotypes were HbSS (n=21, 65.6%) and HbSC (n=10, 31.3%). Majority of patients were male (n=82, 52.3%) and Caucasian (n=113, 73.4%). For those who received imaging (n=57, 37.0%), the majority did not have lymphadenopathy present on imaging (n=152, 98.7%), but for those who did show lymphadenopathy on imaging (n=2, 1.3%) the most common location was axillary (n=2, 100.0%). Pfizer was the most commonly received vaccine (n=130, 84.4%), followed by Moderna, (n=19, 12.3%), and Johnson and Johnson (n=5, 3.2%). The majority of vaccinated patients did not have a documented reaction to the vaccine (n=148, 96.1%). Of those who experienced a reaction (n=4, 3.9%), most commonly seen were myalgias (n=3, 50%), fever (n=1, 16.7%), chills, (n=1, 16.7%), and other (n=5, 83.3%).

Conclusions: The majority of pediatric patients with cancer or SCD who received the COVID-19 vaccine had few side effects, and those who experienced side effects were reflective of those seen in the general population. Children with cancer or SCD are prone to severe COVID-19 infections. Hematologists/oncologists hesitant to recommend the COVID-19 vaccination to patients should be aware of the post-vaccine course to appropriately guide patients and families.

Translational/Clinical Research ***Medical Student***

POSTER #83

DISSECTING RAS ONCOGENE-INDUCED KINOME INVOLVED IN BREAST CANCER METASTASIS

Amna Yaqub[1], Harikrishna Nakshatri[2]

[1] *Indiana University School Of Medicine, Indianapolis, IN*
[2] *Department Of Surgery, Indiana University School Of Medicine, Indianapolis, IN*

Email: *asyaqub@iu.edu*

Background and Hypothesis:

The RAS and PI3K-AKT-mTOR signaling pathways are often dysregulated in cancer. RAS pathway alterations, however, are more common in breast cancer metastasis. The laboratory's recently developed model system demonstrated the ability of RAS but not PIK3CA-induced signals in promoting metastasis of breast cancer. Unbiased kinome analyses of isogenic RAS-transformed primary tumor and metastatic cells and PIK3CA-transformed primary tumor cells enabled identification of RAS-activated kinome, which included FER, PAK4, LIMK1, PIK3CD and Casein Kinase 2 (CK2). We hypothesized that therapeutic targeting of these kinases may reduce breast cancer metastasis. As a proof-of-principle, the effect of the CK2 inhibitor Silmitasertib, which is in clinical trial for COVID-19 and refractory multiple myeloma, was tested.

Experimental Design:

The study included four isogenic cell lines: "normal" (KTB34-hTERT), PIK3CA-transformed (TKTB34-PIK3CA), RAS-transformed (TKTB34-RAS), and RAS-transformed cells metastasized to lungs (MKTB34-RAS). Active kinomes in these cells were identified using phospho-proteomics and functional kinome profiling using multiplexed kinase inhibitor beads. Expression levels of FER, PAK4, LIMK1, and PIK3CD kinases were compared through Western Blot using the phospho-antibodies as an indicator of kinase activation. Sensitivity to Silmitasertib was measured using the BrdU Cell Proliferation Assay.

Results:

FER, PAK4, LIMK1, and PIK3CD were all overexpressed in the TKTB34-RAS and MKTB34-RAS cells compared to KTB34-hTERT and TKTB34-PIK3CA cells. The tested concentration range for Silmitasertib (500 nM to 5 μM) was ineffective in killing the RAS-transformed cells and was overly toxic to "normal" cells.

Conclusion and Potential Impact:

FER, PAK4, LIMK1, PIK3CD, and CK2 are potential therapeutic targets for breast cancer metastasis. However, Silmitasertib may not be a good candidate as it is more toxic to "normal" cells compared to cancer cells. The isogenic "normal" and transformed cell line model system described here may help to discover new targets and drugs that kill cancer but not normal cells.

Translational/Clinical Research *Medical Student*

POSTER #84

PREVALENCE AND MANAGEMENT OF CONSTIPATION IN PEDIATRIC PATIENTS UNDERGOING CHEMOTHERAPY: A RETROSPECTIVE REVIEW

Trisha Reddy[1], Megan Parod[2], Jennifer Belsky [3]

[1] *Department Of Pediatrics, Indiana University School Of Medicine*
[2] *Marian University College Of Osteopathic Medicine*
[3] *Pediatric Hematology Oncology, Indiana University School Of Medicine*

Email: *trreddy@iu.edu*

Background: Pediatric cancer patients suffer from a wide variety of chemotherapy induced side effects, including constipation. Psychological factors, chemotherapeutic medications, and lifestyle changes are anticipated to play a role in the multifactorial development of constipation. Methotrexate is a common chemotherapy agent administered for leukemia treatment and is primarily eliminated by the kidneys and liver. Failure to clear methotrexate from the body results in toxicities including mouth sores, mucositis, and organ dysfunction. Occasionally, resultant hyperbilirubinemia has been demonstrated, and is thought to contribute to delays in clearance. To date, no studies have examined the correlative relationship between constipation and delayed methotrexate clearance. This study aims to examine the characteristics of chemotherapy induced constipation management, and the relationship between methotrexate delays and constipation.

Methods: This single institution, retrospective study, analyzed data from Riley Hospital for Children, including patients aged 0-21 years of age that were diagnosed with acute lymphoblastic leukemia from January xxx-September 2021. Constipation was defined as no stool for 48 hours in the inpatient Cerner documentation.

Results: We investigated 23 unique pediatric oncology patients with acute lymphoblastic leukemia average age 6.7 (range 0-26) years. Majority of patients were male (74%), and Caucasian (87%). We captured 57 unique encounters of patients receiving methotrexate with an average of 5-day admissions. Almost a quarter (n= 11, 24.4%) of patients during inpatient admission met our definition of constipation, without a stool for 48 hours, and 0% of patients had a documentation of constipation in a progress note. Of these patients, 10.5% (n=51) experienced a delay in methotrexate clearance. Only 25% of patients had a scheduled constipation medication prescribed, while 55.4% (n=31) had an as needed constipation medication available. Miralax was the most common (86%) medication scheduled and prescribed as needed. In addition, 16.7% of patients (n=9) had an opioid prescribed during admission. Vincristine was the most common chemotherapy agent received (96.4%) in combination with methotrexate. Prior to admission, 47% of patients had historical constipation during previous admissions, and 90% of patients did not have a last stool documented prior to chemotherapy administration.

Conclusion: Patients admitted for methotrexate clearance suffered from constipation without appropriate bowel regimens. In addition to their prolonged hospitalizations, they received vincristine which is a known cause of chemotherapy induced constipation. Future prospective studies should focus on appropriate bowel regimens and secondary effects of constipation in pediatric oncology patients.

Translational/Clinical Research ***PGY-2 Resident***

POSTER #85

CLINICO-GENOMIC CHARACTERIZATION OF METASTATIC THYMIC EPITHELIAL TUMORS

Fatemeh Ardeshir Larijani[1], Milan Radovich[2], Bryan P. Schneider[2], Patrick J. Loehrer[2]

[1] *Indiana University, Medicine-Division Of Hematology/Oncology, Indianapolis, IN*
[2] *Medicine-Division Of Hematology/Oncology, Indianapolis, IN*

Email: *fardesh@iu.edu*

Background:Thymic epithelial tumors (TET) are one of the rarest adult malignancies. Overall, patients have favorable survival outcomes, however a small subset develop metastatic disease. Genomic characterization of this very rare, clinically aggressive TET subset is lacking. Herein, we evaluated the clinical and genomic characteristics of metastatic TET (mTET) compared to a large cohort (n = 117) of primary TET (pTET) from The Cancer Genome Atlas (TCGA).

Methods: From 2015 to 2020, 52 pts with mTET underwent clinical CLIA-based sequencing using either whole-exome (n = 35), panel-based testing (n = 13) and/or liquid biopsy (n = 22). The specimen was taken from a metastatic organ (n = 34) or relapsed primary mediastinal mass (n = 14); 4 pts had liquid bx only. Data on pTET was derived from the TCGA. Kaplan-Meier and log-rank test was used for assessment of PFS, OS.

Results: The median age was 56 yrs in mTET (range 32-74) vs. 60 yrs (range 17-84) in TCGA data. The M/F (%) was 40/60 in mTET and 48/52 in TCGA, respectively. Of note, 13 mTET pts had other types of cancer prior or concurrent with TET diagnosis (4-breast, 2-bladder, 5-other) in which radiotherapy (n = 4) and/or chemotherapy (n = 3) was administered prior to TET diagnosis. In our cohort, 19 pts had stage IVA and 33 pts had stage IVB (most common metastatic site was liver in 17 pts). WHO histologic classification was: A = 1, A/B = 3, B1 = 4, B2 = 10, B3 = 12, TC = 18, TC with neuroendocrine feature = 3, and lymphoepithelial carcinoma = 1. WHO B3 and TC histologies were more common in our cohort of mTET than in the TCGA cohort (63% (33/52) vs. 17% (20/117), respectively). Pts with TC had worse mOS compare to thymoma (109m vs. 163m, HR = 2.78, P = 0.04). The most common genomic alteration in mTET was TP53 (n = 17, 33%) compared to 3% in TCGA. This was followed by CDKN2A (n = 5, 10%), PIK3CA (n = 4, 8%), CDKN2B (n = 3, 6%) and NF1 (n = 3, 6%). All TP53 missense mut functionality was analyzed with polyphen-2 software and 91.6% (22/24) had 98-100% damaging probability. 70% of pts that harbored TP53 muts were TC (41%) or B3 (29%) histology. Clinically actionable genomic alterations targetable with available or investigational agents (e.g. high TMB; gain-of-function mutations in PIK3CA, CDK4, and mTOR; loss-of-function mutations in NF1) were seen in 23% (12/52) of pts.

Conclusions: Patients with mTET are associated with more aggressive WHO histology (B3 and TC). Greater frequency of TP53 mutations are observed in mTET compared to pTET. Clinically actionable genomic alterations are frequently seen in mTET suggesting value in the routine sequencing of these patients

Translational/Clinical Research ***Post-Doctoral/Medical Fellow***

POSTER #86

MICROENVIRONMENT INDUCED CHROMATIN CHANGES AND C-JUN INDUCTION REGULATES OVARIAN CANCER METASTASIS

Frank H.C. Cheng[1], Ji Wang[1], Subramanyam Dasari[1], Anirban K Mitra[1]

[1] *School Of Medicine-Bloomington, IN, Simon Comprehensive Cancer Center, Indianapolis, IN*

Email: *frcheng@iu.edu*

Ovarian cancer (OC) is the deadliest gynecologic malignancy and high-grade serous OC (HGSOC) is its most prevalent and lethal subtype. About 70% OC patients are diagnosed at a late stage with extensive metastasis, which contributes to the high mortality rate. Ovarian cancer predominantly undergoes transcoelomic metastasis and the omentum – a large fat pad in the peritoneal cavity – is the most common site of metastasis. The regulation of OC metastasis, especially metastatic colonization, which is the rate limiting step, is still poorly understood. Since most OC patients will be treated for metastatic disease, a better understanding of the key regulators would result in more effective therapies. During metastatic colonization, cancer cells must first successfully adapt to the new microenvironment before they can eventually develop into the metastatic tumor. This requires productive cross-talk between the OC cells and the microenvironment of the metastatic site, resulting in adaptive changes in gene regulation. Transcription factors (TFs) and epigenetic changes induced by the metastatic microenvironment would be expected to play a key role. Using an organotypic 3D culture model of the omentum mimicking metastatic colonization combined with the end point analysis of matched primary tumors and metastases from HGSOC patients, we identified a metastasis signature. c-Jun was the key transcription factor regulating these metastasis genes. We also observed that the metastatic microenvironment induces changes in chromatin in the cancer cells when we performed ATAC-seq in HGSOC cells seeded on the 3D omentum culture model. The top TFs predicted to bind to the newly open chromatin regions were Fos and c-Jun. We have performed a c-Jun CUT&RUN in HGSOC cells seeded on the 3D culture model and overlapped the results with the ATAC-seq data, to identify the direct targets of c-Jun that are induced by microenvironmental signals. HGSOC cells co-culture with microenvironment cells and conditioned medium (CM) experiments revealed that paracrine signals from mesothelial cells and cancer associated fibroblasts (CAFs) regulated c-Jun via the activation JNK. Galectin-1, Galectin-3 binding protein and Periostin were identified as the key paracrine factors through proteomic analysis of the secretomes of omental mesothelial cells, CAFs and normal omental fibroblasts. Functional studies revealed that c-Jun regulated migration, invasion through the outer layers of the omentum and colony formation on the omentum. Moreover, knocking out c-Jun significantly decreased metastasis in a mouse HGSOC xenograft model. Taken together, our studies reveal the novel phenomenon of microenvironment-induced upregulation of c-Jun, combined with the microenvironment-induced opening of the chromatin of certain c-Jun binding sites, which together regulate HGSOC metastatic colonization though specific transcriptional targets. Targeting the cross-talk to prevent c-Jun activation and chromatin changes would be a novel approach to effectively treat OC metastasis.

Translational/Clinical Research ***Post-Doctoral/Medical Fellow***

POSTER #87

MICROENVIRONMENT INDUCED EPIGENETIC DOWNREGULATION OF MIR-193B VIA THE ERK/EZH2/DNMT1 AXIS PROMOTES OVARIAN CANCER STEMNESS AND METASTASIS

Subramanyam Dasari[1], Frank Cheng [1], Melissa Halprin[1], Yiming Fang[1], Ji Wang[1], Komal Agarwal[1], David Pepin[2], Anirban K Mitra[1]

[1] *School Of Medicine – Bloomington, Bloomington, IN*
[2] *Harvard Medical School, Boston, Boston, MA*

Email: *sudasari@iu.edu*

Extensive metastasis and frequent relapse are key contributors to the high mortality rate of ovarian cancer (OC) patients. There is a critical need to better understand the mechanism of regulation of metastasis and disease recurrence to develop effective treatment strategies targeting them. Using an organotypic 3D culture model of the human omentum, we have studied the productive cross-talk between metastasizing OC cells and its microenvironment that is critical for establishment of metastasis. To identify the clinically relevant microRNAs that can regulate both early and advanced metastasis, we combined our 3D omentum culture approach with the end point analysis of microRNA expression profiles of matched primary and metastatic tumors from 42 OC patients. miR-193b was a key microRNA thus identified to be downregulated in early and advanced metastasis. The epigenetically downregulated miR-193b promoted metastatic colonization by enhancing the ability of the OC cells to invade through the outer layers of the omentum and increased cancer stem cell-like phenotype. Stably overexpressing miR-193b resulted in a significant decrease in metastases in OC xenografts while stable inhibition had the opposite effect. Moreover, treating a chemoresistant OC patient derived xenograft (PDX) model of metastasis with miR-193b significantly reduced metastasis. We have identified the microenvironmental signals and the resulting mechanism of miR-193b downregulation via the ERK/EZH2/DNMT1 axis, using heterotypic coculture models, conditioned medium experiments, secretome analysis, inhibition, and rescue experiments. Basic FGF (bFGF), IGF binding protein 2 (IGFBP2) and IGF binding protein 6 (IGFBP6) were the key mesothelial factors that were responsible for the downregulation of miR-193b in cancer cells. These factors were found to activate ERK in the cancer cells that induced EZH2 and DNMT1 expression. Using ChIP and MeDIP, we discovered that EZH2 induces H3K27me3 in the miR-193b promoter, which helps recruit DMNT1 that catalyzes DNA methylation at the miR-193b promoter. Having established the mechanism of miR-193b downregulation by the microenvironment, we proceeded to study the mechanism by which its decrease promotes metastasis. By performing RNA-seq in OC cells overexpressing miR-193b, we identified cyclin D1 (CCND1) as a key target, which was validated at RNA and protein levels. Knockdown of CCND1 mimicked the decreased expression of stem cells markers (ALDH1A1, OCT4, SOX2 and Nanog), and spheroid formation, caused by miR-193b overexpression. The induction of OC stem cells upon miR-193b inhibition could be rescued by simultaneous overexpression of CCND1. In conclusion, we have identified the mechanism of microenvironment-induced downregulation of miR-193b in OC cells that helps establish metastatic tumors by inducing cancer stem cells via its target CCND1. Treating a chemoresistant OC PDX with miR-193b significantly reduced metastases, indicating that miR-193b replacement therapy could be a promising approach to treat OC patients, a vast majority of whom succumb to metastatic and chemoresistant disease.

Translational/Clinical Research ***Post-Doctoral/Medical Fellow***

POSTER #88

TARGETING REF-1 REDOX FUNCTION PERTURBS CRITICAL TRANSCRIPTIONAL SIGNALING IN SOFT TISSUE SARCOMA (MPNST) RESULTING IN REDUCED GENE EXPRESSION AND TUMOR CELL SURVIVAL

Silpa Gampala[1], Olivia Babb[1], Steven D Rhodes[1], Reza M Saadatzadeh[1], Christine Pratilas[2], Karen E Pollok[1], Wade D Clapp[1], Mark R Kelley[3], Melissa L Fishel[3]

[1] *Department Of Pediatrics And Herman B Wells Center For Pediatric Research, Indiana University School Of Medicine, Indianapolis, IN*

[2] *The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins School Of Medicine, Baltimore, MD*

[3] *Department Of Pediatrics And Herman B Wells Center For Pediatric Research, Department Of Pharmacology And Toxicology, Indiana University School Of Medicine, Indianapolis, IN*

Email: *sgampala@iu.edu*

Malignant Peripheral Nerve Sheath Tumor (MPNST) is a rare soft tissue sarcoma that can arise from patients with NF1 (neurofibromatosis type 1). Due to intratumoral molecular heterogeneity and altered signal transduction pathways within MPNSTs, existing chemotherapeutic and targeted agents have thus far not been successful. The 5-year patient survival rate of 35%-50% dictates the need for novel and effective targets for this disease. One such target is Ref-1 (redox factor-1) which regulates two transcription factors that are important in driving MPNST, Signal Transducer and Activator of Transcription-3 (STAT3) and Hypoxia Inducible Factor 1 (HIF1).

Ref-1 and STAT3 expression is high in MPNST patient samples. Knockdown of these genes in MPNST lines resulted in decreased proliferation, wound healing, and tumor signaling. Further, there is crosstalk between these pathways as STAT3 knockdown also affected the expression of HIF1a and malignant phenotypes, showing the importance of these transcription factors (TFs) to drive MPNST tumorigenesis. Due to Ref-1's redox regulation of these TFs, we propose to inhibit Ref-1 to block transcription of MPNST survival genes driven by HIF1a and STAT3. We showed in our recent publication that Ref-1 inhibition reduces expression of genes highly expressed in MPNST compared to benign NF1-derived neurofibroma Schwann cells. Inhibitors of Ref-1 redox function, APX3330, APX2009, and APX2014 inhibit cell growth and colony formation *in vitro* and APX2009 blocks *in vivo* tumor growth. To further these findings and confirm this blockade of TFs by Ref-1 redox inhibition, we are screening more potent analogs of APX3330 through multiple strategies including TF-driven luciferase reporter assay, Ref-1 redox functional mutants, Ref-1 redox inactive analog RN7-58, and MPNST patient-derived xenograft (PDX) cells. *In vitro* results are being validated using *in vivo* MPNST orthotopic models (sciatic nerve implant).

Ongoing experiments show that Ref-1 and STAT3 knockdown reduce NFkB activity in MPNST cells, again highlighting significant crosstalk between these pathways. Ref-1 redox inhibitors efficiently inhibited NFkB and HIF1a activity while negative control analog RN7-58 did not, pointing toward on-target activity. Xenoline, RHT-92 established from patient PDX as well as NF90-8 cells were used for RNA sequencing after knockdown of Ref-1 or STAT3 to determine mechanistic effects on MPNST gene expression. Both lines demonstrated a marked decrease in metabolic pathways which is currently under validation. In support of these findings, we used OXPHOS deficient and proficient osteosarcoma cells to confirm Ref-1 redox activity impact on metabolism. Generating patient-derived cell lines from this rare cancer with samples from Riley hospital and Johns Hopkins University will be used to validate and confirm these result and will provide wealth of data to help improve MPNST treatment.

Translational/Clinical Research ***Post-Doctoral/Medical Fellow***

POSTER #89

A PILOT STUDY IN THE USE OF FENTANYL PRE-MEDICATION TO DECREASE BROWN ADIPOSE TISSUE UPTAKE OF 18-FDG TRACER IN PEDIATRIC LYMPHOMA PATIENTS

William Gaylord[1], Jennifer Belsky[1], Justin Sims[2], Lindsay Blazin[1], Courtney Frye[3]

[1] *Pediatric Hematology-Oncology, Riley Hospital For Children, Indianapolis, IN*
[2] *Clinical Radiology And Imaging Sciences, Riley Hospital For Children, Indianapolis, IN*
[3] *Pediatric Critical Care, Riley Hospital For Children, Indianapolis, IN*

Email: *wcgaylor@iu.edu*

Background: Brown fat is a metabolically active tissue, resulting in uptake of the 18-FDG tracer used in PET-CT scans, which may lead to uncertainty on scans in children. Brown fat has been found in 34-40% of children, causing oncologists difficulties interpreting scans. Retrospective studies investigating the use of fentanyl in attempt to decrease Brown fat uptake, reported decreasing incidence from 34-40% to 7-10%. We conducted an institutional pilot study to investigate safety, feasibility, and report characteristics of brown fat with fentanyl pre-medication in pediatric lymphoma patients.

Methods: This single center, prospective study, investigated PET scans performed on patients aged 6 year to 25 years old with a presumed or confirmed diagnosis of lymphoma from April to July 2021 utilizing a fentanyl protocol with the Pediatric Sedation team at Indiana University North Campus. Patients <25kg received 1mg/kg of IV fentanyl and for patients ≥25kg, a dose of 0.75mg/kg up to 50mg maximum was given. Patients received fentanyl 10 minutes prior to tracer injection and vitals were recorded along with times of fentanyl injection, 18-FDG tracer injection, and through patient recovery.

Results: A total of 9 unique pediatric lymphoma (6 Hodgkin's Lymphoma, 1 Burkitt's Lymphoma, 2 Primary Mediastinal Large B-Cell Lymphoma) patients aged 13-18 years old (median 14 years) were eligible to receive fentanyl pre-treatment. 1 patient was deemed ineligible by Sedation Team secondary to superior vena cava syndrome. A total of 10 scans on 8 unique pediatric were performed with fentanyl. Fentanyl was given 10-18 minutes prior to tracer (mean 11.9 min). Patients had no serious adverse events during or following fentanyl intervention. 1 patient reported dizziness after fentanyl injection that resolved after 5 minutes, with normal vital signs during the event. No residual fentanyl sedative effects were observed prior to patients proceeding into the PET scanner. No patients required additional recovery time after the scan. In the group receiving fentanyl, 90% of patients had no reportable brown fat uptake. The brown fat that was observed was present in cervical and thoracis areas and was not seen on a subsequent scan later in the patient therapy that was also done with fentanyl pre-treatment.

Conclusion: In this small cohort, fentanyl was found to be safe and feasible as a pre-treatment agent to mitigate brown fat interference on PET scan. We observed decreased uptake of tracer by brown fat in pediatric lymphoma patient that were similar to previously reported data on fentanyl. There were no serious adverse events and no increased time added to their visit. Large multi-center, randomized control trials are warranted to investigate fentanyl pre-treatment efficacy compared to other agents currently in use.

Translational/Clinical Research ***Post-Doctoral/Medical Fellow***

POSTER #90

PRE-TRANSPLANT M PROTEIN LEVEL PREDICTS MRD STATUS AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA

Zachary Meyer[2], Mohamed Manaa[2], Yan Han[1,2], Magdalena Czader[2], Attaya Suvannasankha[2,3], Rafat Abonour[2,4], Sherif Farag[2], Melissa Stanley[2], Mohammad Abu Zaid[2,4]

[1] *Biostatistics And Health Data Science, Indianapolis, IN*
[2] *Indiana University School Of Medicine*
[3] *Richard L. Roudebush VAMC*
[4] *Indiana University Simon Cancer Center*

Email: *zameyer@iu.edu*

Introduction: Autologous stem cell transplantation has been a staple treatment modality in patients with multiple myeloma for more than 30 years. Multiple studies have shown increased survival among patients who undergo transplant compared to those who receive chemotherapy alone. Despite the efficacy associated with transplant among populations in totality, individual response to therapy is variable. Recent studies have demonstrated that achieving minimal residual disease (MRD) negativity is associated with increased survival in multiple myeloma. In this study, we performed a retrospective analysis on patients with multiple myeloma who underwent autologous stem cell transplantation and investigated potential markers to predict post-transplant MRD status.

Methods: Patients with multiple myeloma that underwent treatment with high-dose melphalan followed by autologous stem cell transplantation at the Indiana University Simon Cancer Center between 2019-2020 were included in the analysis. Patient demographics, disease characteristics, pre-transplant and post-transplant laboratory values, and approximately day +100 post-transplant bone marrow sample results were collected. MRD analysis on post-transplant bone marrow aspirations was performed using an 8 color flow cytometry panel with 10 markers. The limits of quantification and detection were calculated at 5X10-6 and 2X10-6. MRD negativity was defined as having no identifiable M protein via post-transplant serum protein electrophoresis (SPEP) or immunofixation electrophoresis (IFE) and having negative MRD on post-transplant bone marrow testing. Univariate logistic regression was performed to assess the association of pre-transplant variables with post-transplant MRD status. Multivariate logistic regression model was utilized to analyze markers with a p-value <0.25 in univariate analysis.

Results: 133 Patients were included in the analysis with average age at transplant being 60.84 years (range 32.18 years-78.13 years). 83/133 (62.41%) patients were male and 118/133 (88.72%) patients were white. 84/133 (63.16%) patients had achieved a VGPR or less according to the International Myeloma Working Group response criteria prior to transplant. Among all patients, age at transplant, gender, race, body mass index, glomerular filtration rate on day -1, serum albumin on day -1, kappa/lambda ratio on day -1, melphalan dose received, and immunoglobulin subtype were not associated with response to therapy. Pre-transplant M protein positivity was associated with a higher likelihood of post-transplant MRD positive status with an odds ratio of 24.32 ($p<0.0001$). When restricting analysis to include only patients at VGPR status or less prior to transplant, pre-transplant M-protein positivity and increased age at transplant were associated with increased likelihood of MRD positive status with odd ratios of 9.00 ($p=0.0121$) and 1.066 ($p=0.0366$) respectively.

Conclusions: Detectable levels of pre-transplant M protein via serum protein electrophoresis is associated with an increased likelihood of having positive MRD following autologous stem cell transplantation in multiple myeloma. Increased age at transplant may be associated with inferior outcomes in patients achieving a VGPR or less prior to transplantation.

Translational/Clinical Research ***Post-Doctoral/Medical Fellow***

POSTER #91

IDENTIFYING PEDIATRIC HEMATOLOGY/ONCOLOGY PHYSICIAN AND NURSE PRACTITIONER BARRIERS TO HPV VACCINATION

Meagan Miller[1], Scott Coven[1], Mahvish Rahim[2], Seethal Jacob[1], Gregory Zimet[3], Carolyn Meagher[3], Gabi Lozano[4], Mary Ott[5]

[1] *Indiana University School Of Medicine, Indianapolis, IN*

[2] *Indiana University School Of Medicine, Indianapolis , IN*

[3] *Division Of Adolescent Medicine, Dept. Of Pediatrics, Indiana University School Of Medicine, Indianapolis, IN*

[4] *IUPUI, Indianapolis, IN*

[5] *Division Of Adolescent Medicine, Dept. Of Pediatrics, IU Center For Bioethics, Indiana University School Of Medicine, Indianapolis, IN*

Email: *meaemill@iu.edu*

Background: Vaccination against human papilloma virus (HPV) is the most important preventative measure to protect individuals against the development of HPV related cancers. Notably, data suggests that pediatric cancer survivors may be more likely to develop a second primary HPV-related cancer than the general population. Despite this risk, a retrospective chart review of patients 11-26 years of age at Riley Hospital for Children's sickle cell disease (SCD) and oncology survivorship clinics showed suboptimal rates of HPV vaccination, with only 47% and 42% of patients up to date in SCD and survivor clinics, respectively. In community settings, a strong provider recommendation has been shown to increase vaccination rates; therefore, we sought to understand pediatric hematology/oncology physician and nurse practitioner views and experiences with the HPV vaccine.

Methods: We completed 20 semi-structured qualitative interviews with 18 pediatric hematology/oncology physicians and 2 nurse practitioners. Interviews were subsequently transcribed and analyzed via thematic content analysis. The interviews assessed providers' experiences, approaches, attitudes, views, and barriers related to HPV vaccination.

Results: Participants identified barriers at the provider, clinic, and institutional levels. The most common identified provider specific barrier to HPV vaccination was lack of continuing education on this subject. Additionally, most subspecialty providers believe that HPV vaccination should fall under the purview of the PCP and HPV vaccine was not included in stem cell transplant revaccination protocols. Clinic level barriers included lack of time and flow constraints, as well as having the ancillary staff resources and trainings to administer the vaccine. Commonly cited institutional barriers to vaccination included a lack of access to HPV vaccine, capacity to store vaccines in the clinic, and access to vaccine records.

Conclusions: Provider-specific barriers can be improved through continuing education on how to effectively recommend HPV vaccination and through clinic-based policies promoting vaccination. Clinic and institutional barriers can be addressed by improving accessibility of the vaccine in clinic and refining the workflow to administer the vaccine in an efficient manner. Resolving these barriers is of the upmost importance to help increase vaccination rates in this patient population.

Translational/Clinical Research ***Post-Doctoral/Medical Fellow***

POSTER #92

ANALYTICAL VALIDATION OF A COMPUTATIONAL METHOD FOR PHARMACOGENOMICS GENOTYPING FROM CLINICAL WHOLE EXOME SEQUENCING

Tyler Shugg[1], Reynold Ly[2], Ryan Ratcliff[6], Victoria Pratt[3], Bryan Schneider[4], Milan Radovich[4], Steven Bray[6], Benjamin Salisbury[6], Baiju Parikh[6], S. Cenk Sahinalp[7], Ibrahim Numanagić[5], Todd Skaar[1]

[1] *Division Of Clinical Pharmacology , Indiana University School Of Medicine, Indianapolis, IN*

[2] *Division Of Clinical Pharmacology , Department Of Medical And Molecular Genetics, Indiana University School Of Medicine, Indianapolis, IN*

[3] *Department Of Medical And Molecular Genetics, Indiana University School Of Medicine, Indianapolis, IN*

[4] *Division Of Hematology/Oncology, Indiana University School Of Medicine, Indianapolis, IN*

[5] *University Of Victoria, Victoria, BC,*

[6] *LifeOmic Inc*

[7] *National Cancer Institute*

Email: *tshugg@iu.edu*

Background

Germline clinical whole-exome sequencing (WES), which is commonly generated in the clinical workflow of molecular tumor boards, has the potential to be repurposed to support clinical pharmacogenomics (PGx). However, accurately calling PGx alleles from WES remains challenging. In this study, we assessed the accuracy of Aldy, a computational tool to call PGx star alleles and diplotypes from WES, for 13 major PGx genes.

Methods

Germline DNA was obtained from whole blood and used for both CAP and CLIA-accredited WES using custom xGen target capture with Illumina NovaSeq 6000 and OpenArrays™ targeted genotyping for 75 subjects from the IU Health Precision Oncology Clinic. Aldy v3.3 was installed on the LifeOmic Precision Health Cloud™ platform to call PGx star alleles and diplotypes from WES. Aldy could not call copy number from WES, so Aldy was fixed to call two copies per gene. We compared Aldy results from WES with targeted genotyping for 57 star-defining variants within CYP2B6 (3), CYP2C8 (3), CYP2C9 (6), CYP2C19 (9), CYP2D6 (16), CYP3A4 (2), CYP3A5 (3), CYP4F2 (1), DPYD (4), G6PD (3), NUDT15 (3), SLCO1B1 (1), and TPMT (3), yielding a total of 3,737 variant genotypes and 899 diplotype calls.

Results

WES read depth was >100x for all variants except *CYP3A4* c.15389C>T *(*22;>30x). All 3,737 genotypes were concordant between Aldy and targeted genotyping. For the 752 diplotype calls containing only alleles assessed by both platforms, there was also 100% concordance. Aldy identified additional star alleles not covered by targeted genotyping for 147 diplotype calls within *CYP2B6* (28), *CYP2C9* (2), *CYP2C19* (1), *CYP2D6* (12), *CYP3A4* (2), *CYP4F2* (21), *DPYD* (59), *NUDT15* (2), *SLCO1B1* (19), and *TPMT* (1). Eleven diplotypes were excluded from the analysis due to copy number variation in *CYP2D6* that was identified by targeted genotyping. Two *CYP2D6* diplotypes were excluded from the analysis due to ambiguous phasing that prevented precise determination of the diplotype.

Conclusion

Aldy accurately called star alleles anddiplotypes for 13 major PGx genes from clinical WES, thus allowing repurposing of WES to support clinical PGx. A current limitation of Aldy, and other algorithmic-based methods to extract PGx information, is the inability to accurately determine changes in copy number from WES.

POSTER #93

SYNERGISTIC LETHALITY BETWEEN PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) INHIBITION AND B-LAPACHONE-INDUCED OXIDATIVE DNA DAMAGE IN NQO1-POSITIVE CANCER CELLS

Xiaolin Su[1,3], Jiangwei Wang[2,3], Xiumei Huang[2,3]

[1] *Biochemistry And Molecular Biology, Indiana University School Of Medicine, Indianapolis, IN*
[2] *Radiation Oncology, Indiana University School Of Medicine, Indianapolis, IN*
[3] *Simon Comprehensive Cancer Center*

Email: *xiasu@iu.edu*

β-Lapachone is a classic quinone-containing antitumor NQO1-bioactivatable drug that directly kills NQO1-overexpressing cancer cells. However, the clinical applications of β-lapachone are primarily limited by its high toxicity and modest lethality. To overcome this side effect and expand the therapeutic utility of β-lapachone, we demonstrate the effects of a novel combination therapy including β-lapachone and the proliferating cell nuclear antigen (PCNA) inhibitor T2 amino alcohol (T2AA) on various *NQO1*$^+$ cancer cells. PCNA has DNA clamp processivity activity mediated by encircling double-stranded DNA to recruit proteins involved in DNA replication and DNA repair. In this study, we found that compared to monotherapy, a nontoxic dose of the T2AA synergized with a sublethal dose of β-lapachone in an NQO1-dependent manner and that combination therapy prevented DNA repair, increased double-strand break (DSB) formation and induced catastrophic energy loss. We further determined that T2AA promoted programmed necrosis and G1 phase cell cycle arrest in β-lapachone-treated *NQO1*$^+$ cancer cells. Our findings show novel evidence for a new therapeutic approach that combines of β-lapachone treatment with PCNA inhibition that is highly effective in treating *NQO1*$^+$ solid tumor cells.

Translational/Clinical Research ***Post-Doctoral/Medical Fellow***

POSTER #94

TARGETING BET PROTEINS IN COMBINATION WITH SALVAGE CHEMOTHERAPY FOR RELAPSED PEDIATRIC ALVEOLAR RHABDOMYOSARCOMA

Sarah Supples[1], Pankita Pandya[1,2], Barbara Bailey[2], Harlan Shannon[2], Anthony Sinn[3], Melissa Trowbridge[3], Kathryn Coy[3], Courtney Young[2], Erika Dobrota[2], M. Reza Saadatzadeh[1,2], Khadijeh Bijangi-Vishehsaraei[1,2], Jamie Renbarger[1,2,4], Michael Ferguson[1], Karen Pollok[1,2]

[1] *Department Of Pediatrics, Hematology/Oncology, IU School Of Medicine, Indianapolis, IN*
[2] *Herman B. Wells Center For Pediatric Research, IU School Of Medicine*
[3] *Indiana University Melvin And Bren Simon Comprehensive Cancer Center, In Vivo Therapeutics Core*
[4] *Indiana Institute Of Personalized Medicine*

Email: *ssupples@iu.edu*

Outcomes for children with relapsed alveolar rhabdomyosarcoma (ARMS) remain poor with survival rates below 20% with current salvage therapy regimens. More effective therapies are urgently needed. The majority of ARMS tumors harbor the aberrant transcriptional fusion protein PAX3-FOXO1 (fusion-positive ARMS), which confers a particularly poor prognosis. PAX3-FOXO1 mediates the expression of downstream target genes including *IGF1R, MET, FGFR4, ALK, MYOD1,* and *MYCN*. BRD4, a member of the BET protein family of epigenetic readers, directly interacts with PAX3-FOXO1 at enhancer regions and serves as a critical co-activator of target gene transcription. Treatment with small molecule BET inhibitors (BETi), results in loss of this interaction and fusion protein degradation, as well as ARMS tumor suppression both *in vitro* and *in vivo*, rationalizing BET proteins as a therapeutic target in ARMS. However, reports of adaptive resistance mechanisms to BETi monotherapy are emerging and anti-tumor effects in clinical trials are short-lived, suggesting that combination therapy will be required to successfully treat ARMS. To that end, topotecan, a well-established agent used in ARMS salvage therapy, was selected for combination with the highly potent, bivalent BRD4-specific BETi AZD5153. Topotecan is a cytotoxic chemotherapy that inhibits topoisomerase I (TOP1) inducing double-strand DNA breaks and subsequent cell death. BRD4 also plays a role in repair of double-strand breaks, further rationalizing this combinatorial approach. We *hypothesize* that targeting BET proteins with the BETi AZD5153 in combination with the salvage chemotherapy topotecan will serve as an efficacious and well-tolerated therapeutic option in preclinical models of relapsed ARMS. Our preliminary data in ARMS cell lines (Rh30 and Rh41) treated with AZD5153 plus topotecan demonstrate additive to synergistic growth inhibition. We have developed two relapsed ARMS patient-derived xenograft (PDX) models from patients at Riley Hospital. Thus far, dose-finding studies in these models indicated inhibition of tumor growth at clinically relevant doses of AZD5153 and topotecan as single agents. Upcoming experiments will evaluate AZD5153 plus topotecan in combination in our PDX models, and will further investigate the BET/BRD4-dependent anti-tumor mechanisms in ARMS. Information gained in this project will be highly clinically relevant, and should provide preclinical rationale for clinical trials to ultimately improve outcomes for patients with aggressive ARMS.

Translational/Clinical Research ***Post-Doctoral/Medical Fellow***

POSTER #95

DUAL TARGETING OF MYC AND HSF1 DECREASES OVARIAN CANCER TUMOR GROWTH

Imade Williams[1], Bobby Walker[1], Kenneth P. Nephew[2], Richard Carpenter[3]

[1] *Medical Sciences, Indiana University School Of Medicine, Bloomington, IN*

[2] *Medical Sciences, Melvin And Bren Simon Cancer Center, Indianapolis, IN, Department Of Anatomy, Cell Biology And Physiology, Indiana University School Of Medicine, Bloomington, IN, Indiana University School Of Medicine, Indianapolis, IN*

[3] *Medical Sciences, Melvin And Bren Simon Cancer Center, Indianapolis, IN, Department Of Biochemistry And Molecular Biology, Indiana University School Of Medicine, Bloomington, IN, Indiana University School Of Medicine, Indianapolis, IN*

Email: *iimasuen@iu.edu*

Ovarian cancer (OC) has the highest case-to-fatality ratio of all gynecologic malignancies and is the fifth leading cause of mortality in women in the United States. High grade serous ovarian carcinoma (HGSOC), the most common OC epithelial subtype, is a highly aggressive disease characterized by a high frequency of *TP53* driver mutations (50%), genomic instability, and rapid tumor growth with a propensity for widespread peritoneal metastasis and marked chemoresistance. A dismal 20% survival rate for women diagnosed with advanced disease underscores an urgent need to devise novel treatment strategies. The MYC proto-oncogene is amplified in > 60% of HGSOC. In addition to MYC, the gene for heat shock factor 1 (*HSF1*), a master regulator of the heat shock response, is reported to have pro-tumor functions in several cancer types, including OC. In our preliminary studies using data from The Cancer Genome Atlas (TCGA), we have found that HSF1 is amplified in 40% of OC and that 36% (110/302) of OC patients have both *MYC* and *HSF1* amplifications (χ^2=165.8, p<0.0001). We have also observed that the transcriptional activity of MYC and HSF1 are highly correlated in OC patients (r=0.53, p<0.0001), that MYC and active HSF1 protein levels are highly correlated in OC cell lines (r=0.82, p=0.0019), and that patients with *MYC* and *HSF1* co-amplification have worse overall and recurrence-free survival independent of age (multivariable HR=1.549, p=0.013) leading us to **hypothesize** that that MYC and HSF1 cooperate to promote OC tumor growth and progression. Considering there are currently no viable therapeutics targeting MYC or HSF1, we examined potential therapeutic targets in the context of *MYC* and *HSF1* co-amplification. Polo-like kinase 1 (PLK1), which normally regulates mitotic entry, has previously been shown to directly phosphorylate and enhance the activity of both MYC and HSF1. BI-6727 is a highly selective PLK1 inhibitor that has been well-tolerated in clinical trials. Using cell viability, two-dimensional growth, and a three-dimensional spheroid assay we report that *MYC-HSF1* co-amplified ovarian cancer cells had increased sensitivity to BI-6727 compared to non-amplified cells. In a subcutaneous nude mouse model of ovarian cancer, we also observed a significant decrease in *MYC-HSF1* co-amplified OVCAR8 tumor volume after 20mg/kg BI-6727 treatment compared to vehicle control as early as 14 days. In future studies we will assess the effect of BI-6727 alone and in combination with carboplatin on the ovarian cancer stem cell and tumor growth *in vivo.* In addition to interrogating a promising and viable treatment option for a subset of OC patients with *MYC-HSF1* amplification, these results support a role for inhibiting PLK1 in OC patients with *MYC-HSF1* co-amplified tumors.

Translational/Clinical Research ***Post-Doctoral/Medical Fellow***

POSTER #96

PASSAGE NUMBER AFFECTS DIFFERENTIATION EFFICIENCY OF SENSORY NEURONS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS GENERATED FROM PERIPHERAL BLOOD

Erica Cantor[1], Fei Shen[1], Guanglong Jiang[2], Geneva Cunningham[2], Xi Wu[1], Santosh Philips[3], Bryan Schneider[1]

[1] *Hematology/Oncology, School Of Medicine, Indiana University, Indianapolis, IN*
[2] *Medical & Molecular Genetics, School Of Medicine, Indiana University, Indianapolis, IN*
[3] *Clinical Pharmacology, School Of Medicine, Indiana University, Indianapolis, IN*

Email: *ericant@iu.edu*

Human induced pluripotent stem cells (iPSCs) are a valuable resource for neurological disease-modeling and drug discovery, due to their ability to differentiate into neurons reflecting the genetics of the patient from which they are derived. Thus far, neuroscience research has been mostly conducted using poorly translatable animal models and immortalized cell lines due to the limited availability of viable human nerve tissue. iPSC-derived cultures, however, are highly variable due to differences in culture conditions, such as starting iPSC passage number, i.e., the "age" of an iPSC line. In this study, we investigated the correlation of iPSC passage number with differentiation efficiency to optimize the generation of functional, mature peripheral sensory neurons.

Three iPSC lines were differentiated into sensory neurons (iPSC-dSNs) at each of three different passage numbers within the following ranges: low (LP; 5-10), middle (MP; 20-26), and high (HP; 30-38). The morphology and pluripotency of the iPSCs, as well as the maturity and functionality of the differentiated iPSC-dSNs, were compared across passage numbers. Immunofluorescent staining for neuronal markers peripherin and βIII-tubulin was performed. The expression of pluripotency factors Sox2, Oct3/4, and Nanog was assessed by flow cytometry and RNA-sequencing (RNA-seq). Expression of immature neuron markers *NTRK1* and *PAX6*, mature neuron markers *RUNX1* and *PIEZO2*, and sensory neuron markers *TRPM8, CALCA, POU4F3, HCN1, SCN9A,* and *NEFH* was assessed by RNA-seq. Finally, the functional maturity of the iPSC-dSNs was also assessed based on electrophysiological properties.

All parent iPSC lines at each passage number displayed the same expected morphology and there were no statistically significant differences in the pluripotency of these lines, suggesting that passage number does not affect the quality of the starting iPSCs. The differentiated iPSC-dSNs were also morphologically comparable across passage numbers There were no obvious differences in staining pattern or intensity of βIII-tubulin or peripherin, and no statistically significant differences in the expression of *TUBB3* (βIII-tubulin) or *PRPH* (peripherin), except for a significantly higher level of *TUBB3* in the HP iPSC-dSNs. However, compared to MP and HP iPSC-dSNs, LP iPSC-dSNs exhibited significantly higher expression of *NTRK1* at day 3 post-induction, significantly lower expression of both *NTRK1* and *PAX6* at day 33 post-induction, and significantly higher expression of the mature and sensory neuron markers listed above at day 33. LP iPSC-dSNs also demonstrated significantly higher functionality based on electrophysiology data. These data indicate that LP iPSC-dSNs achieved significantly greater maturity and better recapitulated the desired peripheral sensory neuron phenotype.

In conclusion, lower passage numbers may be better suited for differentiation into peripheral sensory neurons. Further studies are warranted to elucidate factors contributing to this variability associated with iPSC passage number, such as genomic stability and epigenetic memory, which may help to improve the reliability and consistency of iPSC-derived models.

Translational/Clinical Research ***Research Technician***

POSTER #97

METHADONE HYDROCHLORIDE BLOCKS EPINEPHRINE-INDUCED PROLIFERATION AND ENHANCES APOPTOTIC EFFECTS OF NAB-PACLITAXEL IN ESOPHAGEAL ADENOCARCINOMA

Nicholas Cwidak[1], Md Sazzad Hassan[1], Chloe Johnson[2], Saisantosh Ponna[2], Niranjan Awasthi[1], Urs Von Holzen[1,3]

[1] *Surgery, Indiana University School Of Medicine - South Bend*

[2] *University Of Notre Dame, Notre Dame, IN*

[3] *Goshen Center For Cancer Care*

Email: *ncwidak@iu.edu*

Introduction: Esophageal adenocarcinoma (EAC) is an aggressive form of cancer with 5-year survival rates under 20%. The low survival rate is largely due to advanced stage detection upon diagnosis. Improved treatment options remain essential in combating the disease and bolstering patient outcomes. Nanoparticle albumin bound paclitaxel (nab-paclitaxel) is a next-generation taxane receiving attention for its potential in EAC treatment. Nab-paclitaxel has exhibited greater specificity for targeting cancer cells while decreasing cytotoxic effects to adjacent healthy populations. Unfortunately, chemoresistance remains an issue surrounding adequate treatment regimes. Epinephrine and its receptor, β2 adrenergic, have been shown to dysregulate various oncogenic pathways and reduce chemotherapeutics efficacy. Typically associated with pain management and addiction treatment, methadone hydrochloride has been investigated for its potential to block the oncogenic effects of epinephrine and strengthen the impact of chemotherapeutics. In this study, we evaluated the enhancement of apoptosis by the combination of nab-paclitaxel and methadone, and the ability of methadone to block epinephrine-induced chemoresistance.

Methods: Immunoblot analyses were performed to elucidate EAC cell lines with the greatest expression of Mu opioid and β2 adrenergic receptors. WST-1 assays were utilized to investigate adequate dosages of methadone and epinephrine for the EAC cell lines and to determine cell growth under co-administration of nab-paclitaxel and methadone as well as methadone and epinephrine. Immunoblotting was performed to assess the apoptotic effect of single drug and combination treatments by cleaved caspase-3 and cleaved poly-ADP ribose polymerase (PARP). We also investigated anti-proliferative effects by evaluating phosphorylation levels of extracellular signal-regulated kinase (ERK) and cAMP-response element binding protein (CREB) via immunoblotting. Propidium iodide cell viability flow cytometry assay was also used to detect apoptosis.

Results: OE19 and OE33 cell lines showed increased expression of Mu and β2 adrenergic receptors, ensuring the cells were capable of binding to methadone or epinephrine. WST-1 assays established 100 nM of epinephrine and 200 nM of methadone as optimal dosages for both cell lines. Our previous studies demonstrated that about 5.85 mM of nab-paclitaxel (5mg/mL) induces apoptosis in OE19 and OE33. Immunoblotting showed enhanced expression of cleaved caspase-3 and cleaved PARP under co-administration of nab-paclitaxel and methadone as well as decreased expression under epinephrine and methadone treatment. Furthermore, immunoblotting revealed decreased phosphorylation of CREB and ERK under combination treatment of epinephrine and methadone compared to single-dose epinephrine. Propidium iodide staining verified the enhancement of apoptosis under combination treatment of nab-paclitaxel and methadone.

Conclusions: The current study confirmed the efficacy of NPT for the treatment of EAC, demonstrated methadone's ability to block epinephrine-induced proliferation and showed an enhanced effect on apoptosis when combining nab-paclitaxel with methadone in EAC treatment.

Translational/Clinical Research ***Research Technician***

POSTER #98

INVESTIGATING THE EFFECTS OF ARSENIC EXPOSURE ON HUMAN BLADDER CELLS AND THE MECHANISMS OF ARSENIC-INDUCED BLADDER CANCER

Haimanti Ray[1], Richard Carpenter[1,2]

[1] *Medical Sciences Program, School Of Medicine, Indiana University, Bloomington, IN*
[2] *Department Of Biochemistry And Molecular Biology*

Email: *rayh@iu.edu*

Arsenic occurs naturally in various forms and can be found in soil, groundwater, air, plants, and animals. Humans are often exposed to arsenic through the food, water, and the air we consume and use. Exposure to arsenic has been linked to several cancers, especially lung, skin, and bladder cancer. Although arsenic is considered carcinogenic to humans with regards to these cancer types, there is a lack of research investigating the mechanisms by which arsenic leads to cancer, particularly in bladder cancer. We found that the transcription factor HSF1 (Heat Shock Factor 1) is activated in response to acute arsenic exposure in human bladder epithelial cells. HSF1 is the master regulator of the heat shock response and helps cells survive in the presence of external stressors. Thus, we hypothesize that HSF1 might be involved in the survival of bladder cells exposed to arsenic. Arsenic exposure can induce DNA alterations and the production of reactive oxygen species (ROS). Increased HSF1 activity may increase the survival of cells with DNA alterations from arsenic exposure and thus may lead to the development of cancer. We will test our hypothesis by examining DNA alterations, such as aneuploidy and DNA damage, in response to arsenic exposure as well as how these effects change in the absence of HSF1 and scavenging of ROS. We will also study the characteristics of arsenic-induced bladder cancer by exposing human bladder cells to arsenic over a period of 26 weeks at doses that correspond to the urine arsenic levels of populations in arsenic-exposed areas around the world. These arsenic-exposed bladder cells have shown evidence of transformation through increased proliferation and clonogenic growth compared to passage-matched control cells. We will use DNA and RNA sequencing of these transformed bladder cells to identify unique patterns of mutations and expression in response to chronic arsenic exposure. These experiments and models will allow us to investigate the mechanisms and potentially the therapeutic targets relevant to arsenic-induced bladder cancer.

Translational/Clinical Research ***Research Technician***

POSTER #99

RHENIUM COMPOUNDS AS ANTICANCER THERAPEUTICS FOR HIGH GRADE SEROUS OVARIAN CANCER

Aneesha Anand[1], Subramanyam Dasari[1], Santosh K Mandal[2], Anirban K Mitra[1]

[1] *Indiana University School Of Medicine-Bloomington, Bloomington, IN*
[2] *Department Of Chemistry, Morgan State University, Baltimore, MD*

Email: *ananan@iu.edu*

Abstract

Ovarian cancer is the fifth leading cause of cancer related deaths among women in the United States and the most fatal gynecologic cancer. The 5-year survival rate for epithelial ovarian cancer is 48% and among them, high grade serous ovarian cancer (HGSOC) is the most prevalent and lethal subtype. The standard of care for ovarian cancer is a combination of cytoreductive surgery and carbo-taxol chemotherapy. The reason for the poor outcome is extensive metastasis and disease relapse. To overcome the clinical limitations of platinum-based anticancer drugs, several rhenium (Re) compounds with potential anticancer properties have been synthesized in recent years. Many of these Re complexes/compounds display promising cytotoxic properties against various cancer cells including brain cancer and breast cancer cells. In the present study, we report our investigation of the anticancer activity of these Re compounds against HGSOC cells. Initially, we screened the anti-proliferative activity of 5 Re compounds (PR7, NIC7, IBU6, TOS7 and INS7) in OVCAR4, OVCAR8 and Kuramochi HGSOC cells. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was done using a range of doses of the Re compounds in these HGSOC cells to determine their IC_{50} dose. The IC_{50} values of the compounds ranged between 792.1nM and 7370nM, with PR7 being the most effective across all cells. It was found to have an IC_{50} of 792.1nM in OVCAR4, 2088nM in OVCAR8, and 946.1nM in Kuramochi. Subsequently, PR7 was chosen to determine the mechanism of its anti-cancer activity. Treatment with PR7 was found to induce apoptosis in the HGSOC cells. This was determined by western blotting for caspase 3 and PARP cleavage using lysates of the HGSOC cells treated with PR7 compared to vehicle control. We will also determine its effects on cell cycle and the potential of combination therapy with carboplatin. Our long-term goal is to test its efficacy as an adjuvant therapy for HGSOC.

Translational/Clinical Research ***Undergraduate Student***

POSTER #100

BIOSPECIMEN COLLECTION AND BIOBANKING CORE (BC-SQUARED)

Anna Maria Storniolo[1]

[1] *IU Simon Comprehensive Cancer Center, Indianapolis, IN*

Email: *astornio@iu.edu*

POSTER #101

GENOMICS CORE

Yunlong Liu[1]

[1] *IU Simon Comprehensive Cancer Center, Indianapolis, IN*

Email: *yunliu@iu.edu*

POSTER #102

CLINICAL PHARMACOLOGY ANALYTICAL CORE

Andi Masters[1]

[1] *IU Simon Comprehensive Cancer Center, Indianapolis, IN*

Email: *argrove@iupui.edu*

POSTER #103

PRECLINICAL MODELING AND THERAPEUTICS CORE (PMTC)

Tony Sinn[1]

[1] *IU Simon Comprehensive Cancer Center, Indianapolis, IN*

Email: *alsinn@iupui.edu*

POSTER #104

BIOINFORMATICS CORE

Jun Wan[1]

[1] *IU Simon Comprehensive Cancer Center, Indianapolis, IN*

Email: *junwan@iu.edu*

POSTER #105

FLOW CYTOMETRY CORE

Andy Canciamille[1]

[1] *IU Simon Comprehensive Cancer Center, Indianapolis, IN*

Email: *acancia@iu.edu*

POSTER #106

HYPOXIA-MEDICATED SUPPRESSION OF PYRUVATE CARBOXYLASE CREATES AN IMMUNOSUPPRESSIVE TUMOR MICROENVIRONMENT

Eylem Kulkoyluoglu Cotul[1], Michael Francis Coleman [2], Violet Abigail Kiesel[3], Muhammad Hassan Safdar[1], Dorothy Teegarden[4], Stephen David Hursting [3,5], Michael K. Wendt[1,6]

Medicinal Chemistry And Molecular Pharmacology, West Lafayette, IN[2]
Department Of Nutrition, Chapel Hill, NC[3]
Department Of Nutrition, North Carolina, NC[4]
Department Of Nutritional Sciences, West Lafayette, IN[5]
Lineberger Comprehensive Cancer Center, Nutrition Research Institute, North Carolina, NC[6]
Purdue University Center For Cancer Research, West Lafayette, IN

Email: ***ekulkoyl@purdue.edu***

Based on the seed and soil hypothesis, the tumor microenvironment has a pivotal role to determine the metastatic fate of breast cancers. Metabolic adaptation can be dramatic for breast-originated lung metastases due to differences in oxygen and nutrient availability. Hypoxia is a selective low-oxygen condition in the tumor microenvironment that enables aggressive cancer cells to survive and expand their colonies. Patients with hypoxic tumors have a poor prognosis and increased likelihood of metastasis. They also experience resistance to both chemotherapy and radiotherapy. Studies have shown that hypoxia supports cancer growth by using different mechanisms, such as transforming fibroblasts into tumor-prone cancer-associated fibroblasts (CAFs), remodeling extracellular matrix, and vascularization elements favoring metastases, and attenuating anti-tumor immune function. Although a lot is known about hypoxia, the mechanistic link between hypoxia and metabolic reprograming is still not well understood.

In this study, we focused on a gluconeogenic enzyme called pyruvate carboxylase (PC) which converts pyruvate into oxaloacetate. Previously, we have shown that PC expression is higher in oxygen-rich lung metastases compared to hypoxic primary breast tumors. Thus, herein we utilized RT-PCR, immunoblot, and reporter assays to demonstrate that PC expression is inhibited by hypoxia. To recapitulate loss of PC in a hypoxic TME we utilized an shRNA approach. Metabolomic characterization of these cells using mass spectroscopy demonstrated that depletion of PC caused cells to produce more lactate compared to control cells. Importantly, this directly correlated with the amount of CD4 and CD8 positive cells in the tumor microenvironment, leading to a more immune suppressed TME. As a result depletion of PC increases primary tumor growth, a result that can be reversed by inhibition of the lactate transporter MCT1. Together with our previous studies, these results demonstrate the importance of PC in regulating immune suppression in hypoxic tumor microenvironments. In addition, our studies suggest a novel treatment strategy to overcome metabolic plasticity.

Keywords: Metabolic reprogramming, metastatic breast cancer (MBC), pyruvate carboxylase (PC)

Basic Science ***Post-Doctoral/Medical Fellow***

POSTER #107

MIMICKING TUMOR MICROENIVIRONMENT OF PANCREATIC DUCTAL ADENOCARCINOMA THROUGH CLICK CHEMISTRIES

Chun-Yi Chang[1], Chien-Chi Lin[2]

Weldon School Of Biomedical Engineering, Purdue University, Indianapolis, IN
Department Of Biomedical Engineering, Purdue School Of Engineering & Technology, Indiana University-Purdue University Indianapolis, Indianapolis, IN

Email: ***chang676@purdue.edu***

The tumor microenvironment (TME) of pancreatic ductal adenocarcinoma (PDAC) is characterized by a fibrotic stroma and elevated stiffness over time. The dynamic changes in matrix properties affect cancer cell migration (e.g., durotaxis and chemotaxis). However, it is still unclear how the interplay between stiffness heterogeneity and chemokine/growth factor gradient govern the migration and invasion of pancreatic cancer cells. To understand how cancer cells respond to the stiffness increase in the TME, we have previously developed a gelatin-based hydrogel. Gelatin was modified with click chemistry 'handles' - norbornene and hydrazide - that enables gelation and dynamic stiffening of the gel matrix, respectively. As shown in our previous research, oxidized dextran (oDex) was an effective stiffening reagent, thus it was utilized for creating stiffness gradient in the modified gelatin hydrogel to mimic the heterogeneous stiffness of the TME. On the other hand, heparin, a sulfated glycosaminoglycan that sequestrates growth factors/cytokines, was used to create a stable gradient of growth factor/cytokine. Our results show that oDex was able to diffuse into the hydrogel matrix and react with the chemical motif, thus creating a stiffness gradient. Similarly, heparin gradient was created in the same manner, as indicated by the fluorescence intensity of the fluorescent dye-labeled heparin, and dimethylmethylene blue staining. Future direction includes establishment of growth factor/cytokine gradient and evaluate the influence of multi-physicochemical gradients on PDAC cell migration.

Translational/Clinical Research ***Graduate Student***

POSTER #108

Characterizing Intra-Tumoral Heterogeneity Of Human Brain Tumors Using Multimodal And Multiparametric In Vivo Imaging

Mahsa Servati[1], Emily E Diller[2], Jason G Parker[3]

Indiana University, School Of Medicine, Department Of Radiology And Imaging Sciences, Purdue University, School Of Health Sciences, INDIANAPOLIS, IN
Indiana University, School Of Medicine, Department Of Radiology And Imaging Sciences, Indianapolis, IN
Indiana University, School Of Medicine, Department Of Radiology And Imaging Sciences, Purdue University, School Of Health Sciences, Indianapolis, IN

Email: ***mservati@iu.edu***

Human brain tumors exhibit non-uniform somatic and genomic composition across space and time. This intra-tumoral heterogeneity (ITH) enables treatment resistance and disease recurrence, leading to the dismal prognosis and low survival rates of high grade brain tumors. The conventional diagnostic work-up for human brain tumors uses pre-surgical imaging and biopsy to assess cellular and molecular properties so that subsequent chemotherapy and radiation treatments can be optimized for patient-specific mutation profiles. However, these tools cannot identify all of the various somatic and genomic mutations present within a tumor. We hypothesize that cellular and molecular ITH can be described by mapping multimodal and multiparametric *in vivo* imaging signatures to underlying somatic and genomic aberrations. In this project, we use multiparametric magnetic resonance imaging (MRI) and perfusion and amino acid positron emission tomography (PET) to scan treatment-naïve patients with radiologic-confirmed WHO grade II-IV glioma. A minimum of two tumor samples will be collected from each subject using a stereotactic core biopsy for accurate spatial registration with the pre-surgical image datasets. All collected tumor tissues then undergo whole exome sequencing (WES), array methylation, and RNA sequencing to extract genome-wide association information. The genomic features will be co-registered to pre-surgical MR and PET images, and evaluated using logistic regression, generalized linear models, and non-parametric machine learning. The result will be a generalized patient-independent model linking cross-modality voxel intensity patterns to cellular and molecular information, addressing ITH within the tumor. This clinically validated model will impact the tumor-wide treatment response and improve overall clinical outcomes.

Translational/Clinical Research ***Graduate Student***