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POSTER #1

AUGMENTING NAB-PACLITAXEL CHEMOTHERAPY RESPONSE THROUGH TARGETED INHIBITION OF THE HGF/C-MET PATHWAY BY MERESTINIB IN PRECLINICAL MODELS OF GASTRIC CANCER

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Combination chemotherapy regimens are commonly used to treat gastric adenocarcinoma (GAC), but the median survival time remains less than one year. *Nab*-paclitaxel (NPT) has demonstrated high antitumor activity in preclinical GAC studies. Many growth factors and their receptors are overexpressed in GAC and have been implicated in its pathophysiology. Aberrant activation of the HGF/c-Met pathway has been reported in up to 50% of GAC patients. We hypothesize that merestinib (Mer), a novel small-molecule inhibitor of c-Met, with additional inhibition of Axl and DDR1/2 pathways, will enhance *nab*-paclitaxel chemotherapy response in GAC. Animal survival studies were performed in peritoneal dessimination xenografts using GAC MKN-45 cells. Tumor growth studies were performed in GAC subcutaneous xenografts using MKN-45 and SNU-1 cells in NOD/SCID mice. Immunohistochemistry analyses were performed using Ki67 and endomucin to examine tumor cell proliferation and microvessel density. Animal survival was 17 days in controls, 37 days in the *nab*-paclitaxel group (118% increase), 24 days in the merestinib group (41% increase), and 43 days in the combination group (153% increase), indicating an additive effect. In high c-Met expressing MKN-45 xenografts , tumor growth in control, *nab*-paclitaxel, merestinib, and combination was 503 mm^3, 115 mm^3, 91 mm^3, and -9.7 mm^3 (tumor regression). In low c-Met expressing SNU-1 xenografts, tumor growth in control, *nab*-paclitaxel, merestinib, and combination was 219 mm^3, 105 mm^3, 131 mm^3, and 57 mm^3. Immunohistochemistry analysis of tumor cell proliferation and microvessel density corroborated tumor growth study results. *Nab*-paclitaxel and merestinib decreased *in vitro* cell proliferation in GAC-associated cells, with an additive effect in combination. Reduction in cell proliferation by *nab*-paclitaxel (10 nM), merestinib (100 nM), and their combination was 87%, 82%, and 94% (MKN-45), 59%, 50%, and 82% (SNU-1), and 53%, 19%, and 66% in gastric fibroblasts. In MKN-45 cells, merestinib treatment increased expression of pro-apoptotic proteins and decreased expression of phospho-c-Met, phospho-EGFR, phospho-IGF-1R, phospho-ERK, and phospho-AKT. These findings suggest that merestinib has strong antitumor activity in GAC and exhibits an additive effect when administered with *nab*-paclitaxel. These results provide compelling evidence that this therapeutic approach might lead to a clinically relevant combination to improve GAC patients' survival.

Basic Science ***Faculty***

POSTER #2

THERAPEUTIC TARGETING OF HIF-1 ALPHA AND CD73 BY ACRIFLAVINE IN EXPERIMENTAL ESOPHAGEAL ADENOCARCINOMA

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Introduction: Esophageal adenocarcinoma (EAC) is one of the most aggressive human cancers with poor prognosis, and the overall 5-year survival rate is less than 20 percent. Prognosis for EAC remains poor even with modern combination therapies due to high resistance to chemotherapy. Therefore, new therapeutic approaches for EAC treatment improvements are urgently needed. Hypoxia or insufficient tissue oxygenation contributes to cancer aggressiveness and poor clinical prognosis. Overexpression of hypoxia-inducible factor 1-alpha (HIF-1 alpha) and immunosuppressive CD73, an ecto-5'-nucleotidase enzyme in cancer can give rise to tumor progression with drug resistance. CD73 has never been proposed as a therapeutic target in EAC and its relationship with hypoxia or HIF-1 alpha has not also been investigated in EAC. In this study, we therefore investigated the therapeutic targeting of HIF-1 alpha and CD73 by acriflavine in experimental EAC. **Methods:** Hypoxia in EAC cells were induced by 3D culture and hypoxic exposure. NanoCulture® plates and dishes were used for 3D cultures. For hypoxic exposure, cells were placed in a sealed modular incubator chamber flushed with a gas mixture containing 1% O2, 5% CO2 and 94% N2. Hypoxic status was detected by adding hypoxia probe LOX-1 and fluorescent microscopy. Nanoparticle albumin-bound paclitaxel (NPT) was used as chemotherapeutic agent, whereas acriflavine was used as hypoxia-targeting agent. In vitro cell growth was detected by WST-1 and Cell Titer-Glo (CTG) luminescent assays, in vivo tumor growth was detected by measuring subcutaneous xenografts, apoptosis was detected by cleaved caspase 3/PARP expressions and hypoxia-targeting was detected by HIF-1 alpha/CD73 expressions. **Results:** We observed overexpression of both HIF-1 alpha and CD73 in 3D culture and hypoxic exposure of EAC cells. Interestingly acriflavine treatment drastically inhibited both HIF-1 alpha and CD73 expression in EAC 3D culture and hypoxic exposure. 3D culture was more resistant to antiproliferative effect of chemotherapeutic agent NPT over 2D monolayer culture. Contrary to that, hypoxia-targeting agent acriflavine showed stronger antiproliferative effects in 3D culture than in 2D culture. We also observed hypoxia inside the 3D culture spheroids. In addition, acriflavine showed significant in vivo antitumor efficacy both as monotherapy and in combination with NPT. In subcutaneous xenografts using OE19 EAC cells, acriflavine monotherapy exhibited a significant decrease in relative tumor volume to 55.02% compared to control (p=0.04) and addition of NPT with acriflavine also showed a significant enhancement effect of tumor regression as tumor size decreased to 32.70% compared to control (p=0.002). **Conclusion:** These results support the potential of acriflavine as HIF-1 alpha and CD73 targeting and its combination with chemotherapy NPT as an effective option for EAC therapy.

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POSTER #3

THE PNPLA3 I148M VARIANT PROMOTES HEPATOCELLULAR CARCINOMA VIA ACTIVATION OF YAP/TAZ SIGNALING PATHWAYS

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Background & Aims: Patatin-like phospholipase domain–containing 3 (PNPLA3) in the genetic polymorphism of I148M is deeply related to various spectrum fatty liver diseases including steatosis, steatotic hepatitis and its progression stage of fibrosis, and cirrhosis. As an end stage of chronic liver diseases, hepatocellular carcinoma (HCC) is one of the serious medical issues globally, but there is no therapeutic available, and still uncovered an exact pathophysiological mechanism. Here we explored how the PNPLA3 I148M variant contributes to promoting HCC development.

Methods: Mice were fed 12 weeks of a 5% ethanol feeding diet with 10 weeks of CCl4 injection (0.32 μg/g for once a week) and mice were sacrificed then liver tissue and serum samples we examined. Primary hepatocytes were isolated by 2 weeks of ethanol-feeding diet mice.

Results: The PNPLA3 I148M variant promoted an early stage of HCC that was evidenced by enhancement of alpha-fetoprotein (AFP), arginase 1 (Arg1), CD44, and CD133, respectively. Interestingly, our data showed that HCC was developed without cirrhosis. We observed that the PNPLA3 148M group showed hepatic oxidative stress parameters including excessive generations of hepatocellular reactive oxygen species (ROS), overproduction of lipid peroxidation, and DNA fragments by the development of HCC. Regarding hepatic inflammation, we observed the hepatic resident macrophages, Kupffer cells, were markedly altered as M2 polarizations, which are associated with tumor associated macrophages (TAMs) through the liver tissues of the PNPLA3 148M group. To address the underlying mechanisms that how the variant PNPLA3 I148M affects to HCC development, we examined Yes associated protein (YAP)/WW domain containing transcription regulator 1 (WWTR also known as TAZ) signaling pathway is deeply implicated in our model. We further figured out the Wnt/β-Catenin pathway, especially by activation of non-canonical signaling pathway is activated in the PNPLA3148M mice liver tissue. These alterations are well supported by RhoA activities, lysophosphatidic acid (LPA) metabolism, and Autotaxin (ATX) expression in the liver tissue. Using primary hepatocyte and human hepatoma cell line (Huh7 cells) of in vitro assay also well supported how the PNPLA3I148M variant regulates the above alterations by regulation of YAP/TAZ-signaling pathways.

Conclusion: PNPLA3148M overexpression in the liver tissue promotes HCC development, particularly at the initial stage, by activation of the YAP/TAZ signaling pathway. This pathological alteration may attribute to evoking other molecular pathways such as partial activation of the Wnt/β-catenin signaling pathways, RhoA activities, lysophosphatidic acid (LPA) metabolism, and Autotaxin (ATX) expression in the liver tissue and in vitro experiment.

Key words: Patatin-like phospholipase domain–containing 3, alcoholic liver diseases, hepatocellular carcinoma, Yes-associated protein, WW domain containing transcription regulator 1

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POSTER #4

OBESITY INDUCED INFLAMMATION EXACERBATES CLONAL HEMATOPOIESIS

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Abstract

Characterized by the accumulation of somatic mutations in blood cell lineages, clonal hematopoiesis (CH) of indeterminate potential (CHIP) is frequent in ageing, involves expansion of mutated hematopoietic stem and progenitor cells (HSC/Ps) that leads to an increased risk of hematologic malignancy. However, risk factors that contribute to CHIP-associated CH are poorly understood. Obesity induces a pro-inflammatory state and fatty bone marrow (FBM), which may influence CHIP-associated pathologies. We analyzed exome sequencing and clinical data from 47,466 individuals with validated CHIP in the UK Biobank. CHIP was present in 5.8% of the study population and was associated with a significant increase in waist-to-hip ratio (WHR). We also examined the association between baseline obesity measured as BMI (n=46,460), WHR (n=47,405) and the risk of developing future myeloid leukemia using the Cox proportional hazards model. The hazard ratio (HR) (95% confidence interval (CI)) incident of myeloid leukemia per 1-SD increase in BMI was 1.71 (1.23, 2.38) with a p value of 1.31E-03, and that of WHR was assessed to be 1.59 (0.91, 2.79) with a

p value of 1.05E-01. These results demonstrate that obesity is associated with myeloid leukemia. In mouse models of obesity and CHIP driven by heterozygosity of *Tet2*, *Dnmt3a*, *Asxl1* and *Jak2* resulted in exacerbated expansion of mutant HSC/Ps due in part to excessive inflammation. Calcium channel blocker, nifedipine or SKF-96365, either alone or in combination with metformin, MCC950 or anakinra (IL-1 receptor antagonist), suppressed the growth of mutant CHIP cells and partially restored normal hematopoiesis. Our results show that obesity is highly associated with CHIP and a pro-inflammatory state can potentiate progression of CHIP to more significant hematologic neoplasia. Targeting CHIP mutant cells individually with metformin, nifedipine, SKF-96365, MCC950 or anakinra or combinations of these drugs could be a novel approach to treat CH and its associated abnormalities in obese individuals.

Key Words: Obesity, CH, CHIP, MPN, Calcium, Inflammation.

Basic Science ***Faculty***

POSTER #5

TARGETING YAP-TEAD INTERACTION TO INHIBIT TUMOR CELL SURVIVAL

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The Hippo signaling pathway controls tissue growth and is a critical organ size checkpoint. This pathway is comprised of a series of kinases that ultimately regulate the transcriptional coactivators, YAP and TAZ. Without phosphorylation, YAP/TAZ translocate into the nucleus where they associate with TEAD transcription factors to direct gene expression programs that promote cell proliferation, survival signals, and cell fate decisions. In addition to its potential direct contribution to cancer progression, upregulation of YAP-TEAD activity has been associated with drug resistance in human malignancies and genetically engineered mouse models of cancer. Consequently, we sought to develop small molecule inhibitors of YAP-TEAD interaction to both disrupt tumor progression and prevent relapse.

Structural analyses have demonstrated that YAP wraps around the globular structure of TEADs

and forms extensive interactions via three highly conserved interfaces. We anticipated that disruption of this YAP-TEAD interaction would suppress tumor growth and sought assistance from the biotech company, Atomwise Inc. to use its AI-directed scanning algorithms to predict molecules that bind to a hydrophobic pocket in TEAD that interacts with an a-helical region of YAP.

A collection of 76 small molecules were screened for ability to (i) blocks YAP/TEAD-induced gene expression using a TEAD-responsive luciferase reporter plasmid and (ii) inhibit the growth of pancreatic ductal adenocarcinoma (MIA-PaCa2 and Pa14c) or NSCLC (H460) 3-D spheroid cultures. Three related sulfonamide compounds were identified to be most effective and two, compounds 22 and 59, were further investigated. Compound 22 was the most effective at inhibiting TEAD-induced gene expression (IC_{50} = 0.74 uM in HeLa cells) and pilot fluorescence polarization-based measurements using recombinant TEAD and a fluorescent Yap peptide confirm that the compound directly disrupts YAP-TEAD interaction. Meanwhile, Compound 59 inhibited human tumor cell growth with an IC_{50} of 1-10 uM, acting more effectively on pancreatic cancer cells than HPNE, a normal pancreatic epithelial line. To test pharmaco-stability in animals, compound 59 was formulated in DMSO/PEG 400/corn oil and injected i.p. at 75 mg/kg and mouse plasma levels monitored over 24 hr. The compound had a $t_{1/2}$ of >5 hr. Compound 59 treatment had only a modest effect on subcutaneous Pa03C PDAC tumor growth but this same batch of compound was subsequently found to also be less effective in vitro (IC_{50} = 14 vs 5 uM). Future studies will use co-crystallization of compounds with TEAD to design more potent inhibitors of YAP-TEAD interaction, evaluate specificity for TEADs 1-4, ability to disrupt TEAD binding to YAP paralog TAZ, and address whether these compounds can cooperate with traditional and targeted cancer therapies to inhibit tumor formation.

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POSTER #6

ERG FUNCTION IN DIFFERENT CELL TYPES IN PROSTATE TUMORS INFLUENCES CELL FATE DECISIONS

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Prostate cancer is the second most common cancer among men. An estimated 50% prostate cancer harbors a chromosomal rearrangement that causes *TMPRSS2:ERG* fusion. TMPRSS2 is a prostate specific, androgen responsive, transmembrane serine protease. ERG is a member of ETS family of transcription factors that contain a highly conserved ETS DNA binding domain. Fusion of the promoter and 5' UTR of the *TMRPSS2* gene to the open reading frame of ERG results in aberrant expression of either full-length or N-terminally truncated ERG protein in prostate epithelial cells. ERG is otherwise silent in adult epithelial cells. The prevalence of this common genetic event makes it attractive as potential therapeutic target. In different models ERG can promote either luminal epithelial fates, or epithelial to mesenchymal transition. 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. Hence, we hypothesize that, in basal/mesenchymal prostate cancer cells, ERG is activated by TLR4 signaling and functions to promote stemness as in hematopoietic stem cells, and in luminal-epithelial prostate cancer cells ERG is activated by VEGF signaling and functions to promote luminal differentiation similar to the role in endothelial cells. Identification of cell specific ERG regulation is critical for the development of targeted drugs and ensure the translatability of our findings.

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POSTER #7

GCN2 EIF2 KINASE AND P53 COORDINATE AMINO ACID HOMEOSTASIS AND METABOLISM IN PROSTATE CANCER

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Activation of the integrated stress response (ISR) contributes to the progression of many cancers, including prostate cancer (PCa). The ISR features a family of protein kinases that phosphorylate the eukaryotic translation initiation factor 2 (eIF2) during different stress conditions, resulting in the repression of global protein synthesis. In parallel, eIF2 phosphorylation also enhances the translation of select gene transcripts, such as ATF4, which directs the transcription of ISR-target genes critical for cancer stress adaptation. We recently reported that the eIF2 kinase GCN2 drives the ISR in PCa and is crucial for maintaining essential amino acid (EAA) homeostasis (Cordova et al., 2022 eLife 11:e81083). GCN2 is activated in PCa due to EAA limitation, resulting in increased expression of key amino acid transporters which provide for nutrient import to facilitate protein synthesis and metabolism that drive PCa proliferation. Genetic loss or pharmacological inhibition of GCN2 results in lowered expression of amino acid transporters, leading to severe depletion of intracellular EAAs, and reduced proliferation in PCa cell lines and xenograft tumors. These results support the therapeutic potential of targeting GCN2 in PCa.

Recently we found that loss of GCN2 in PCa triggers a G1 nutrient-sensitive cell cycle checkpoint that is dependent on p53. Induced G1 arrest and p53 signaling by GCN2 inhibition was reversed by supplementation with EAAs, suggesting amino acid limitation is critical for activating p53. Metabolic stresses, such as amino acid starvation, are suggested to activate p53 in different cancers, and p53 regulates metabolic pathways essential for cancer cell adaptation to stresses. Using transcriptomic and metabolomic analyses, we found that GCN2 and p53 coordinately regulate multiple metabolic pathways, specifically those involved in amino acid and nucleotide metabolism. Both GCN2 and p53 are activated by reduced amino acids and are critical for PCa adaptation to nutrient-deprived conditions. During amino acid starvation, GCN2 phosphorylates eIF2 resulting in repression of global protein synthesis, and paradoxically, induces the expression of genes involved in amino acid synthesis, transport, and reclamation. In parallel, p53 induces a G1 nutrient-sensitive cell cycle checkpoint required for cells to survive amino acid starvation. Interestingly, both GCN2 and p53 are required for optimal mitochondrial respiration in PCa, and loss of GCN2 or p53 severely impacted ATP production. Importantly, inhibition of GCN2 in combination with loss of p53 resulted in increased cell death in PCa.

We propose that GCN2 and p53 are activated in parallel and are critical for PCa adaptation to amino acid limitation by coordinating metabolism, translation, and cell cycle control. Targeting both these stress pathways may provide enhanced efficacy for the treatment of PCa.

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POSTER #8

REPLICATION PROTEIN A TARGETED THERAPY: IN VIVO ANTICANCER ACTIVITY AND CELLULAR TARGET ENGAGEMENT.

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Replication protein A (RPA) plays essential roles in DNA replication, repair, recombination and the DNA-damage response (DDR). Retrospective analysis of lung cancer patient data demonstrates high RPA expression as a negative prognostic biomarker for overall survival in smoking-related lung cancers. These observations are consistent with the increase in RPA expression serving as an adaptive mechanism that allows tolerance of the genotoxic stress resulting from carcinogen exposure. We have employed chemical synthesis, in vitro analyses and in vivo xenograft studies to assess mechanism of action, cellular engagement and therapeutic activity of RPA-targeted agents. We have discovered, developed and characterized a novel small molecule RPA inhibitor (RPAi) NERx-329 that blocks the RPA-DNA interaction. We have optimized formulation for in vivo analyses and cellular engagement studies. NERx 329 elicits single agent in vitro anticancer activity across a broad spectrum of cancers which allowed the identification of genetic predictors of RPAi efficacy. A genetic knock out screen also identified additional genetic alterations that increase RPAi activity. A series of these genetic models were pursued, and results demonstrate specific genetic alterations increase RPAi activity in vitro and in vivo. Chemical RPA inhibition is shown to potentiate the anticancer activity of a series of DDR inhibitors as well as traditional DNA damaging cancer therapeutics. The analysis of cell cycle, chromatin-bound RPA and DDR pathway activation demonstrate on-target cellular engagement of RPA by NERx-329. These data demonstrate that targeting the RPA-DNA interaction elicits a state of chemical RPA exhaustion that results in in vivo anticancer activity.

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POSTER #9

IL-9 IMMUNOTHERAPY FOR LUNG TUMOR GROWTH

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BACKGROUND: Interleukin 9 (IL-9) is produced by T cells, mast cells, and innate lymphoid cells, and has pleiotropic activities in enhancing immune cell recruitment and function. Despite the anti-tumoral property of IL-9 in various cancers, we and others have reported endogenous IL-9 signaling has pro-tumorigenic effects on tumors growth in the lung, although the therapeutic potential of IL-9 blockade is not well studied.

METHODS: In the present study, we evaluate the therapeutic efficacy of IL-9 blockade by IL-9-neutralizing antibodies in a melanoma lung metastasis model and a carcinogen-induced spontaneous lung cancer model, perform the analysis on tumor burden and cellular components in lung tissue.

RESULTS: The mice receiving anti-IL-9 treatment have a significantly lower number of lung tumor foci compared to control IgG-treated mice. Cellular analysis indicates IL-9 blockade inhibits immunosuppressive lung interstitial macrophage (IM) expansion, decreases arginase-1 production from lung IMs and dendritic cells. Moreover, IL-9 blockade reduces the frequency of regulatory T cells, and diminishes their proliferation and activation in the lung.

CONCLUSIONS: Anti-IL-9 treatment reduces the lung tumor burden by altering the immunosuppressive tumor microenvironment in both models, and IL-9 blockade may represent a potential immunotherapy strategy for lung tumor growth.

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Basic Science ***Faculty***

POSTER #10

THE HEPARAN SULFATE MIMETIC PG545 INHIBITS TUMOR GROWTH IN BOTH MALE AND FEMALE MOUSE MODEL OF PANCREATIC DUCTAL ADENOCARCINOMA (PDAC) WITH SEX DIFFERENCE IN THE EFFECT ON CACHEXIA.

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BACKGROUND: Cachexia occurs in up to 80% of PDAC patients and is characterized by weight loss mainly due to muscle and fat wasting. Cachexia leads to functional impairment, reduced anticancer therapy tolerance and increased mortality. PG545, a heparan sulfate (HS) mimetic, shows anticancer activity; however, it is unknown whether sex affects the anticancer activity and what effect PG545 has on cancer associated muscle and fat wasting. Given that many cytokines induced in patients with cancer cachexia can bind HS, the aim of this study was to assess PG545 effects on PDAC cachexia with emphasis on sex difference.

METHODS: PDAC cells were injected into the pancreas of 10-week C57BL/6 male and female mice. The orthotopic tumor-bearing mice were treated with PG545 (10 mg/kg; i.p.). Body weight and body composition were monitored, and tumor and organs were collected. Skeletal muscles were cryosectioned for evaluation of cross-sectional area (CSA). C2C12 myotubes, the *in vitro* model of muscle wasting, were treated with PDAC cell-derived conditioned medium (CM) without or with PG545 and analyzed for the changes in size. PDAC cell proliferation was assessed by MTT assay.

RESULTS: PG545 treatment markedly reduced tumor mass in both males and females compared to the vehicle controls. PG545 also reduced body weight but the difference gradually dissipated over time despite repeated dosing. However, male mice recovered from the negative effects more slowly than female mice. Similar sex difference was observed in the changes in the PG545-induced lean and fat mass loss. Of note, fat mass in PG545-treated females at the end of experiment was higher than in the vehicle controls. Furthermore, males had more reductions in muscle and fat weights than females in response to PG545. Grip strength in males was weakened by PG545 but returned to the level like that in the vehicle controls at the end, while PG545 did not weaken grip strength in females at the same time points. In addition, males, but not females, had smaller muscle fiber CSA after PG545 treatment versus their vehicle controls. In *in vitro* assay, PG545 inhibited PDAC cell proliferation, indicating a direct effect on cancer cells. Opposed to the *in vivo* negative effect on cachexia, PG545 partly prevented myotube wasting induced by PDAC CM and the effect was likely through inhibiting the enhancement of catabolism as the induction of atrogin1 mRNA expression by PDAC CM was significantly attenuated. Lastly, to reveal whether tumor was involved in the *in vivo* negative effect of PG545, the same treatment approach was used on no-tumor-implanted sham control mice. Negative effects were observed; however, unlike the tumor-bearing mice, the male and female no-tumor mice had similar muscle and fat wasting after PG545 treatment.

CONCLUSION: PG545 has strong anti-PDAC activity in both male and female mice, at least partly through inhibition of cancer cell proliferation. While PG545 negatively affects cachexia in the early stage in both sexes, female tumor-bearing mice reverse the negative impact faster than the male counterpart whereas the no-tumor mice do not show this sex difference. Thus, the difference may be mediated by the stress condition posed by tumor. Because of the protective effect on C2C12 myotubes treated with PDAC CM, PG545 might induce some counteracting factors *in vivo* in the initial treatment and identification of these factors may offer

molecular targets for combination therapy to counteract the negative effects while take advantage of the strong anti-cancer potential.

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Basic Science ***Faculty***

POSTER #11

OXYGEN TENSION - DEPENDENT SIGNALING BY PDGFRB DRIVES RESPONSE TO TARGETED THERAPIES IN BREAST CANCER

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Current approaches to preclinical cancer research often fail to consider the negative impact of maintaining cancer cells under ambient oxygen (O_2; ~21%). This is also true for hypoxia studies that typically involves cancer cells previously grown in ambient O_2 before subsequent transfer to hypoxia. However, the tumor microenvironment is characterized by significantly lower O_2 levels. We have previously demonstrated the impact of ambient O_2 on cancer stem cell populations, signaling pathways and resistance to therapy. We developed an approach that allows us to collect and process mammary tumor tissues from transgenic mouse models under physioxia (3% O_2) such that they are never exposed to ambient O_2. In this study, our goal was to explore signaling pathway alterations that occur due to physioxic and ambient O_2 tensions and to determine how these pathways influence response to targeted drugs.

Our studies revealed increased basal phosphorylation levels of EGFR (Y1068) in cells processed and propagated in ambient air (AA), relative to physioxia. However, EGFR's downstream signaling effectors AKT and ERK showed higher phosphorylation levels under physioxia, compared to AA, suggesting that their activation under physioxia is independent of EGFR signaling. These findings correlate with decreased sensitivity of the tumor cells under physioxia to targeted drugs lapatinib and alpelisib. We then sought to examine basal, and drug induced kinome changes in tumor cells under physioxia and AA via Multiplexed Inhibitor Beads (MIBs) kinome assay. This assay revealed significant differences in the kinome of the tumor cells under physioxia compared to AA. Although direct comparisons between control and lapatinib treated cells under physioxia and AA showed minimal changes, pairwise comparison between lapatinib treated physioxia cells and vehicle treated AA cells showed an increase in the activity of PDGFRB in lapatinib treated physioxia cells. Similarly, a receptor tyrosine kinase array and western blotting showed increased basal and lapatinib induced PDGFRB phosphorylation (Y751) under physioxia. Next, we determined the potential role of PDGFRB in downstream signaling pathway activation of AKT and ERK and resistance to lapatinib. Knockdown of PDGFRB led to a simultaneous decline in the phosphorylation of AKT and ERK under physioxia, suggesting a role for PDGFRB in the activation of AKT and ERK under physiologic conditions. We found that Sunitinib, a multitarget RTK inhibitor with high affinity for PDGFR, effectively decreased PGDFRB activity under physioxia, with a concurrent decrease in the phosphorylation of AKT. Moreover, tumor cells under physioxia were more sensitive to sunitinib treatment, relative to ambient air. Importantly, a combination of lapatinib and sunitinib rendered tumor cells under physioxia more sensitive to treatment than with lapatinib alone. These findings suggest that ambient and physioxic oxygen tensions differentially impact cancer relevant signaling pathways and that oxygen dependent PDGFRB signaling influences response to targeted therapies.

Basic Science ***Graduate Student***

POSTER #12

PDGFRA UPREGULATION CONTRIBUTES TO FGFR INHIBITOR RESISTANCE IN BREAST CANCER

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Breast cancer is the most diagnosed cancer in females in the United States and the second leading cause of cancer related deaths among women. Fibroblast growth factor receptor (FGFR) signaling pathway is frequently aberrantly expressed in breast cancer, making it an attractive therapeutic target, especially among metastatic disease. However, despite initial promise, clinical trials of FGFR inhibitors in breast cancer have been disappointing in comparison to other cancer types treated with FGFR inhibitors. Several factors contribute to the limited success of FGFR inhibitors in breast cancer. One of the major hurdles is the development of drug resistance which is common in targeted cancer therapeutics. Resistance to FGFR inhibitors can occur through multiple mechanisms, including upregulation of alternative signaling pathways, mutations in FGFR genes, or alterations in the tumor microenvironment. To improve the efficacy of FGFR inhibitors in breast cancer, several strategies are being investigated, such as the development of more selective and potent inhibitors, the identification of biomarkers for patient selection, and the combination with other targeted therapies or immune checkpoint inhibitors. Our group has shown that upregulation of platelet-derived growth factor receptor alpha (PDGFRA) may be responsible for resistance to FGFR inhibitors in breast cancer. PDGFRA is commonly co-expressed with FGFR in breast cancer and can activate downstream signaling pathways that circumvent FGFR inhibition. In summary, the development of FGFR inhibitors for the treatment of breast cancer has faced several challenges, including drug resistance, and toxicity. Further research is needed to overcome these limitations and to identify novel therapeutic strategies to improve the treatment of breast cancer.

Basic Science ***Graduate Student***

POSTER #13

ACTIVATION OF PP2A-B56α PROMOTES ABERRANT MACROPINOSOME PROCESSING IN PANCREATIC CANCER CELLS LEADING TO METABOLIC VULNERABILITIES AND CELL DEATH.

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Pancreatic Ductal Adenocarcinoma (PDAC) is the fourth leading cause of cancer related deaths in the US, with the lowest five-year survival rate of all cancers.Nutrients in the PDAC microenvironment are commonly depleted, with the vital amino acid glutamine among the most deficient metabolites. To circumvent this deprivation, PDAC cells initiate KRAS dependent macropinocytosis, an actin-driven nutrient scavenging pathway. Macropinosomes fuse with lysosomes in a process mediated by the kinase PIKfyve. This process allows cells to replenish nutrients required for survival. As glutamine is essential for PDAC cell survival, therapeutic inhibition of macropinocytosis represents a novel strategy to suppress nutrient acquisition and drive cell death in PDAC.

Protein phosphatase 2A (PP2A) is a heterotrimeric Serine/Threonine phosphatase known to inhibit downstream targets of the KRAS signaling cascade and is implicated in macropinocytosis regulation. However, the mechanism of regulation by PP2A and the regulatory subunit involved is not understood. PP2A holoenzyme comprises of A, B and C subunits, where the regulatory B subunit provides substrate specificity to the enzyme. We demonstrate that pharmacological activation of PDAC cell lines with the small molecule activator of PP2A-B56α, DT061, leads to significant accumulation of intracellular vesicles. Using high molecular weight TMR-Dextran and scanning electron microscopy, we confirmed that these vesicles are macropinosomes. Through immunofluorescence and DQ-BSA assays, we established that PP2A-B56α activation prevents the colocalization of macropinosomes with lysosomes leading to macropinosome accumulation. This disruption is most likely through inhibition of the kinase PIKfyve therefore limiting the nutrient supply to PDAC cells.

RNA sequencing analysis showed that glutamine deprivation genes are significantly enriched with acute activation of PP2A-B56α by DT061, and the oxidative phosphorylation pathway was observed to be significantly inhibited. Steady state metabolite analysis also showed significant downregulation of amino acid glutamine with PP2A-B56α activation or overexpression. Further, combination of DT061 with glutamine transport inhibitor V-9302 was found to be synergistic in inhibition of the survival of PDAC cells.

Together, these findings indicate that activation of PP2A-B56α in late stage PDAC promotes aberrant macropinocytosis by preventing lysosomal fusion and establishes a novel role of PP2A in nutrient scavenging and cell death. These pathways can be further exploited to explore potential combination therapeutics in Pancreatic cancer.

Basic Science ***Graduate Student***

POSTER #14

QUANTITATIVE PROFILING OF THE LYSINE METHYLOME IN BREAST CANCER

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Lysine methylation (Kme) is a dynamic post-translational modification that has been detected on thousands of human proteins and is recognized by specific reader proteins to mediate signaling and downstream functions. The family of proteins that add or "write" this modification, lysine methyltransferases (KMTs), are often dysregulated in diseases such as cancer. As a result, many cancers have well-documented changes in histone methylation, but the impact of methylation on non-histone proteins has not been studied. Indeed, it is a major technical challenge to reproducibly quantify changes in the lysine methylome. We recently reported a new method to obtain deep coverage of lysine methylation using mass spectrometry. In this study, our main objective was to apply these advances to reproducibly quantify changes in the lysine methylome in triple-negative breast cancer cells. We used tandem mass tag (TMT) labeling and quantitative mass spectrometry (qMS) to quantify and compare the lysine methylome of two triple negative breast cancer cell lines (MDA-MB-231 and HCC1806), an ER+ breast cancer cell line (MCF7), and a normal mammary epithelial cell line (MCF10A). Our results provide both technological insight into the best practices for quantitatively comparing lysine methylation and more importantly reveal significant Kme changes between MCF10A, TNBC, and ER+ breast cancer. We also identified changes in KMTs and KDMs protein expression between the two breast cancer subtypes and the normal control cells, allowing us to connect changes in specific Kme sites with candidate KMTs and KDMs. Future work will focus on studying the role of specific methylated sites identified in this study on TNBC proliferation.

Basic Science ***Graduate Student***

POSTER #15

IL-9 RESPONSIVE MACROPHAGES UTILIZE ARGINASE 1 TO ENHANCE LUNG TUMOR GROWTH

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In cancer, the immunosuppressive function of myeloid cells that support tumor progression is controlled by secreted factors in the tumor microenvironment. In the tumor microenvironment, arginine and arginine-derived metabolites are crucial factors in tumor development. Within macrophages, arginine metabolism influences the polarization of macrophages and therefore, tumor growth by eliciting an anti-tumoral or pro-tumoral phenotype. Interleukin 9 (IL-9) is a pleiotropic cytokine that signals through the IL-9 receptor (IL-9R) and can function as a positive or negative regulator in tumor immunity. Recently, our lab has demonstrated that IL-9 signaling promotes tumor progression in the lung by expanding pulmonary interstitial macrophage populations and inducing Arginase 1 (ARG1), an enzyme associated with pro-tumoral macrophage function, activity. However, the mechanism by which IL-9R/ARG1+ interstitial macrophages promote tumor development remains unknown. Here, using a B16F10 model of metastatic lung cancer, we demonstrate that knockdown of ARG1 using macrophage targeting *Arg1* siRNA containing nanoparticles results in altered pulmonary T-cell and macrophage proportions. Moreover, attenuation of IL-9 signaling and ARG1 expression in macrophages impacts arginine and arginine-derived metabolite concentration in lung tissue and BAL fluid, correlating with decreases in CD4+ T Cell exhaustion marker expression and a concomitant reduction in PDL1 expression on pulmonary interstitial macrophages in tumor-bearing mice. Thus, our work suggests that the IL-9R/ARG1 axis in macrophages is sufficient to alter arginine in the tumor microenvironment and promote tumor growth.

Basic Science ***Graduate Student***

POSTER #16

DEVELOPMENT OF GEFITINIB PRODRUGS TARGETING NON-SMALL CELL LUNG CANCER BRAIN METASTASES VIA INHIBITION OF P-GLYCOPROTEIN AT THE BLOOD-BRAIN BARRIER

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The prognosis of non-small cell lung cancer (NSCLC) patients with brain metastases is very poor. Targeted therapeutic agents that are used as the first-line treatment of oncogene-driven NSCLC have limited effectiveness in treating brain metastases of mutant NSCLC due to efflux transporters, such as P-glycoprotein (P-gp), that limit drug penetration across the blood-brain barrier (BBB). To address this limitation, we present the development of a dimeric prodrug P-gp inhibitor based on the P-gp substrate and first-line NSCLC targeted therapeutic gefitinib. With the goal of improving gefitinib's therapeutic brain penetration, the gefitinib-based dimer **GFTB-Q** was designed to have a dual role: to inhibit P-gp efflux at the BBB and to regenerate the monomeric therapy within cellular reducing environments. **GFTB-Q** exhibited potent inhibition of P-gp-mediated efflux in cell culture, including for human brain endothelial cells. Additionally, reduction of **GFTB-Q** yielded the regeneration of its monomeric components, validating its design as a dimeric prodrug inhibitor of P-gp.

Basic Science ***Graduate Student***

POSTER #17

ELUCIDATION OF THE MECHANISM OF FIBRINOGEN DEPLETION IN THE PANCREATIC DUCTAL ADENOCARCINOMA MICROENVIRONMENT THAT IMPEDES TUMOR PROGRESSION

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Pancreatic ductal adenocarcinoma (PDAC) exhibits one of the poorest prognoses of all solid cancers and is associated with very low overall and progression-free survival rate. Clinically, PDAC patient tumors show high expression levels of multiple components of the coagulation system and fibrinolytic system. Pancreatic cancer patients show elevated levels of circulating fibrinogen (Fib) and the fibrin degradation product D-dimer in plasma, with increased correlation in patients with distant metastasis. Immunofluorescence and histopathology of PDAC tumor tissues have shown dense fibrin networks in the PDAC stroma compared to healthy pancreas in which fibrin networks are practically undetectable. Dense fibrin networks in the extracellular matrix (ECM) can contribute to drug resistance by acting as a physical barrier, while serving as a molecular bridge between receptors on tumor cells and the vascular endothelial cells, platelets, and leukocytes to promote cancer cell metastasis. To investigate the contribution of Fib in the microenvironment, I used Fib-specific antisense oligonucleotide (ASO) treatment to specifically deplete fibrinogen levels in mice bearing human PDAC tumors. Treatment with Fib-ASO significantly decreased tumor burden in both orthotopic and subcutaneous models bearing human tumors. In an aggressive orthotopic model of PDAC, Fib-ASO treatment decreased progression of primary tumors and subsequent spontaneous metastatic burden. Histology of lung and liver tissue from Fib-ASO-treated mice demonstrated significantly fewer metastatic lesions in both liver and lung. To further investigate the applicability of Fib-ASO as a treatment option in PDAC, an additional orthotopic patient-derived xenograft (PDX) model in which the patient tumor stained strongly for fibrin was used. Fib-ASO treatment again significantly diminished growth of these tumors compared to Control ASO treatment. Collectively, our data demonstrate that depleting Fib in circulation reduces tumor growth and metastasis. To delve into the mechanism behind the dramatic impact on tumor growth and metastatic burden, we used western blotting and a global proteomics approach to evaluate expression of various ECM proteins, and observed enhanced expression of basement membrane proteins such laminin and fibronectin on depletion of fibrinogen. Therefore, I hypothesize that depletion of fibrinogen increases the expression of other ECM proteins such as laminin and fibronectin which strengthens the basement membrane and impedes metastasis by inhibiting the cancer cells from breaking through the basement membrane. PDAC is highly metastatic which significantly impacts treatment outcome of patients, and only 10-20% of PDAC patients that are diagnosed with borderline resectable disease are eligible for curative surgery. We have shown that Fib-ASO treatment impedes metastasis in PDX models. In the long run, treatments such as Fib-ASO can potentially be useful in controlling the metastatic spread of PDAC to keep the tumor localized to the pancreas, making more patients eligible for curative surgery.

Basic Science ***Graduate Student***

POSTER #18

THE ROLE OF GCN2 KINASE IN LEUKEMOGENESIS AND THERAPEUTIC RESPONSE TO L-ASPARAGINASE

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Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer, affecting the lymphoid cells of both B and T lineages. Most ALL cells are auxotrophic for asparagine, a nonessential amino acid for protein synthesis, due to the low expression of asparagine synthetase (ASNS), a rate limiting enzyme for de novo biosynthesis of asparagine. As a result, standard ALL treatment takes advantage of this vulnerability by giving patients L-asparaginase, a bacterial enzyme that depletes the circulating asparagine. However, previous work from our lab and others have shown that some ALL cells become resistance to L-asparaginase treatment through the induction of ASNS expression. Mechanistically, amino acid starvation actives the general control nonderepressible 2 (GCN2) kinase, leading to the accumulation of ATF4 transcriptional factor. ATF4, in turn, is recruited to the promoter of the *ASNS* gene to activate its transcription. However, the role of GCN2 kinase in the process of leukemogenesis under nutrient limiting environment has not been established. Using a mouse T-ALL model driven by a mutant KRas, we found that the integrated stress response (ISR) was activated by the mutant KRas during leukemogenesis. Genetic inactivation of GCN2 or a small molecule inhibitor of GCN2 sensitized the leukemic cells to asparaginase treatment in vitro. Our goal is to determine the therapeutic potential of GCN2 inhibition in the context of asparagine restriction in vivo.

Basic Science ***Graduate Student***

POSTER #19

ALTERNATE-DAY FASTING REDUCES ANDROGEN RECEPTOR EXPRESSION AND ENHANCES THE ACTIVITY OF ANTI-ANDROGEN THERAPY IN PROSTATE CANCER MODELS

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Prostate cancer (PCa) is the most common cancer diagnosed in men in Western countries. Epidemiological studies have suggested that environmental factors such as the Western diet, characterized by the consumption of high-caloric food containing large amounts of animal protein and fats, play a key role in the pathogenesis of PCa. We propose that dietary intervention, by reducing caloric or protein intake, may be beneficial for the treatment of PCa.

We recently demonstrated that dietary protein restriction and alternate-day fasting reduced tumor growth in a LuCaP23.1 patient-derived xenograft (PDX) model of PCa. Interestingly, only alternate-day fasting reduced the expression of androgen receptor (AR), a major driver of PCa growth. Therapies targeting AR activity, termed androgen deprivation therapies, include the AR antagonist Enzalutamide (ENZ), are the current standard of care treatments for PCa. To address the therapeutic potential of combining alternate-day fasting with anti-androgens, we utilized the syngeneic mouse PCa models Myc-CaP, PTEN$^{-/-}$ RB$^{-/-}$, and the human LuCaP23.1 PDX model. These AR-positive models harbor the oncogenic drivers most frequently found in PCa, including MYC amplification and loss of the tumor suppressor PTEN.

We show that alternate-day fasting enhances the *in vivo* activity of ENZ in these PCa models. Interestingly, the combination of alternate-day fasting with ENZ treatment reduced AR expression and signaling and decreased proliferation and tumor growth compared to either single treatment alone. Consistent with these findings, the reduction of AR activity by the depletion of endogenous androgens following surgical castration was enhanced with alternate-day fasting. Conversely, dietary protein restriction had no effect on the in vivo activity of ENZ, suggesting that reducing AR expression by alternate-day fasting enhances the effect of anti-androgens. Transcriptomic analysis of tumors from fasted mice confirmed decreased AR transcriptional activity and revealed that several nutrient-sensitive pathways were decreased by this dietary regimen, including PI3K/AKT signaling, mTORC1 signaling, and glycolysis.

Of importance, we found that translation is reduced in fasted tumors. Experiments using PCa cells cultured in media with reduced amino acids and glucose recapitulated the decrease in AR expression and reduction in translation observed in fasted tumors. Using translation inhibitors, our data suggest that AR has a short half-life and is sensitive to translation inhibition. We propose that nutrient limitations by fasting inhibits translation in PCa tumors, and this results in a reduction in AR levels that enhances anti-androgen therapy.

In conclusion, we demonstrate that alternate-day fasting reduces AR signaling and enhances the activity of ENZ in several PCa models. This study suggest that alternative fasting may improve the efficacy of anti-androgen therapy for the treatment of PCa.

Basic Science ***Graduate Student***

POSTER #20

DEFINING THE MECHANISMS OF TGLI1'S ONCOGENIC FUNCTIONS

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Glioblastoma (GBM) is the most common malignant brain and other central nervous system tumor in adults with a five-year survival rate of less than 7%. Discovered in GBM, tGLI1, a novel alternative splicing isoform of GLI1, is highly expressed in both GBM cell lines and primary specimens but not in normal tissue. Despite an in-frame deletion of 123 base pairs, tGLI1 has retained all known GLI1 functional domains, continues to respond to Sonic Hedgehog signaling, and translocates into the nucleus, similar to GLI1. Moreover, tGLI1 operates as a gain of function transcription factor with the ability to bind to and activate genes unique from GLI1 to promote invasion, migration, angiogenesis, and stemness in both glioblastoma and breast cancer. Despite the advancements in our understanding of tGLI1, a genome wide DNA binding pattern of tGLI1 has not been established and the mechanism by which tGLI1 gains access to these genes is unknown. To establish a more robust understanding of the differential DNA binding patterns of GLI1 and tGLI1, we carried out ChIP-sequencing (ChIP-seq) and found tGLI1 to be significantly enriched at 1313 unique sites while GLI1 was significantly enriched at 1197 unique sites. As only 417 regions were bound by both proteins, we concluded that GLI1 and tGLI1 have distinct DNA binding patterns and show unique binding from one another beyond the previously established genes. Next, to determine whether variations in protein interactions of GLI1 and tGLI1 contribute to the unique binding pattern of tGLI1, we carried out immunoprecipitation (IP) followed by mass spectrometry. Results showed only 8 of 35 proteins are associated with both GLI1 and tGLI1. Based on these results, we concluded GLI1 and tGLI1 have a large set of distinct interacting partners, which suggests GLI1 and tGLI1 are likely to function differently in the cell.

Basic Science ***Graduate Student***

POSTER #21

EWS/ETS FUSION PROTEINS AVOID REPRESSION BY EZH2 IN EWING SARCOMA TUMORS

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ETS transcription factors play essential roles throughout development, aiding in hematopoiesis, blood vessel formation and cellular fate. The ETS family is composed of 28 members, all of which share a C-terminal DNA-binding domain that bind core GGAAG(A/T) motifs. Chromosomal rearrangement events can lead to the overexpression of specific ETS in tissues, promoting oncogenic transformation and tumor development. Four ETS members become expressed in prostate tumors: ERG, ETV1, ETV4 and ETV5. Our lab has demonstrated that these "oncogenic ETS" form essential interactions with the ubiquitous RNA-binding protein EWS to promote tumorigenesis. Similarities exist between prostate cancer and Ewing Sarcoma (ES). ES affects young males at the onset of puberty due to increased androgen signaling. ES tumors are also driven by rearrangement events that lead to the expression of EWS/ETS fusion proteins, namely EWS/FLI1 and EWS/ERG.

The closest homolog of ERG within the ETS family is FLI1. The finding that ERG functions with EWS to promote prostate cancer suggests that this complex might function similarly to the EWS/FLI1 fusion in ES. The similarities and differences between ERG and EWS/FLI1, in both their regulation and downstream effects, have not yet been unexplored.

Knockdown of endogenous EWS/FLI1 in the ES cell line, A673, reduces anchorage-independent colony formation in soft agar. Rescue with either EWS/FLI1 or EWS/ERG restores colony formation relative to control. Interestingly, expression of exogenous ERG is unable to rescue colony formation. This observation suggests that ERG is differentially regulated in A673 cells. Previous results from our lab demonstrate interactions between the N-terminus of ERG and EZH2, a component of polycomb repressive complex 2. Pulldown assays from A673 cell lysate reveal that ERG interacts with EZH2. Neither EWS/FLI1 nor EWS/ERG interact with EZH2; it is worth noting that these clinically-relevant fusions result in the N-terminal truncation of both FLI1 and ERG. Additionally, expression of phosphomimetic ERG(S96E) disrupts its interaction with EZH2 and promotes colony formation upon EWS/FLI1 knockdown. ChIP-seq analyses have revealed limited overlap between ERG binding sites and EWS/FLI1 binding sites in A673 cells. Given the quality of our sequencing, we aim to repeat these experiments in A673-ERG and A673-ERG(S96E) cells upon EWS/FLI1 knockdown.

Future directions aim to evaluate the role of EZH2 as a repressor of ERG in A673 cells. EZH2 inhibitors can be utilized to evaluate whether non-repressed ERG can restore colony formation upon EWS/FLI1 knockdown. Additional constructs, including FLI1 and EWS/flERG, will be used to evaluate the contributions of EWS' fusion to FLI1 and the possible interaction between EZH2 and EWS/flERG in A673 cells, respectively. I also plan to explore any interactions between EZH2 and the other oncogenic ETS found in prostate cancer (ETV1, ETV4, ETV5) as well as discern their role in A673 colony formation.

Basic Science ***Graduate Student***

POSTER #23

IDENTIFICATION OF NON-HISTONE SUBSTRATES OF THE LYSINE METHYLTRANSFERASE PRDM9

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Lysine methylation is a dynamic, post-translational mark that regulates the function of histone and non-histone proteins. Many of the enzymes that mediate lysine methylation, known as lysine methyltransferases (KMTs), were originally identified to modify histone proteins but have also been discovered to methylate non-histone proteins. In this work, we investigate the substrate selectivity of the lysine methyltransferase PRDM9 to identify both potential histone and non-histone substrates. Though normally expressed in germ cells, PRDM9 is significantly upregulated across many cancer types. The methyltransferase activity of PRDM9 is essential for double-strand break formation during meiotic recombination. PRDM9 has been reported to methylate histone H3 at lysine residues 4 and 36; however, PRDM9 KMT activity had not previously been evaluated on non-histone proteins. Using lysine-oriented peptide libraries (K-OPL) to screen potential substrates of PRDM9, we determined that PRDM9 preferentially methylates peptide sequences not found in any histone protein. We confirmed PRDM9 selectivity through in vitro KMT reactions using peptides with substitutions at critical positions. A multisite λ-dynamics computational analysis provided a structural rationale for the observed PRDM9 selectivity. The substrate selectivity profile was then used to identify putative non-histone substrates, which were tested by peptide spot array, and a subset were further validated at the protein level by in vitro KMT assays on recombinant proteins. Finally, one of the non-histone substrates, CTNNBL1, was found to be methylated by PRDM9 in cells.

Basic Science ***Graduate Student***

POSTER #24

THE IMPACT OF PP2A-B56A REGULATED CELL PLASTICITY IN EGFR-ALTERED NSCLC

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Lung cancer is the leading cause of cancer related deaths, with a 5-year survival rate of 23 percent. Of lung cancer cases, non-small cell lung cancer (NSCLC) is the most prevalent subtype (85%). Approximately 30% of patients have activating mutations in epidermal growth factor receptor, EGFR. EGFR is a main driver of NSCLC and has been a promising therapeutic target; however, resistance to current therapies is common and occurs in patients in under a year. A unique mechanism of resistance that can occur is histological transformation. This type of resistance includes epithelial-to-mesenchymal transition and NSCLC to small-cell lung cancer transformation, both of which highlight the cell's ability to alter pathway regulation and transition between cell states. Despite accounting for 15% of all resistance, the mechanisms behind this transformation are poorly understood.

One way in which cell state is regulated is through posttranslational modifications by phosphatases. Phosphatases are critical regulators of cell signaling pathways. Protein phosphatase 2A (PP2A) is a heterotrimeric serine/threonine phosphatase and is composed of an A (scaffolding), B (regulatory) and C (catalytic) subunit. The combination of subunits can form over 90 distinct complexes, with the B subunit incorporated determining substrate specificity. The PP2A-B56a complex regulates many of the downstream targets of EGFR and has been implicated in EMT and cell plasticity regulation. Our data indicates that suppression of PP2A-B56a leads to morphological shifts to more a mesenchymal cell state, increased vimentin and decreased e-cadherin expression, and increased migration and invasion. By flow cytometry, we see a population of cells emerge that express CD44 and CD24, indicating a shift toward stem cell state. Our data suggests that these phenotypes can be reversed with PP2A-B56a overexpression. Together these results implicate PP2A-B56a as a key regulator of cell state in EGFR mutant NSCLC and a possible target to combat resistance mechanisms.

Basic Science ***Graduate Student***

POSTER #25

POLO-LIKE KINASE 1 (PLK1) AS A KEY COMPONENT IN ANGIOSARCOMA PATHOGENESIS

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Cancers affecting 1 in 40,000 people are categorized as "rare cancer" according to NCI (National Cancer Institute) categorization. Even though rare cancers are infrequent, they account for 22% of all cancers diagnosed when all rare cancers are taken into one group. All rare cancers pose a common challenge: the low number of available biospecimens for research. Due to this scarcity, most rare cancers are understudied and pose a significant knowledge gap in understanding the molecular drivers and therapeutic targets.

Our study explores Angiosarcoma (AS) pathogenesis and progression, a rare and aggressive vascular sarcoma arising from endothelial cells with an abysmal prognosis of 30-40% 5-year survival. To develop novel therapeutics for AS, we need to understand the molecular and cellular mechanisms of the disease and the main molecular drivers of AS pathogenesis.

PLK1 is a prominent serine/threonine kinase that regulates several critical steps in the cell cycle and is overexpressed in several human and murine cancers. PLK1 overexpression is also associated with poor prognosis in many cancers. Furthermore, KRAS-driven cancers have exhibited hypersensitivity towards PLK1 inhibition.

In this study, we have genetically and pharmacologically inhibited PLK1. Upon doxycycline-inducible shRNAs knockdown of *Plk*1, we observed a significant reduction in cell viability. Furthermore, in a KRAS-driven genetically engineered AS mouse model, *Plk1* deletion significantly prolongs survival. Moreover, we are examining the therapeutic potential of PLK1 pharmacological inhibition to lead to the development of novel treatments for this highly heterogeneous cancer. This research aims to narrow the knowledge deficit on AS development and explore novel aspects of PLK1 biology. The ultimate goal of our project is to identify the role of PLK1 and its therapeutic potential in AS.

Basic Science ***Graduate Student***

POSTER #26

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

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Centrosome amplification is a hallmark of many types of cancer that correlates with poor patient outcomes. It is estimated that in breast cancer, centrosome amplification is found in 75-100% of solid tumors. Cells with centrosome amplification form multipolar spindles, which lead to multipolar cell divisions that generate lethal levels of aneuploidy. However, many cancer cells can cluster centrosomes and form bipolar spindles, leading to lower levels of aneuploidy and cancer cell survival. Kinesin-14 molecular motor proteins that are localized to the mitotic spindle 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 non-essential in normal cells. Therefore, targeting Kinesin-14s could provide a novel mechanism to selectively kill cancer cells. Kinesin-14s are minus-end directed molecular motors that cross-link and slide both parallel and anti-parallel microtubules in the spindle. Previous work from our lab showed that Kinesin-14s can cross-link microtubules using their ATP-dependent kinesin-like motor domain and a second ATP-independent microtubule binding domain in the tail domain. How a single microtubule binding domain in the tail could be involved in cross-linking of microtubules of opposite orientation is not known. To address this question, we mapped the regions of the tail domain 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. To test which domains contribute to spindle morphogenesis and centrosome clustering in cells, we did knockdown/rescue experiments using wild-type and mutant Kinesin-14s to ask the function of both MBD1 and MBD2. Both MBD1 and MBD2 were needed for proper spindle localization, suggesting that each microtubule binding domain contributes to the proper spatial distribution. MBD1 function was necessary for pole clustering, suggesting that anti-parallel microtubule cross-linking through MBD1 may be essential for centrosome clustering. MBD2 parallel cross-linking activity was necessary for maintenance of spindle length, suggesting that the individual microtubule binding domains make distinct contributions to spindle morphogenesis and function. We postulate that loss of MBD1 activity will reduce anti-parallel microtubule cross-linking between supernumerary centrosomes, leading to multipolar divisions and cancer cell death. Therefore, targeting this domain for therapeutics should promote cancer cell death and not harm normal cells due to previously shown non-essential roles in cells that do not have centrosome amplification. 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 #28

INTERACTOME ANALYSIS REVEALS TRANSCRIPTIONAL AND RNA BIOSYNTHESIS REGULATORY FUNCTIONS OF THE IMMORTALIZING ONCOGENE TONSL

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Genetic aberrations initiate malignant transformation of normal cells. Chromosome 8q24.3 (Chr. 8q24.3) amplification is one such recurrent genomic aberrations found in ~20% of breast cancer. Typically, amplification leads to up-regulation of a proto-oncogene residing in the amplicon and consequent aberration of downstream signaling. Genes within chr. 8q24.3 amplicon with oncogenic function were not reported until recently. We identified Tonsoku Like, DNA Repair Protein (TONSL) located within this amplicon as an immortalizing oncogene with an ability to transform primary breast epithelial cells when combined with defined oncogenes (Cancer Research in Press). We observed that TONSL amplified breast cancer cells are dependent on TONSL for tumor growth and these $TONSL^{High}$ tumors had upregulated homologous recombination (HR) DNA repair pathway, which may confer resistance to chemotherapy. Consistent with this possibility, higher level of TONSL protein in primary breast cancer, particularly Estrogen Receptor-positive (ER+) breast cancers, is associated with poor outcome. Although TONSL itself is not druggable because of lack of enzymatic activity, small molecules that disrupt TONSL interactome can be developed. Towards this goal, we performed immunoprecipitation of TONSL in TONSL-immortalized breast epithelial cells with and without DNA damage and subjected the immunoprecipitate to mass spectrometry. We identified two proteins that interacted with TONSL; ALYREF and ETV6. ALYREF is a RNA-binding protein involved in alternative splicing and cytoplasmic transport of specific mature mRNAs including short isoform of NEAT1, which promotes breast tumorigenesis. We also observed that TONSL itself induces the expression of ALYREF revealing previously uncharacterized role of TONSL in RNA biosynthesis machinery. ETV6 is a transcriptional repressor, and it is known to repress RAS signaling in acute lymphoblastic leukemia. ETV6 also binds to NCoR1, a component of the nuclear receptor corepressor complex, which keeps ER inactive in the absence of estrogen. Sensitivity of breast tumors to the antiestrogen tamoxifen is dependent on NCoR1. We are currently pursuing the hypothesis that by sequestering ETV6-NCoR1 complex, TONSL may increase ER and RAS activity. Study of these interactions will contribute to understanding of the role of chr. 8q24.3 amplicon in general and TONSL in particular in initiating/promoting breast tumorigenesis and development of drugs that target TONSL interactome.

Basic Science ***Graduate Student***

POSTER #29

ALCOHOL CONSUMPTION GENETICALLY ALTERS BREAST CANCER CELLS DRIVING THE BREAKAGE OF SYSTEMIC TUMOR DORMANCY

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Breast cancer remains a major cause of cancer-related deaths among women despite several advances in the field due to the problem of metastasis with a 5-year survival rate of less than 30% when the cancer has metastasized to distant organs. Dissemination of tumor cells to future metastatic sites has been shown to begin as early as the primary tumor is diagnosed at just 4mm in size. These disseminated cells remain dormant for extended periods of time evading immune surveillance and later turn into therapy resistant metastases which contribute to the poor prognosis in breast cancer patients. Hence, there is a **critical need** to improve our understanding of the cancer cell dormancy and metastatic programs and its contributors to develop better therapy options.

One such contributor is alcohol which is listed as a carcinogen by the National Toxicology Program. Alcohol consumption is a risk factor for several cancers and has been shown to increase the risk in a dose dependent manner in breast cancer. We have observed in preliminary studies, that alcohol consumption causes increased neutrophil extracellular trap (NET) formation in the lungs and outgrowth of previously dormant cancer cells in mice. Further, NETs have been shown to increase cancer cell seeding and play a role in metastasis. Hence, we **hypothesized** that alcohol consumption breaks cancer cell dormancy by activating neutrophils.

In this study, we have broken cancer cell dormancy and generated a novel cell line, **Alcohol-D2.OR**, by inducing outgrowth of the dormant D2.OR cells in mice through alcohol consumption. Reinjection of the Alcohol-D2.OR cells, into alcohol-naïve mice results in aggressive outgrowth of the cells suggesting these cells have now been modified on a genetic level. Indeed, RNA sequencing analysis of the gene expression in the cells shows that these cells have significantly modified gene expression as well as modified morphology and surface marker expression than the parental D2.OR cells. Importantly, from our analysis we have identified a novel tumor suppressor, SPINK5 which was significantly downregulated in the alcohol line. Further, SPINK5 expression in cancer cells suppressed neutrophil activity in vitro and knockdown of this gene in the parental D2.OR dormant line resulted in outgrowth of the cells in-vivo with increased lung NETs highlighting the importance of this gene for maintenance of dormancy by suppression of neutrophil activity.

Hence, we have successfully identified a novel gene responsible for dormancy maintenance, SPINK5 which will aid in not only therapeutic intervention but also in identification of potential metastatic progression in breast cancer patients. Further, the newly established formerly dormant **Alcohol-D2.OR** cells have a unique gene expression pattern providing us a novel tool to study other genetic mediators of dormancy breakage and initiators of metastasis in breast cancer.

Basic Science ***Graduate Student***

POSTER #30

THE ROLES OF FUMARASE IN CELLULAR RESPONSES TO DNA REPLICATION STRESS

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Conserved from bacteria to humans, the Krebs cycle enzyme fumarase catalyzes conversion of fumarate to malate in the mitochondria. Surprisingly, fumarase also functions in DNA replication stress responses in the nucleus. DNA replication stress activates the intra-S phase checkpoint either through the DNA replication checkpoint (DRC), which is triggered by intact stalled replication forks, or via the DNA damage checkpoint (DDC), which is activated by double stranded DNA breaks that can result from collapsed forks. Our lab has previously demonstrated that yeast fumarase is induced in the presence of replication stress and becomes enriched in the nucleus.The fumarase's "extra-mitochondrial"metabolic product fumarate suppresses DRC-dependent hypersensitivity to replication stress in yeast lacking the histone variant Htz1p. This suppression is mediated through the inhibition of the H3K4-specific, α-ketoglutarate-dependent demethylase Jhd2p. Our present studies demonstrate that fumarase similarly translocates to the nucleus in human cells upon exposure to replication stress, implying a conservation of function of fumarase during replication stress responses across evolution. To understand the roles of fumarase in cellular responses to DNA replication stress, we have employed DNA fiber analyses to assess individual replication forks. Our studies indicate that when cells expressing catalytically inactive fumarase are exposed to HU, replication forks become heavily resected compared to cells expressing wild-type fumarase. However, fumarase mutants retain the ability to restart stalled forks. We have identified chromosomal sites affected by fumarase in replication stress-dependent manner as well as nuclear factors that interact with fumarase. These and related findings on participation of fumarase in cellular responses to DNA replication stress will be presented.

Basic Science ***Graduate Student***

POSTER #31

ELUCIDATING THE MECHANISM BY WHICH LSD1, COREST2, AND STAT3 PROMOTE ENTEROENDOCRINE DIFFERENTIATION IN MUCINOUS COLORECTAL CANCER

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The colon epithelium contains a diversity of different cell types, each exhibiting unique functions to maintain homeostasis within the colon. Emerging from multi-potent stem cells are absorptive colonocytes, mucus secreting goblet cells, and hormone producing enteroendocrine cells (EECs). While much is known about how these cell types maintain homeostasis, how these cell types are altered in gastrointestinal diseases is not well understood. Mucinous colorectal cancer (CRC) accounts for upwards of 20% of CRC cases and is characterized by tumors with mucous accounting for at least 50% of the tumor volume. Interestingly, EEC progenitors have been shown to be enriched in mucinous CRC and promote cancer cell survival via secreted factors. Differentiation within the colon is regulated by a combination of epigenetic alterations and transcription factor activation. Our lab has previously discovered that the chromatin modifier and demethylase LSD1 promotes EEC differentiation in mucinous CRC; however, without a defined mechanism. To carry out its enzymatic function, LSD1 is typically found in transcriptional regulatory complexes. One such complex is the CoREST complex, which contains LSD1, HDAC1/2, and one of three CoREST paralogs. Using an enzymatic inhibitor that targets LSD1 and HDAC1/2 while in the CoREST complex, my preliminary data suggests that LSD1 promotes EEC differentiation while in the CoREST complex. The CoREST paralogs serve two primary functions within the complex: to act as a scaffold for LSD1 and HDAC 1/2 and to bind to DNA. Interestingly, single-cell RNA sequencing data from our lab indicates that CoREST2 is enriched in EECs. Furthermore, my preliminary data shows that CoREST2 promotes EEC differentiation, suggesting that LSD1 promotes differentiation in the CoREST complex with CoREST2. STAT3 is a transcription factor with known roles in promoting cell differentiation, including in spermatogonia and pancreatic beta cells. Interestingly, in both cases STAT3 promoted differentiation by binding to and increasing transcription of *NEUROGENIN-3*, a transcription factor that is essential for EEC differentiation. In my preliminary data, I have demonstrated that STAT3 promotes EEC differentiation. STAT3 can be regulated by various post-translational modifications including methylation. LSD1 was shown to demethylate STAT3 in response to interleukin-6, resulting in increased STAT3 transcriptional activity. My preliminary data demonstrates that STAT3 interacts with LSD1 and CoREST2 and is demethylated in a LSD1 and CoREST2 dependent manner. Therefore, I hypothesize that LSD1 and CoREST2 promote EEC differentiation by potentiating the binding of STAT3 to target genes through demethylating STAT3. Mucinous CRC is an aggressive and chemotherapeutically intractable form of CRC that is enriched for EEC progenitors. Since EEC progenitors promote cancer cell survival and LSD1 and STAT3 are therapeutically targetable, elucidating the mechanism by which LSD1 and STAT3 promote EEC differentiation may lead to new therapeutic options for patients with mucinous CRC.

Basic Science ***Graduate Student***

POSTER #32

TUMOR EPITHELIAL SECRETORY CELL AND MAST CELL INTERACTIONS IN BRAF MUTANT COLORECTAL CANCER.

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Colorectal cancer (CRC) is the third most common cancer worldwide and second most common cause of death in the US. CRC can be classified into different subtypes based on morphological and molecular differences. Activating mutations in the *BRAF* gene occur in approximately 10% of cases and represent one of the most aggressive subtypes of CRC. *BRAF* mutant CRC is characterized by an altered tumor immune microenvironment (TiME) and poor response to standard treatments. However, the causal connection between mutant *BRAF* and the TiME has not been fully explored. Our long-term goal is to find better therapeutic strategies to treat *BRAF* mutant CRC. Towards this goal, my objective is to understand how the TiME is regulated in *BRAF* mutant CRC. Interestingly, we have found preliminarily that human *BRAF* mutant CRC is enriched for mast cells (MCs), a type of granulocytic immune cell. Upon activation, MCs secrete factors that modulate the recruitment and activation of other immune cells leading to a change in composition of the TiME. A positive correlation between an altered TiME and tumor progression has been established in many cancers, suggesting a possible relationship between MCs, TiME and aggressiveness of BRAF mutant CRC. Our laboratory has previously shown that BRAF mutant CRC is enriched for secretory cell populations. These secretory cells, namely goblet cells and enteroendocrine cells, are known to interact with immune cells, including MCs, under normal physiological conditions in the colon, suggesting that secretory cells present in BRAF mutant CRC may also interact with immune cells in the TiME. My preliminary data demonstrates that secretory cells are involved in the recruitment of MCs towards media conditioned by *BRAF* mutant CRC cells in vitro. Based on our laboratory's published work and my preliminary data, **I hypothesize that, in BRAF mutant CRC, secretory cells alter the infiltration and activation of MCs, which in turn promote immune suppression and tumor progression**. To test this hypothesis, I will investigate the mechanisms by which secretory cells recruit and activate MCs in BRAF mutant CRC. Further, I will study the potential pro-tumorigenic role of MCs in BRAF mutant CRC while questioning the importance of secretory cell and MC interactions in this regard.

Basic Science ***Graduate Student***

POSTER #33

IDENTIFICATION OF TUMOR SUPPRESSIVE MICRORNAS IN ANGIOSARCOMA THROUGH CRISPR-CAS9 SCREEN

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Angiosarcoma is a rare and aggressive tumor resulting in a very poor prognosis for patients. MicroRNAs (miRNAs) are small non-coding RNAs, which can regulate gene expression and play important roles in a variety of cancer. Our previous study verified the involvement of miRNAs in angiosarcoma by showing that the loss of *Dicer1*, an enzyme involved in miRNA biogenesis, can lead to the development of angiosarcoma. In this study, we are performing a CRIPSR-Cas9 gRNA screen on a miRNA-focused library called LX-miRNA library, which can target 85% of human annotated miRNAs, to identify tumor-suppressive miRNAs in angiosarcoma. The LX-miRNA library will be transduced into human angiosarcoma cell lines expressing doxycycline (dox)-inducible Cas9. The cells will be treated with or without dox. For the in vitro screen, the cells will be allowed to grow for 25 days. For in vivo screen, Cas9 cells transduced with the library will be subcutaneously injected into immunodeficient mice. Mice will then be randomly divided and treated with and without dox for Cas9 expression. Resulting tumors will be monitored for tumor growth and potentially metastasis. For both the in vitro and in vivo screens, genomic DNA will be collected at the starting point and end timepoint. SgRNA amplicon sequencing will be performed to determine the change in the frequency of gRNAs to identify enriched sgRNAs for the identification of tumor-suppressive miRNAs. This work will provide a list of candidate tumor suppressing miRNAs for future investigation to understand the mechanisms of angiosarcoma development.

Basic Science ***Graduate Student***

POSTER #34

FGFR1 SIGNALING INHIBITS STAT1 PHOSPHORYLATION IN DORMANT BREAST CANCER CELLS

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Tumor cells can disseminate to different parts of the body and may either metastasize or undergo dormancy. Dormant cells are clinically undetectable and may remain asymptomatic for up to 20 years. Dormant cells can awaken to form metastases that may be resistant to therapeutics leading to a poor prognosis. Hence it is important to understand the factors related to dormancy and metastatic outgrowth. Immune cells play a vital role in preventing the metastatic outgrowth of dormant cells. IFN-γ released by T-cells binds to the IFN-γ receptor on dormant cells leading to STAT1 phosphorylation, dimerization, nuclear localization, and transcriptional activation of interferon-stimulated genes thereby maintaining the dormant phenotype. To study dormancy we have used D2.OR cells derived from mouse tumors that disseminate to different parts of the body but do not form metastases. From our preliminary experiments, we have found that the FGFR1 signaling can break the dormancy of D2.OR cells by activating SHP-2 a phosphatase that downstream activates ERK and Akt signaling pathways. Along with the activation of mitogenic signaling, SHP-2 inhibits STAT1 phosphorylation. Pharmacologically targeting SHP2 enhances STAT1 phosphorylation levels. We hypothesize that FGFR1 signaling harnesses SHP-2 signaling to counter-act the tumor-suppressive effects of immune cells to break dormancy.

Basic Science ***Graduate Student***

POSTER #35

CHARACTERIZATION OF THE PRC2-REGULATED PROTEIN ZNF423 IN NF1-RELATED MALIGNANT PERIPHERAL NERVE SHEATH TUMORS

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Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominant disorders with a predisposition to cancer, caused by inactivating mutations in the tumor suppressor gene *NF1*. The leading cause of death in NF1 patients is the development of malignant peripheral nerve sheath tumors (MPNST), a rare and highly aggressive sarcoma subtype, which typically emerge from the progression of benign plexiform neurofibromas (PNF) to pre-cancerous atypical neurofibromas (ANF) and is characterized by loss of the *CDKN2A/B* tumor suppressor loci. In addition to the loss of *NF1* and *CDKN2A/B*, MPNST patients exhibit a high frequency of loss-of-function mutations in either *EED* or *SUZ12*, which are genes encoding subunits of the polycomb repressive complex 2 (PRC2), a chromatin regulator that maintains gene silencing. To investigate the effect of PRC2 loss on MPNST gene expression, we restored *SUZ12* in two PRC2-deficient MPNST lines and performed RNA-sequencing, identifying fourteen common transcription factors downregulated by PRC2 reconstitution. Amongst those downregulated was *ZNF423*, a transcription factor expressed in numerous immature cell populations including neuronal and olfactory precursors, where it functions as a lineage-specific transcription factor by exerting differentiation-promoting functions. In our murine cells-of-origin, when only *Nf1* was lost, *ZNF423* (mouse *Zfp423*) was repressed, however, when both *Nf1* and *Arf* were lost, which occurs during progression of PNF to MPNST, *Zfp423* was significantly de-repressed. It remains unclear how the de-repression of ZNF423 is associated with malignant transformation to MPNST. We hypothesize that ZNF423 expression contributes to stemness and viability of MPNST. Hence, the objective of this study is to modulate the activity of ZNF423 to investigate its association with tumor progression from PNF to MPNST. Our preliminary experiments using shRNA targeting *ZNF423* in human MPNST lines indicated that ZNF423 reduction significantly reduced their viability and proliferation. Ongoing studies will further delineate the role of ZNF423 in human and murine MPNST models using omics approaches, cell-based phenotypic assays, and *in vivo* studies.

Basic Science ***Graduate Student***

POSTER #36

CRITICAL ROLE OF OGT-MEDIATED NOVEL NF-KB O-GLCNACYLATION IN PANCREATIC CANCER

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Pancreatic ductal adenocarcinoma (PDAC) has one of the highest mortalities of all malignancies, with a mere 5-year survival of ~10%. However, the poor outcomes of the current first-line treatment approach highlight the urgent need for innovative therapeutics. The nuclear factor κB (NF-κB) is a crucial transcription factor frequently activated constitutively in PDAC. It mediates the transcription of oncogenic and inflammatory genes that facilitate multiple PDAC phenotypes. Thus, a better understanding of the mechanistic underpinnings of NF-κB activation holds substantial promise in PDAC diagnosis and new therapeutics. The purpose of this study is to identify novel regulation of NF-κB, with the aim of providing new diagnostic and therapeutic strategies for PDAC. Here, we report protein O-GlcNAc transferase (OGT) - mediated NF-κB activation through novel serine O-GlcNAcylation of its p65 subunit. We show that overexpression of serine-to-alanine (S-A) mutant at the O-GlcNAcylation site impaired NF-κB nuclear translocation and transcriptional activity in PDAC cells. Moreover, these S-A p65 mutants downregulate NF-κB-target genes important to major cancer hallmarks and inhibit cellular proliferation, migration, and anchorage-independent growth of PDAC cells compared to WT-p65. Interestingly, this modification happens downstream of the inhibitor of NF-κB (IκBα) degradation. Collectively, we have identified OGT-mediated serine O-GlcNAcylation of NF-κB and determined its mechanistic and cellular function in driving PDAC phenotypes. Thus, our study holds great significance as it uncovers a brand-new strategy for PDAC diagnosis and effective therapeutics development.

Basic Science ***Graduate Student***

POSTER #37

IDENTIFICATION OF UNDERLYING MECHANISM OF NEUROENDOCRINE PROSTATE CANCER DEVELOPMENT

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Prostate cancer is the second most leading cancer disease in men. Due to the disease's dependent sensitivity of androgen receptor (AR) signaling pathway, androgen deprivation therapy (ADT) and androgen signaling pathway inhibitors (ASI) are the main clinical treatment for prostate cancer (PCa) patients. Along with the emergence of ADT/ASI treatments, 17-30% of patients had recurred disease, called neuroendocrine differentiated prostate cancer (NEPC). Treatment-induced neuroendocrine prostate cancer (t-NEPC) is the most aggressive variant of prostate cancer that may arise in prostate cancer patients treated with ADT/ASI as a mechanism of resistance. Unfortunately, the mechanism behind resistance to ADT/ASI treatment and the development of NEPC is still unknown. To have a better molecular understanding of NEPC development, a novel NEPC mouse model is in urgent need. Hu Lab aims to develop a novel t-NEPC mouse model to mimic clinical settings and identify the role of protein arginine N-methyltransferase 5 (PRMT5) in the initiation and maintenance of neuroendocrine differentiation (NED) in PCa after hormone therapy.

Basic Science ***Graduate Student***

POSTER #38

STIFF AND FAST-RELAXING HYDROGEL TO PROBE PANCREATIC DUCTAL ADENOCARCINOMA CELL BEHAVIOR

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Pancreatic adenocarcinoma (PDAC) has been reported to exacerbate as the stiffness of the tumor increases over time. The dense extracellular matrix (ECM) enhanced cancer cell proliferation and induced chemoresistance. Additionally, the viscoelastic and stress-relaxing nature of the ECM facilitates migration and invasion of some cancer types, but the impact of matrix viscoelasticity on PDAC cell behavior remains elusive. The goal of this work is to prepare hydrogels with high stiffness and fast relaxation to mimic PDAC stromal tissue. To this end, gelatin was dually functionalized with norbornene (NB) and boronic acid (NB), yielding GelNB-BA. The NB moieties readily crosslink with 4-arm PEG-thiol via thiol-norbornene chemistry to create hydrogels with tunable stiffness. On the other hand, the BA motifs complex with diol-containing polymers (e.g., poly(vinyl alcohol) or PVA) to afford tunable stress-relaxation rate. However, we found that the use of 1,3-diol containing PVA did not yield fast relaxation in GelNB-BA hydrogels. In this work, we synthesized a new 1,2- diol-containing linear polymer via Reverse-Addition Fragmentation Chain-Transfer (RAFT) polymerization. Briefly, N-hydroxyethyl acrylamide (HEAA) was polymerized into poly-HEAA (PH), which afforded hydroxyl groups for reacting with dihydroxyphenyl acetic acid (DOPAC) to yield PolyDOPAC (PHD). The 1,2-diols on PHD readily formed boronate diols with BA on GelNB-BA to significantly increase the viscoelasticity of the hydrogels. Utilizing the new dynamic viscoelastic hydrogel system, we demonstrated how fast-relaxing hydrogels could influence pancreatic cancer cell fate. We hypothesize that viscoelastic matrix allows dissipation of cell traction forces which might lead to less mechanically activated and aggressive cancer phenotype than those encapsulated in elastic hydrogels.

Basic Science ***Graduate Student***

POSTER #39

PP2A-B56A DRIVES EGFR ACTIVATION IN A NOVEL ROLE IN PANCREATIC CANCER

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Pancreatic ductal adenocarcinoma (PDAC) stands to become the second most deadly cancer by 2030. Over 90% of PDAC patients have driver mutations in the small GTPase, KRAS. The most common KRAS mutation in PDAC, KRASG12D, has yet to be successfully therapeutically targeted, thus there is a critical need for alternative therapeutic targets to improve patients outcomes. Protein phosphatases are master regulators of cellular signaling cascades. Protein phosphatase 2A (PP2A) is a large family of Ser/Thr phosphatases which negatively regulate many downstream targets of KRAS. Small molecule activators of PP2A have emerged as promising anti-cancer agents. Studies have shown that the pharmacological activation of PP2A suppresses oncogenic PDAC signaling pathways, however this response is heterogeneous, suggesting that PP2A may have unique functions in PDAC. Historically, studies have approached PP2A as one entity despite the fact that PP2A encompasses over 90 distinct complexes. However, as the individual roles of specific subunits are interrogated, tissue and context specific roles for PP2A have now emerged. The subunit B56a has been previously implicated as a tumor suppressor but has remained understudied in PDAC.

Using pharmacological activation of PP2A, as well as overexpression and knockdown studies, we have identified a novel role for PP2A-B56a in epidermal growth factor receptor (EGFR) signaling in PDAC. EGFR signaling is a significant signaling node in PDAC: EGFR expression is increased during PDAC progression and loss of EGFR prevents mutant KRAS-driven pancreatic tumorigenesis. Activation of PP2A-B56a in PDAC cell lines leads to the suppression of specific oncogenic pathways, however, is this accompanied with an increase in oncogenic phenotypes and EGFR activation through increased expression and processing of EGFR ligands.

Basic Science ***Graduate Student***

POSTER #40

THE TARGET GENE REGULATORY NETWORK OF MIR-497 IN ANGIOSARCOMA

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Angiosarcoma (AS) is an aggressive, highly metastatic vascular cancer with a 5-year survival rate of 30 percent. Our lab previously found that endothelial-specific deletion of *Dicer1* drives AS development in mice. Given the essential role of DICER1 in canonical microRNA (miRNA) biogenesis, this finding suggests that miRNAs may be important in AS development, therefore warranting further research into their role. MiRNAs have been implicated in several other cancers, however, their role in AS has not been studied extensively. After testing several miRNAs previously suggested to have a tumor-suppressive role in AS, microRNA-497-5p (miR-497) suppressed cell viability most significantly in AS cell lines. Therefore, we hypothesized that miR-497 overexpression would reduce other cancer phenotypes, and downregulate target genes that promote AS pathogenesis. In other cancers, miR-497 is generally reported to be downregulated in malignant tissue compared to normal and has been suggested to have a tumor and metastasis-suppressing role. In addition to the observed effects on cell viability, we also observed that miR-497 expression leads to increased apoptosis and inhibited cell migration in AS cells and that miR-497 expression reduced tumor formation in a subcutaneous murine AS model. To better understand the mechanism of how miRNAs elicit phenotypes, it is pertinent to identify clinically relevant target genes. Using a combination of RNA-sequencing data in an AS cell line overexpressing miR-497, expression data from AS patient tumors, and target prediction algorithms, we identified four clinically relevant miR-497 target genes for further study, and have validated that miR-497 directly regulates these target genes. Based on previous literature and our preliminary gain- and loss-of-function experiments, we have found that one of the miR-497 target genes, *Vat1* may play a role in migration and metastasis in AS. This work will give insight into the role of miR-497 and its target genes in AS pathogenesis.

Basic Science ***Graduate Student***

POSTER #41

ROLE OF RAN-GTP GRADIENT IN KINESIN-14 MEDIATED SPINDLE ORGANIZATION

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The mitotic spindle is a dynamic structure that facilitates chromosome segregation during cell division. Spindle assembly is tightly controlled as errors in this process can result in cancer development. The spindle is composed of microtubules (MTs), MT-associated proteins, and molecular motors, which are important for spindle organization, spindle MT dynamics, and aligning/segregating chromosomes. An important feature in spindle assembly is the Ran-GTP gradient. High Ran-GTP levels promote MT nucleation and organization near chromatin by releasing the spindle assembly factors (SAFs) from importin-mediated inhibition. One important Ran-regulated SAF is the Kinesin-14 (K-14) motor that crosslinks and slides anti-parallel and parallel MTs. K-14s have an ATP-sensitive motor domain that walks toward MT minus ends and a tail domain that binds MTs. We previously showed that importins bind to a nuclear localization sequence (NLS) in the tail that inhibits anti-parallel MT cross-linking. Recently, we identified two independent MT binding domains (MBD1 and MBD2) within the K-14 tail with MBD1 being regulated by the importins. These results suggest antiparallel cross-linking is mediated by MBD1 and that the two modes of K-14 MT crosslinks are differentially regulated by the Ran gradient. Ran levels are higher in cancer cells with increased genomic content, and we showed previously that high Ran recruits more K-14 to the spindle. In addition, loss of K-14 has little effect on normal cells but is lethal to cells with centrosome amplification, suggesting it may be an effective therapeutic target. To ask whether there are K-14-specific cancer mutations, we analyzed the cancer patient mutation database, cBioPortal, and identified two mutations in MBD1, which we postulate could disrupt K-14 MT crosslinking. These mutations were generated in the human K-14 (hK-14) MBD1 and used to determine how they affected MT binding and importin affinity. Surprisingly, we found that these mutants had reduced importin affinity, without greatly compromising MT binding. These mutations could thus provide us with a tool to dissect how Ran regulation specifically impacts K-14 function. One powerful system to understand Ran regulation is the *Xenopus* spindle assembly system where Ran levels can be manipulated and its role in spindle assembly can be separated from nuclear/cytoplasmic transport. We hypothesized that mutations in hK-14 would be functionally conserved in *Xenopus* K-14 (xK-14). To test this idea, we generated the corresponding mutations in the xK-14 MBD1, determined their importin and MT binding, and found that xK-14 mutants had reduced importin affinity without significantly inhibiting MT binding similar to hK-14. We are currently generating these mutations in full-length hK-14 for expression in cells and in full-length xK-14 for use in *Xenopus* spindle assembly to understand how the Ran-GTP gradient modulates K-14 activity to provide insights into how these patient mutations may impact spindle organization and function.

Basic Science ***Graduate Student***

POSTER #42

FIBROBLAST GROWTH FACTOR RECEPTOR 1 AS A DRIVER OF OBESITY-ASSOCIATED MAMMARY EPITHELIAL CELL TRANSFORMATION IN BREAST CANCER

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Breast cancer is driven by dysregulation of cell signaling pathways via acquisition of genetic and epigenetic alterations. Among these alterations, receptor tyrosine kinases (RTKs) are important targets of therapeutic intervention. Indeed, the clinical success accompanying the inhibition of the amplified RTK HER2 in breast cancer not only recognized it as an oncogenic 'driver', but also encouraged the development of drugs targeting other amplified RTKs. Like HER2, Fibroblast growth factor receptor 1 (FGFR1) is amplified in ~13% of breast cancers. However, unpredictable clinical response to FGFR1 inhibitors suggests that other factors besides receptor amplification need to be considered when selecting patients who could best benefit from these recently approved therapeutics. The long-term objective of our study is, therefore, to understand the factors which regulate FGFR1-amplification induced mammary cell transformation and consequent metastases. Our preliminary in-vitro findings suggest the necessity of FGF2 ligand in regulating FGFR1-induced transformation. However, pathological risk factors contributing to FGF2 ligand secretion in-vivo need further investigation. One such factor potentially contributing to FGF2 ligand secretion is obesity. Indeed, accumulating evidence suggests that obesity increases the risk of breast cancer progression, but the underlying mechanisms remain unclear. Previous studies have suggested elevated secretion of FGF2 by visceral adipose tissues. Considering the ligand induced transformation observed in our in-vitro experiments, we hypothesize that obesity is a contributing factor to FGFR1-regulated transformation via elevating the serum levels of FGF2. This study will assist in identification of patient population which could best benefit from the use of FGFR kinase inhibitors in clinic.

Basic Science ***Graduate Student***

POSTER #43

GCN2 INHIBITION SENSITIZES PCA CELLS TO MCL-1 INHIBITION

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The Integrated Stress Response (ISR) enhances cellular adaptation and survival in response to a diverse set of stresses. This stress response pathway is mediated by four eIF2 kinases, each of which sense distinct cellular stresses and phosphorylate eIF2α to facilitate translational and transcriptional reprogramming of gene expression to enhance adaptation to the underlying cellular stress. Our lab previously reported that prostate cancer (PCa) cells rely on the eIF2 kinase GCN2 for maintenance of amino acid (AA) homeostasis and cell growth. GCN2 regulates the expression of AA transporters allowing for enhanced import of essential amino acids critical for PCa growth. Inhibition of GCN2 through genetic or pharmacological means results in decreased expression of AA transporters, reduced levels of intracellular AAs, and decreased proliferation in both in vitro and in vivo PCa models. However, despite a reduction in proliferation following GCN2 inhibition, loss of GCN2 function resulted in senescence and not substantial cell death. As such, we hypothesize that GCN2 inhibition will sensitize PCa cells to senolytic agents and that this combination potentially provides a novel therapeutic treatment strategy for PCa.

We demonstrate that genetic or pharmacological inhibition of GCN2 induces the expression of known senescent markers in cell-line and patient-derived PCa xenograft models. Furthermore, we show increased SA-beta-galactosidase staining in GCN2 KO tumors compared to WT tumors supporting the idea that loss of GCN2 results in a senescence phenotype in PCa tumors. To test the hypothesis that GCN2 inhibition may sensitize PCa cells to senolytic agents, we utilized models of androgen-sensitive and castration-resistant PCa and non-tumorigenic prostate epithelial cell lines. GCN2 inhibition induced a static phenotype in PCa cell lines, but not non-tumorigenic prostate epithelial cells, and this was accompanied by expression of senescent markers. Using small molecular inhibitors and siRNAs that target the Bcl-2 family of anti-apoptotic proteins (BCL-2, BCL-w, BCL-xL, and MCL-1), we found that knockdown or inhibition of MCL-1, and not the other Bcl-2 family proteins, robustly decreased cell growth in GCN2-null PCa cell lines. Additionally, combination of MCL-1 and GCN2 inhibition resulted in increased cell death and apoptosis compared to either single treatment alone.

Our results suggest that GCN2 inhibition results in a senescent phenotype in PCa cells, which renders these cells vulnerable to selective inhibition of MCL-1. We propose that targeting GCN2 in combination with MCL-1 inhibition may be an effective strategy for treatment of PCa.

Basic Science ***Graduate Student***

POSTER #44

UNDERSTANDING THE ROLE OF CIS-FACTORS REGULATING TRANSLATION OF MYC IN RESPONSE TO ASPARAGINE STARVATION

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Our previous study in acute lymphoblastic leukemia (ALL) cells showed that asparagine bioavailability (through *de novo* biosynthesis or exogenous sources) is essential to maintain the expression of the critical oncogene c-MYC. c-MYC is a potent transcription factor and is dysregulated in over 60% of cancers, including hematopoietic malignancies. We showed that this regulation by asparagine is primarily at the translation level and c-MYC expression is rescued only when exogenous asparagine is available or when cells can undertake *de novo* biosynthesis. At the biochemical level, asparagine depletion also causes an induction of ATF4 mediated stress response and suppression of global translation mediated by decreased mammalian target of rapamycin complex 1 (mTORC1) activity. However, we found that neither inhibition of the stress response or rescuing global translation rescued c-MYC protein expression.

To understand the role of cis-factors regulating c-MYC translation we used SF188 cells, a pediatric glioblastoma cell line driven by *c-MYC* amplification. We knocked-out asparagine synthetase gene (*ASNS*) to make these cells auxotrophic for asparagine. Unlike the parental cells, these $ASNS^{KO}$ SF188 cells die when asparagine is limiting in the media. Similar to our observations in ALL cell lines, $ASNS^{KO}$ SF188 cells also cannot sustain c-MYC protein expression in asparagine limiting conditions due to a translation defect. Using these cells as a model, we built a fluorescent reporter system which has the of c-MYC N-terminal leader sequence fused to an enhanced yellow fluorescent protein (eYFP) reporter. Lack of charged tRNAs has been shown to inhibit ribosomal movement and activate the stress response pathway and we believe the high frequency of asparagine residues in the N-terminal of c-MYC could be rate-limiting for c-MYC mRNA translation. Our reporter also has a destabilizing domain attached to the eYFP which ensures rapid proteasomal degradation. This fluorescence is sustained only when a small molecule is added to stabilize the construct. This will allow us to ensure that the fluorescence signal is a readout of translation. We will also use the reporter without N-terminal leader sequence as a readout for global translation.

Our initial results using this system showed an acute and remarkable decrease in fluorescence of the MYC-eYFP reporter when asparagine is limiting (4.4 fold decrease). Our results with the eYFP reporter showed only a moderate decrease in global translation (1.4 fold). This suggests that we c-MYC N-terminal sequence is an important cis-factor regulating c-MYC mRNA translation. Our ongoing work includes to correlate these changes at the molecular level by looking at c-MYC protein levels in these cells. We will also verify some of the other molecular signatures that we observed in ALL cells, including stress pathway activation and mTORC1 suppression. We will eventually use this model for a CRISPR screen to identify trans acting factors regulating c-MYC translation.

Basic Science ***Graduate Student***

POSTER #45

KRAS-MEDIATED SUPPRESSION OF PP2A-B56A PROMOTES PANCREATIC TUMORIGENESIS

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Protein phosphatase 2A (PP2A) is a major serine/threonine phosphatase that regulates many cellular pathways including KRAS, a protein 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 expression of PP2A inhibitors, in PDAC cell lines. These studies suggest that suppression of PP2A activity may be important in PDAC maintenance. While global PP2A has tumor suppressive capabilities, the regulation of specific pathways by PP2A can 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. Together, these studies identify PP2A as a critical regulator of KRAS-induced tumorigenesis and suggest that therapeutic reactivation of PP2A may be a novel therapeutic strategy in PDAC patients.

This project is interdisciplinary through incorporation of molecular signaling, cell biology, use of in vivo mouse models, and addressing of potential therapeutics. In this project, I have interrogated how KRAS mutation effect oncogenic signaling and PP2A protein-protein interactions. Additionally, how these effects are conserved in cells as well as in mouse tissues from genetic mouse models and ex vivo 3D cultures. Finally, I have tested the therapeutic efficacy of an activator of PP2A tumor suppressor activity in ex vivo pancreatic explants.

Basic Science ***Graduate Student***

POSTER #46

TARGETING HSF1 TO PREVENT ITS SUPPORT OF ONCOGENES IN OVARIAN CANCER CELL LINES

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Heat shock factor 1 (HSF1) is a stress response transcription factor that upregulates heat shock proteins (Hsps). HSF1 cooperates with pro-tumor proteins such as HSP90 and MYC wherein HSF1 is activated in response to Hsp90 inhibitors and HSF1 is critical to MYC transcriptional activity. One HSF1 partner is c-MYC in ovarian cancer where these two genes are co-amplified in 36% of patients according to data from The Cancer Genome Atlas (TCGA). We observed that inhibition of PLK1, which is a kinase that enhances both MYC and HSF1 activity, led to the downregulation of MYC and HSF1 and suppressed cell viability when these genes are amplified. HSP90 inhibitors have largely failed but the Carpenter lab has found that certain Hsp90 inhibitors lead to HSF1 activation. One such inhibitor, BI6727 (Volasertib), is an ATP-competitive PLK1 inhibitor that was employed on 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). We have also found that inhibition of HSF1 led to enhanced sensitivity to Hsp90 inhibition. To test this inhibition, we employed a combinatorial therapeutic approach using leading drug candidates DTHIB and 17-DMAG that inhibit HSF1 and could reduce ovarian cancer cell viability. To do this, MTT cell viability assays were also conducted on ovarian cancer cell lines with MYC and HSF1 gene amplification (OVCAR8) and combinatorically treated with DTHIB and 17-DMAG to determine if they work synergistically to reduce ovarian cancer growth. These results have important implications for the dual targeting of MYC and HSF1 in ovarian cancer and can be applied for testing combinatorial treatment strategies with the current standard of care chemotherapeutic carboplatin to combat chemoresistance. HSF1 is likely to be a significant contributor to therapies targeting its cooperating partners. Consequently, blocking HSF1 will likely aid in the efficacy of therapies targeting these HSF1 cooperating partners.

Basic Science ***Graduate Student***

POSTER #47

EFFECT OF PEPTIDERGIC SENSORY NERVE ABLATION ON THE PROSTATE.

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The prostate cancer tumor microenvironment (TME) contains a heterogenous and diverse cell population that contributes to treatment resistance. Often-ignored within this microenvironment is innervation of the tissue by sensory and autonomic nerves. Previous studies have shown that the prostate is densely innervated by autonomic nerves, which control normal prostate function and have been implicated in promoting prostate cancer proliferation and metastasis. While sensory nerve-associated neuropeptides, such as tachykinins and calcitonin gene-related peptide (CGRP) are present in the prostate, no studies have demonstrated contiguous sensory innervation of the prostate, nor have they examined a functional role of peptidergic sensory nerves (PSNs) in the prostate and in prostate cancer. PSNs are critical for many physiological processes outside of nociception, including immune regulation, vascular patterning, stem cell regulation, bone homeostasis, and tissue repair and recovery. Therefore, we hypothesize that peptidergic sensory nerves are integral to the homeostatic regulation of the prostate.

Methods

To evaluate the microarchitecture of PSNs, prostates were harvested from transgenic mice expressing EGFP driven by the CGRP promoter (Calca-fEGFP) and fixed with 4% paraformaldehyde. Tissues were immunolabeled and processed through a modified ethyl cinnamate-based optical tissue clearing protocol and imaged by confocal microscopy. To determine the functional significance of PSNs, we selectively ablated PSNs by administering diphtheria toxin to transgenic mice that specifically express the diphtheria toxin receptor on PSNs. Sensory nerve ablation was confirmed by deficiencies in response to nociceptive thermal stimuli and by immunofluorescence imaging of dorsal root ganglia (DRG). Prostates from PSN-ablated animals were harvested for tissue clearing and paraffin embedding followed by hematoxylin and eosin staining for histomorphological assessment.

Results

Continuous, tortuous, GFP+/CGRP+ nerves fibers are seen in 50–100μm thick volumes in immunolabeled, cleared, prostate lobes. Punctate CGRP signals are dispersed along continuous GFP+ fibers indicative of large, peptidergic dense core vesicles in PSNs. PSN-ablated animals showed increased latency to thermal stimuli and decreased number of CGRP+ DRG cell bodies compared to wildtype control animals.

Conclusions

Immunofluorescence labeling with ethyl cinnamate-based tissue clearing has enabled us to clearly delineate the microarchitecture of PSN-neuronal tracts interwoven around prostatic acini. The highly organized structure of innervating PSNs proximal to the epithelial glands suggests that PSNs play a role in normal prostatic function. In accordance with previous findings in 2D tissue sections, PSNs are also in the stroma between acini, however, the abundance and organization of PSN fibers was a surprising finding. We have been able to successfully ablate PSNs while the effects of PSN-ablation on prostate histomorphometry warrants further characterization.

Basic Science ***Graduate Student***

POSTER #49

POSTDOCTORAL RESEARCH FELLOW

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Integrin-linked kinase regulates ovarian cancer stemness through interaction with the frizzled 7 receptor

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Abstract

Ovarian cancer (OC) is the fifth leading cancer among women in the United States, and the first leading cause of cancer-related deaths from gynecologic malignancies. OC cells have high proliferative capacity, are invasive, resist apoptosis, and tumors often display rearrangement of extracellular matrix (ECM) components, contributing to accelerated tumor progression. The metastatic progression is mediated by exfoliation of tumor cells into the peritoneal cavity, the formation of compact OC spheroids, and their implantation onto the overlying peritoneal surface. Spheroid formation is promoted by a change of cell adhesion properties, enhanced secretion of ECM components, such as fibronectin (FN), that stiffen the tumor stroma, promote the aberrant activation of integrins, and recruit the adaptor protein integrin-linked kinase (ILK). ILK drives cytoskeletal assembly and modulates key processes, including survival, invasion, and stemness. However, the functional role of ILK in chemoresistance of ovarian cancer stem cells (OCSCs) remains incompletely understood. The hypothesis is that the formation of FN-integrin complexes at the cell membrane promotes survival of OCSCs and supports OC spheroid formation by activating ILK and downstream oncogenic signaling.ILK, FN, and integrin β1 *m*RNA expression levels were increased in OCSCs compared with non-CSC ($P<0.001$) and enriched in OC spheroids compared with monolayers ($P<0.01$). ILK blockade with the specific inhibitor cpd-22 suppressed spheroid proliferation, tumor initiating capacity in xenograft mouse model, and altered key oncogenic signaling pathways, in particular decreased β-catenin signaling essential to sustaining cancer cell stemness. The Wnt receptor Frizzled 7 (Fzd7) showed the greatest difference in gene expression, indicating a direct correlation between ILK activation and Wnt signaling. By co-immunoprecipitation and proximity ligation assay, we demonstrated that ILK co-localizes with Fzd7 in OC spheroids. Mechanistically, the combination of carboplatin and ILK-Fzd7 blockade decreased the β-catenin signaling, inhibited phospho-Akt at Ser473 and increased levels of cleaved-caspase-3 compared to single agent alone, resulting in apoptosis. By regulating β-catenin signaling, Fzd7 expression and ILK activation are essential for OCSCs survival. Targeting Fzd7/ILK clusters may represent new therapeutic approach to eradicate OCSCs.

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

POSTER #50

TUMOR MICROENVIRONMENT INDUCED C-JUN PROMOTES OVARIAN CANCER METASTASIS

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Ovarian cancer (OC) is the most lethal 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. However, the regulation of OC metastasis, especially metastatic colonization, which is the rate limiting step, is still poorly understood. The regulation of OC metastasis, especially metastatic colonization, which is the rate limiting step, is still poorly understood. 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 the c-Jun as a key transcription factor increased in metastasizing OC cells when they interacted with the tumor microenvironment. ATAC-seq revealed that the microenvironment induced changes in OC chromatin accessibility. The top TFs predicted to bind to the newly open chromatin regions were Fos and c-Jun, further confirming the important role of c-Jun during metastasis. Fos and Jun combine to form AP-1 to regulate transcription. A c-Jun CUT&RUN-seq in HGSOC cells seeded on the 3D culture model identified 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. Mass spectrometric analysis of the secretome of omental mesothelial cells (HPMC), CAFs, and normal omental fibroblasts (NOF), identified key factors involved. 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 vivo*. Taken together, our studies reveal that microenvironment-induced upregulation of c-Jun, combined with the microenvironment-induced opening of the chromatin of certain c-Jun binding sites 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 HGSOC metastasis.

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

POSTER #51

RNA-BASED MULTIFUNCTIONAL NANOPARTICLES FOR SPECIFIC DELIVERY OF THERAPEUTICS INTO CANCER CELLS

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One of the bottlenecks in cancer treatment is the lack of specific drug delivery approaches. In clinical treatment, chemotherapy is usually administered orally or injected into the cavity, vein, or muscle. However, the disadvantages of poor bioavailability from biological barriers and ubiquitous chemotoxicity limit the optimal therapeutic effects. In our lab, we seek to develop an innovative delivery system based on RNA Three-Way-Junction (3WJ) core loaded with multiple therapeutics for specific delivery into cancer cells.

In our design, the delivery system comprises 4 essential modalities: 1) 3WJ domain based on the bacteriophage phi29 packaging as a multifunctional core; 2) aptamer which is a single-stranded oligonucleotide (tridimensional folded structure) that can bind specifically to its target biomarkers on the cell surface and initiate the internalization process; 3) siRNA gene therapy, and 4) chemically modified FDA-approved chemotherapy drug.

As a demonstrative application, we utilize multiple myeloma (MM) cancer cell lines. CD38 is a known therapeutic target for MM treatment. The CD38-specific single-stranded aptamer was developed and showed specific binding to MM cells with high affinity[1]. So, we generate CD-38 aptamer conjugated to RNA 3 WJ core (R-a38) and assess the binding of the nanoparticles to the cells by flow cytometry. In our experiment, we apply the JJN3 MM cell line that highly expresses CD38 and K562 cells that do not express CD38 as a control group.

Next, it has been reported that siRNA molecules can still be recognized by Dicer after being linked to RNA nanoparticles.[2] Therefore, we expect that Dicer will recognize the conjugated dsRNA, and the processed single-stranded siRNA-Dicer complex will bind to the targeted complementary mRNA and form the RISC complex, leading to the mRNA degradation and gene silencing. To validate the system, we apply a well-studied *β2 macroglobulin (siB2M) mRNA as a model and synthesize both sense and anti-sense strands. Consequentially, we generate CD38 aptamer-conjugated RNA nanoparticle (R-a38) loading siRNA for gene silencing. The knockdown of siB2M will be verified by western blot.* As a complementary assay, we will conjugate the RNA nanoparticle with Cy5 fluorophore to track the specific delivery using a confocal microscope and flow cytometry.

Finally, we will conjugate the R-a38 with the FDA-approved proteasome inhibitor Bortezomib for MM treatment (R-a38-Bort). Based on the active boronic acid moiety in Bortezomib, we will perform organic synthesis to make the Bortezomib prodrug bearing boronic ester group. On the other hand, the modified RNA oligonucleotides will be solid-phase synthesized, and the 2′-alkyne derivatizations will be introduced to selective positions. Under appropriate metal-facilitated catalysis, the classic Click reaction will occur between the azido and the alkyne groups to form a 5-membered heteroatom ring[3], and subsequently, Bortezomib prodrug is covalently linked to the alkyne-modified RNA to form Prodrug-RNA conjugate.

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

POSTER #52

TARGETING LOSS OF THE TUMOR SUPPRESSOR TENT5C IN MULTIPLE MYELOMA

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Introduction: Multiple myeloma (MM) is a genetically complex and heterogeneous neoplasm in which multiple genomic events lead to tumor development and progression.

TENT5C mutation or deletion on chromosome 1p results in its loss in more than 20% of newly diagnosed MM patients and is associated with a poor prognosis. Patients with abnormalities of *TENT5C* have a decreased progression free survival and overall survival. In addition, these abnormalities are not present in smoldering MM patients indicating a role in disease progression. Although, many studies have identified *TENT5C* as a tumor suppressor gene in MM, the impact of gene loss on MM physiopathology remain to be elucidated.

Our previous RNA-seq data analysis of patient samples identified an association of biallelic *TENT5C* abnormalities with increased alternative RNA splicing and high expression of a non-homologous end joining (NHEJ) DNA repair signature that is also associated with poor prognosis.

Methods: To further investigate these results, we generated CRISPR/Cas9 knockout (KO) of *TENT5C* in wild type (wt) U266 and KMS-11 MM cell lines and over-expressed (oe) *TENT5C* in MM cell lines with biallelic inactivation (H929 and LP-1). Cell cycle analysis was performed on the modified cells. Targeted and high-throughput drug screens (HTS) were performed as we hypothesize that targeting synthetic lethal pathways related to loss of *TENT5C* in MM will result in specific cell death.

Results: MM cell cycle analysis, 24 hours after the release from cell synchronization in G2/M phase using nocodazole, showed that cells with loss of *TENT5C* had an increased G2/M proportion compared to cells with *TENT5C* present (U266 $TENT5C^{wt}$ 2.6% vs. $TENT5C^{ko}$ 19.86%, $p<0.001$; H929 $TENT5C^{-/-}$ 8.22% vs. $TENT5C^{oe}$ 1.18%, $p<0.001$), indicating an increase in cell cycle progression in *TENT5C* negative cells.

Given our prior data indicating an increase in splicing abnormalities and NHEJ activation in biallelic loss *TENT5C* patient samples, we performed specific growth inhibition curves using appropriate drugs. The spliceosome inhibitor pladienolide B affected KO cells at lower concentrations than wt cells (U266 $TENT5C^{wt}$ GI_{50}=1.34 vs. $TENT5C^{ko}$ GI_{50}=0.68 nM, $p<0.001$) as did NHEJ inhibitors (U266 $TENT5C^{wt}$ GI_{50}=113.83 vs. $TENT5C^{ko}$ GI_{50}=42.93 μM, $p<0.01$). A HTS has also identified several hits that are undergoing structural and purity analysis. Hits will be validated in other cell lines to ensure target specificity.

Conclusion: Our results indicate a tumor suppressor role of *TENT5C* in MM, with antiproliferative properties and a susceptibility to spliceosome and NHEJ inhibitors. The HTP screen will identify more potential targets leading to the identification of the molecular mechanisms underlying this function.

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

POSTER #53

FGFR1 SIGNALING FACILITATES OBESITY-DRIVEN PULMONARY OUTGROWTH IN METASTATIC BREAST CANCER

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Distant organ metastasis drives breast cancer mortality. Almost 30% of women originally diagnosed and treated for early-stage breast cancer will eventually develop distant organ metastasis. Survival of dormant and therapeutic resistant disseminated tumor cells contributes to the prolonged tumor relapse and metastatic disease progression. Our understanding of the factors that contribute to tumor dormancy and metastatic outgrowth remain incomplete, preventing our ability to advise patients on lifestyle habits that could contribute to sustained tumor dormancy and prolonged periods of remission. Recent meta-analyses suggest that obese women (BMI>35) have 40% more risk to develop metastasis at 10 years follow up compared to normal weight women. Therefore, we sought to investigated the effect of diet-induced obesity (DIO) on primary tumor growth and metastatic progression formation using metastatic and dormant breast mouse models. We developed a novel protocol to induce obesity in Balb/c mice by combining dietetic and hormonal interventions with outside temperature adjustment strategy. Our findings show that in contrast to standard housing conditions, ovariectomized Balb/c mice fed a 60% fat diet under thermoneutral conditions with become obese over period of 10 weeks resulting in a 250% gain in fat mass. This

approach led to increased primary tumor formation and pulmonary metastasis using the 4T1 model. More importantly, obese mice injected with dormant D2.OR cells developed macroscopic pulmonary nodules compared to mice fed a lean diet. Analysis of the serum from lean and obese mice revealed increased levels of the fibroblast growth factor 2 (FGF2). Using dormancy-inducing 3D culture and *in vivo* approaches we demonstrate that serum from obsess animals, exogenous FGF2 stimulation, or constitutive expression of FGF2 are sufficient to break dormancy and drive metastatic progression in FGFR1 expressing D2.OR cells. Overall, our studies have resulted in a novel model of diet-induced obesity that allowed to define the importance of FGF2:FGFR1 signaling as a key molecular mechanism connecting obesity to the breakage of tumor dormancy, leading to metastatic progression.

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

POSTER #54

IMPACT OF A NEW GENERATION OF KU-DNA BINDING INHIBITORS ON THE DNA DSBS-INDUCED DNA DAMAGE RESPONSE.

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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). We have pursued chemical inhibition of DNA-PK kinase activity as a therapeutic strategy for the treatment of cancer by targeting the Ku 70/80 heterodimer interaction with DNA. 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 therapies including bleomycin, etoposide and ionizing radiation. In this study, we have expanded our structure activity relationship analyses to focus on optimizing cellular uptake and reducing protein binding. We present the synthesis and analysis of a series of novel compounds. The data demonstrate that chemical modifications of the X80 core structure can be implemented to achieve these goals while retaining Ku inhibitory activity. In addition, novel relationships with *in vitro* inhibitory activity were identified as a function of new pharmacophore additions. These novel compounds enable cellular studies to be conducted in serum contain media and the pursuit of *in vivo* analysis and represent a significant advance in the development of Ku-DNA binding inhibitors.

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

POSTER #55

INVESTIGATING THE INTERACTION BETWEEN ETS FAMILY MEMBERS AND MUTANT P53

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Mutations in genes often result in tumor formation. TP53 encodes p53, a tumor suppressive transcription factor, and is mutated in nearly 50% of all cancer cases. Once mutated, p53 loses its tumor suppressive function while simultaneously gaining oncogenic function. p53 mutations disrupt direct p53 binding to the DNA of tumor suppressor genes, but promote p53 binding to regulatory regions of oncogenes via protein-protein interactions with other transcription factors. One of these interacting partners is ETS2. ETS2 belongs to the 28 member ETS transcription factor family. ETS binding sites are present in 50% of all mutant p53 occupied regulatory elements. Other ETS family members have also been linked to mutant p53 but these interactions have either been deemed weak (ETS1) or have yet to be identified as direct (ERG). It is my aim to determine the scope of interaction between the ETS family and mutant p53 and to elucidate whether this interaction has genetic and/or phenotypic consequences.

To determine which ETS proteins interact with mutant p53, I conducted affinity pull-down assays using 26 purified ETS proteins. Several ETS proteins interacted with mutant p53 better than ETS2 and the entirety of the ETS family interacted to some degree. Truncation studies were used to determine interaction domains. ERG and ETS2, both strong mutant p53 interactors, had two interaction interfaces. One of the interacting regions was the DNA binding domain, which may explain interactions with the entire family. I hypothesize that the second interaction interface defines strong interactors. My next step was to determine if interacting ETS are responsible for targeting mutant p53 to the genome. I performed chromatin immunoprecipitation sequencing studies in the presence or absence of different ETS factors to determine differences in mutant p53 binding. These data indicate that each of the conditions resulted in different p53 binding patterns in the ChIP-Seq and that there is a requirement for ETS in mutant p53 binding. Lastly, I wanted to determine if there was a correlation between p53 mutation and expression of strong mutant p53 interactors in certain cancer types. Analysis of TCGA samples showed that in ovarian cancers with p53 mutations, strong interactors were upregulated in a mutually exclusive pattern, while weak interactors tended to be downregulated, or unchanged. Similar trends were observed for lung squamous cell carcinoma, prostate cancer, breast cancer, and a pan-cancer analysis.

My studies have demonstrated that ETS proteins interact with mutant p53 and that this interaction seems to be relevant in mutant p53 driven cancers and required for mutant p53 binding to the genome. Additionally, studies are ongoing to determine phenotypes related to the ETS/mutant p53 interaction. Ultimately, if ETS/mutant p53 interactions are deemed important for oncogenesis, these will be attractive targets for drug development.

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

POSTER #56

CENTENMK: AN EFFICIENT APPROACH OF BIOMARKER DISCOVERY FOR NEUROENDOCRINE PROSTATE CANCER

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Abstract

Neuroendocrine prostate cancer (NEPC) is a highly aggressive subtype of prostate cancer with resistance to therapies. The identification of biomarkers in NEPC continues to be challenging. In this study, we present a simple and robust computational approach called Centrality-Ensemble Marker (CentEnMK) to identify biomarkers of NEPC. This approach steps through a gene list initially chosen according to the degree of differential expression shown in two profiling experiments, NEPC and non-NEPC, followed by an information gain filter strategy. Then our approach integrates phenotype-specific co-expression correlations with a protein-protein interaction (PPI) network to select the top-rank biomarkers.

The CentEnMK takes advantage of two different types of centrality measures on sample-specific co-expression PPI networks: path-like centrality (betweenness) and degree-like centrality (eigenvector) to find the genes that correspond to dysregulated genes between NEPC and non-NEPC. It then uses the method of rank ensembles for the final prioritization of candidate genes ranked by alterations of both betweenness and eigenvector. Using the proposed method, a small subset of key genes is discovered. While 40% of them have been previously reported as potential biomarkers for the diagnosis of NEPC, among which, AR, KLK3, NKX3-1, and CHGA are the most common biomarkers across previous studies and ranked at the top in our gene set, the other 60% of our biomarkers have not been reported yet. The enrichment analysis on signaling pathways associated with identified NEPC biomarkers uncovers that the NEPC biomarkers are significantly enriched in the "Hippo" signaling pathway, which has the unique ability to contribute to tumorigenesis and is a critical regulator of several hallmarks of prostate cancer. A linear regression model is finalized given 12 significant key biomarkers identified by the CentEnMK. The classification by our model demonstrates a good performance on different testing sets collected, indicating the high potential and good application of our marker genes to predict NEPC or non-NEPC.

In conclusion, our novel approach, CentEnMK, integrates the ensemble rank method with the results of path and degree centrality measures on phenotype-specific co-expression PPI networks to identify 67 key marker genes for NEPC. We further propose a linear regression model with only 12 key genes to predict the probability of NEPC based on the gene expression profile. The CentEnMK has low computational complexity and a high ability to identify biomarkers, which can be expanded to other biomarker studies.

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

POSTER #57

THE ROLE OF OXYGEN IN NORMAL AND MALIGNANT HEMATOPOIETIC CELL GROWTH

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Hematopoietic stem (HSC) and progenitor cells (HPCs) are responsible for the ongoing replenishment of blood/immune cells, are a source of treatment for malignant and non-malignant diseases, and when dysregulated are a source of hematological neoplasia. HSCs/HPCs and malignant hematopoietic cells such as acute myeloid leukemia (AML) are exposed to differing oxygen tensions ranging from <1% to 21% as they reside in different tissues or are harvested for clinical study or utility. Healthy HSCs and AML cells in mouse tissues with varying oxygen levels exhibit differing characteristics, including cell cycle variations. Here, we examine properties of cord blood (CB) HSCs/HPCs and mouse AML cells grown in varying oxygen tensions and show that manipulating oxygen levels can be used to alter the expansion and functional properties of HSCs/HPCs or growth/self-renewal of AML cells *ex vivo*.

To examine the effects of varying oxygen tensions on normal and malignant hematopoietic cell growth, human CB CD34+ cells or primary mouse MLL-AF9 AML cells were grown in stimulating culture in 1%O_2, 3%O_2, 5%O_2, 14%O_2, or 21%O_2. Immunophenotypically defined HSCs/HPCs and functional HPC colony forming units (CFUs) expanded significantly more after 4 or 7 days of growth in higher oxygen tensions (5%O_2-21%O_2) compared to lower (1%O_2-3%O_2). Using flow cytometry analysis, we found that HSCs/HPCs grown in low oxygen tensions had significantly lower ROS levels, a significantly higher percentage of cells in G0, and were slightly but reproducibly smaller and less granular than those grown in high oxygen levels. HSC/HPC numbers were reduced in high oxygen tensions 1-2 days after plating but were better maintained in low, suggesting cells undergo a culture shock/stress after plating that is mitigated by reduced oxygen. In the presence of UM171, a potent agonist of HSC growth, HSCs expanded significantly better at 14%O_2 and 21%O_2, but HPCs are better maintained in 5%O_2. MLL-AF9 AML cells recovered significantly better after freeze-thaw by growth and CFU numbers in 5%O_2 compared to 3% and 21%, suggesting 5% is an ideal tension for recovery of stressed AML cells. After equilibrating to various O_2 tensions, MLL-AF9 cells grow more rapidly and form more CFUs at higher oxygen levels compared to lower. Finally, MLL-AF9 cells grown in lower O_2 tensions have lower oxygen consumption rates.

Together, our data shows that HSCs/HPCs and AML cells proliferate rapidly in high oxygen but have fewer quiescent cells, higher ROS, are larger and more granular, and exhibit higher oxygen consumption, which are all markers associated with exhaustion. While high oxygen allows for faster growth, low tensions may mitigate cell stress (i.e. chemotherapy) and allow for prolonged growth (i.e., HSC/HPC expansion) while maintaining functional properties. Manipulating oxygen tension or oxygen sensing pathways could be used to improve HSCs/HPCs expansion or to treat AML.

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

POSTER #58

INTEGRATED GENETIC AND GENOMIC ALTERATIONS REVEALED THE ROLE OF CALCIUM REGULATING PROTEINS IN THE BASAL CELL CARCINOMA SKIN CANCER

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Objective: In this study, utilizing both Whole Exome Sequencing (WES) and RNA-seq data of tumor samples and adjacent normal tissues from 22 basal cell carcinoma (BCC) patients, we aim to uncover the key players that coordinate genetic mutations and gene expression alternations in the BCC with the potential biological functions.

Method: We proposed a novel approach to leverage information of gene mutations and gene expression to explore their coordination. The fold changes (FCs) of all genes were calculated between each pair of the tumor and the normal tissue. Given the mutation status of each gene, we then separated all 22 patients into two groups, named mutated or unmutated groups. The Wilcoxon test was performed to compare the differences of FCs (ΔFCs) of every gene between these two groups to identify mutations with significant impacts on gene expression changes.

Result: 11,152 genes were identified with at least one somatic mutation in one tumor sample. Our integrative analysis recognized 159 genes whose mutations were associated with ΔFC of 2,075 genes between two groups, with and without such mutations. Of these 159 genes, 26 mutated genes formed 49 mutation pairs, orchestrating at least four common target genes. The mutations on these 26 genes and corresponding target genes which showed significant ΔFC between mutated and unmutated groups can be categorized into multiple groups with distinguished biological functions. For example, mutations of *CALML6* down-regulated 442 genes associated with an immune and inflammatory response in tumor tissue. Mutations in *DUSP19*, *EXOSC1*, and *NBAS* up-regulated 164 genes significantly enriched in differentiation and proliferation of keratinocytes, and epidermis development, while these 164 genes were down-regulated by mutations in *GLB1*, *HGS*, *CAMKK1*, *ZBP, CCDC91*, *SMARCAD1*, and *EFHC1*. Interestingly, CALML6, CASQ2, CAMKK1, and EFHC1 are known to regulate the intracellular concentration of calcium ion or calcium signaling cascade, which in turn activates the differentiation of keratinocytes.

Conclusion: Calcium-regulating proteins at different stages of calcium signaling pathway may work in concert to regulate the proliferation and differentiation of keratinocytes. Our genetic and genomics data indicated that mutations of these pathways may disrupt such regulation, causing or exacerbating BCC skin cancer.

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

POSTER #59

TARGETING DIFFERENTIAL METABOLISM IN AN AGED MODEL OF PANCREATIC CANCER CACHEXIA

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Cancer cachexia is a multifactorial syndrome entailing muscle and fat wasting, inflammation, and metabolic dysfunction. Pancreatic cancer has the highest incidence of cachexia at around 80%. However, there are no FDA approved therapy for cachexia in the clinic yet. This is mostly because of the failure of preclinical therapies to demonstrate success in the clinic. Most preclinical studies utilize 6-8 week old mice which roughly corresponds to a human age of ~ 20 years. However, the median age of diagnosis of pancreatic cancer is ~70 years. Given widespread physiology, microenvironment, and metabolism differences in young and aged skeletal muscle, we sought to study cancer cachexia in mice aged to >78 weeks, which corresponds to a human age of greater than 60 years. Our previously published study reported little difference in tumor growth or survival between the two cohorts, yet significant alterations in the muscle transcriptome. Building on this work, we show significant differences in the muscle metabolome of young/aged control and tumor bearing mice. Notably, histamine was higher in aged versus young control mice and even higher in the aged tumor-bearing cohort. We show higher activity of histamine decarboxylase (HDC), the enzyme that converts histidine to histamine in the muscles of aged tumor-bearing mice. Addition of cancer conditioned-media (CM) to C2C12 myotubes was able to induce histamine production and HDC activity *in vitro,* which demonstrates the ability of muscle cells to induce histamine independent of mast cells. We further show that addition of histamine to myotubes is sufficient to increase atrophy-associated markers. Inhibition of HDC activity by α-fluoromethylhistidine (FMH) in the *in vitro* model of cachexia protected against myotube thinning. Finally, we treated tumor-bearing aged mice with FMH and observed a) decreased histamine in the muscles 2) decreased HDC activity in the muscles 3) decreased muscle wasting, 4) decreased atrophy markers. Together, we present a novel wasting-associated metabolic pathway and corresponding therapeutic avenue for cancer cachexia that may have been overlooked if not for the use of a more-age-appropriate model of pancreatic cancer.

Basic Science ***Research Assistant Professor***

POSTER #60

PATIENT DERIVED XENOGRAFTS PROVIDE A PLATFORM TO COMPREHENSIVELY INVESTIGATE CLONAL EVOLUTION AND GENOMIC DIVERSITY OF MULTIPLE MYELOMA

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Introduction: Multiple myeloma is a genetically heterogeneous disease composed of clonally diverse subsets of malignant plasma cells. To comprehensively evaluate the genomic landscape of multiple myeloma, a patient derived xenograft (PDX) model was used. PDX models have become an important tool in translational cancer research to evaluate drug treatments and understand tumor biology. A PDX model system of multiple myeloma will provide valuable insights into the interplay of genomics, biology, and treatments on disease progression.

Methods: Bone marrow aspirates collected from multiple myeloma patients were provided by the Indiana Myeloma Registry. CD138+ cells were injected into mice using the SCID-Rab model. Briefly, rabbit kit femur halves were implanted under the flank of NOD-SCID-γ (NSG) mice, followed by injection of cells into the rabbit bone after vascularization. Mice (n=48) were injected with nine unique patient samples. At the endpoint, PDX cells were collected and underwent CD138+ MACSorting. An average of 61 million CD138+ cells were isolated per mouse. Seven PDX mice were evaluated for bone disease, using X-Ray and CT scans, and organ infiltration by histology.The PDX cells and the original patient sample underwent whole genome and transcriptome sequencing to identify mutations, copy number abnormalities, structural variants, and gene expression. Also, CD138+ patient samples underwent single-cell multiome sequencing and copy number variation was determined. Cells from eight PDX mice were analyzed for genome topology interactions by Micro-C.

Results: Of the nine patient samples injected into mice three molecularly defined myeloma subgroups were represented, and all samples bore high-risk markers including *TP53* abnormalities, 1p32 (*CDKN2C*) deletion, gain/amp of 1q, or least one *RAS* mutation.

In one patient sample injected into two mice, both PDX clones had a similar copy number profile, but one clone had acquired gain 1q, resulting in congenic lines. Single cell data was unable to identify these high-risk cells in the original patient sample indicating that the PDX cells derived from a low abundance clone in the patient or had subsequently evolved in the mouse. In another patient sample, there was a dominant *NRAS* mutation and a subclonal *KRAS* mutation which was injected into four mice resulting in a dominant *NRAS* clone in one mouse, a dominant *KRAS* clone in two mice, and subclonal *NRAS/KRAS* mutations in the last mouse. Not only did the PDX model reveal complex genomic alterations, but CT scans of the mice revealed lytic lesions in clinically relevant bone regions of the PDX mice as well as splenomegaly.

In conclusion, our PDX model provides insight into the clonal evolution of multiple myeloma demonstrating the accumulation of high-risk markers and private mutations undetectable in the original patient samples and recapitulates the progression to myeloma bone disease commonly seen in human patients.

Basic Science ***Research Technician***

POSTER #61

TOWARDS UNDERSTANDING THE ANGIOMOTIN MEMBRANE FUSION ACTIVITY

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Carcinomas are tumors that originate from epithelial cells and make up 80-90% of all cancers. Expression of some Angiomotins (Amots) have been shown to be oncogenic as they play an important role in the localization, and therefore regulation, of many cellular polarity, differentiation, and proliferation proteins. This important function has been accredited to the Amot coiled-coil homology (ACCH) domain ability to selectively bind, deform, fuse and reorganize phosphatidylinositol (PI) lipids containing membranes. However, it is unclear as to how this function leads to tumorigenesis and metastasis. Based on our previous work, we hypothesized that disruption of the ACCH domain ability to fuse membranes will lead to loss of normal cellular polarization, adhesion, and therefore increase the proliferation and migration (metastasis) rates. To test this hypothesis, we follow up on our previous mutation screens that also identified mutations in the Cancer Genome Atlas (TCGA) - gastric adenocarcinoma R153H, head and neck cancers R103I/R140H, and endometrial cancer K111H/R234C. Here, we present our in vitro approach to understanding the relationship between ACCH domain activity and downstream cellular effects. In this work, we use fluorescence microscopy and cellular fractionation to demonstrate how these mutations affect cellular trafficking by measuring: 1) Amot80 membrane association with plasma membrane and ER vesicles; 2) membrane fusion within the cytosol and/or vesicles with the plasma membrane; 3) localization of polarity proteins that are in the plasma membrane/ER trafficking pathway; and 4) localization PI lipid known to be in abundance within the ER and golgi and/or driving plasma membrane polarity.The results presented provide insight into the role of these individual ACCH domain residues and their roles in maintain normal phenotypes and how mutation may initiate epithelial cell cancers.

Basic Science ***Research Technician***

POSTER #62

TARGETING GCN2 REGULATION OF AMINO ACID HOMEOSTASIS IN PROSTATE CANCER

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Prostate cancer (PCa) is the second leading cause of cancer-related deaths in men in the United States. The androgen receptor (AR) is considered the primary driver of PCa growth, and therapies targeting AR are the current standard of care. Unfortunately, patients develop resistance, termed castration resistance, and fail therapy. Identifying novel therapeutic targets independent of AR signaling axis is needed and clinically relevant.

A stress response pathway termed the Integrated Stress Response (ISR) is critical for the adaptation and survival of tumor cells to different stresses. The ISR features four protein kinases (PERK, GCN2, PKR, and HRI), each activated by different stress conditions, that phosphorylate the eukaryotic translation initiation factor eIF2, resulting in inhibition of protein synthesis. At the same time, eIF2 phosphorylation enhances the translation of gene transcripts, such as ATF4, which is central for ISR-directed gene transcription. The ISR has been shown to be activated in multiple cancers including PCa, however, which ISR kinase is the major driver of the ISR in PCa was not known.

Using a wide range of PCa cell lines, xenograft models, and patient samples, we demonstrate that the eIF2 kinase GCN2 is activated in PCa and contributes to enhanced eIF2 phosphorylation and ATF4 expression. We show that GCN2 is activated in PCa due to essential amino acid limitations. Genetic or pharmacological inhibition of GCN2 reduced growth in androgen-sensitive and castration-resistant PCa cell lines in culture and cell line-derived and patient-derived xenograft mouse models *in vivo*.

Analysis of the transcriptome in cells treated with a GCN2 inhibitor revealed that GCN2 is critical for expression of SLC genes, including amino acid transporters (ASCT1, ASCT2, 4F2, CAT1, LAT1, LAT3, and xCT) previously implicated in different cancers including PCa. Loss of GCN2 function in PCa cells results in downregulation of amino acid transporters resulting in amino acid starvation and reduced proliferation. Using CRIPSR-based drop-out screens, we identified SLC3A2 (4F2) as a key SLC gene induced by GCN2 that is essential for PCa growth. SLC3A2 interacts with multiple amino acid transporters, ensuring their proper localization to maximize amino acid transport. Importantly, knockdown of SLC3A2 phenocopied GCN2 inhibition resulting in amino acid starvation and decreased proliferation of PCa cells, and over-expression of SLC3A2 in GCN2 knock-out cells partially restored amino acid levels and growth.

We propose that PCa relies on GCN2 to maintain the expression of key amino acid transporters to adapt and survive amino acid starvation. Inhibition of GCN2 reduces growth in both androgen-sensitive and castration-resistant PCa models, and we conclude that targeting GCN2 may be a novel therapeutic approach for the treatment of PCa.

Basic Science ***Research Technician***

POSTER #63

LOSS OF PEROXIREDOXIN-1 IN HUMAN PANCREATIC DUCTAL ADENOCARCINOMA (PDAC) SIGNIFICANTLY REDUCES CELL SURVIVAL IN COMBINATION WITH INHIBITION OF REF-1 REDOX SIGNALING

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PDAC is inherently resistant to therapy and can proliferate under conditions of hypoxia and nutrient deprivation. Innovative approaches including combinatorial therapies against new targets are needed. Redox factor-1 (APE1/Ref-1, or Ref-1) is a regulator of multiple transcriptional factors (TFs) involved in cancer cell signaling through its redox signaling activity. Ref-1 redox status is itself regulated by other redox proteins such as peroxiredoxin (PRDX) and thioredoxin (TRX); referred as the PRDX-Ref-1-TRX redox cycling axis. The PRDXs scavenge H_2O_2, and like Ref-1, are reduced by TRX when oxidized. PRDX proteins have an exposed Cysteine involved in the catalytic cycle and a resolving Cysteine, similar to Ref-1. Studies have demonstrated an interaction of peroxiredoxin-1 (PRDX1) and Ref-1. The redox interactions between PRDX1, Ref-1, and NF-κB are responsible for overproduction of inflammatory cytokine, IL-8. PRDX1 is a member of a family of peroxidases comprising six isoforms that differ in their H_2O_2 scavenging, organelle/conditional expressions, and chaperone activity; yet, their relationship with Ref-1 is largely unknown. We explored the PRDX-Ref-1-TRX axis in a human patient PDAC cell line, Pa03c, with siRNA knock-down of PRDX1 ($PRDX1^{KD}$) or TRX (TRX^{KD}) in combination with our second-generation Ref-1 inhibitor, APX2014. We found that both $PRDX1^{KD}$ and TRX^{KD} conditions resulted in significant sensitivity to Ref-1 inhibition. We then had engineered CRISPR/Cas9 PRDX1 knock-out cells ($PRDX1^{KO}$) and tested APX2014 with both a mixed population of $PRDX1^{KO}$ cells and isolated $PRDX1^{KO}$ clones. We discovered that $PRDX1^{KO}$ cells were even more sensitive to Ref-1 inhibition compared to the $PRDX1^{KD}$ cells. Since we observed lower rates of cell growth with $PRDX1^{KO}$ and dissimilar morphology, we measured growth and cell-colony formation and confirmed statistical distinctions. We are also testing $PRDX1^{KO}$ in combination with the other five PRDXs and APX2014. Initial studies with PRDX2 knockdown ($PRDX2^{KD}$) had no increased effect in combination with Ref-1 inhibition. Additionally, Ref-1's second major function as a DNA repair protein apurinic/apyrimidinic endonuclease was not impacted by $PRDX1^{KO}$. Since PRDXs are major scavengers and regulators of H_2O_2, we are investigating $PRDX1^{KO}$ ROS generation and metabolic NADP/NADPH activity in combination with APX2014. We are also testing $PRDX1^{KO}$ under tumor conditions with cancer associated fibroblasts (CAFs) in 3D cell assays as well as in an orthotopic tumor mouse model. Ourhypothesis is that the activity of Ref-1 and interplaying redox proteins such as PRDX1 in PDAC tumor microenvironment (TME) correlates with resistance to treatment and thus could be a biomarker of response to the currently available standard of care regimens. The eventual goal is to select the subset of patients who could benefit most from APX compounds, which are currently in clinical development and could be an important addition to the current arsenal of treatments either in the upfront setting or upon failure.

Basic Science ***Research Technician***

POSTER #64

UNDERSTANDING THE CONNECTION OF KRAS ACTIVATION AND CIP2A EXPRESSION IN INITIATING PDAC EVENTS

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Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related deaths in the United States and has one of the worst five-year survival rates at only 12%. In over 90% of PDAC cases, there is an activating mutation in the GTPase KRAS, which signals to downstream effectors such as c-Myc and Erk to drive cell proliferation and survival. Although KRAS-targeted drugs are in clinical trials, conferred drug resistance is inevitable; therefore, alternative therapeutic targets are of interest. Protein phosphatase 2A (PP2A), a major serine-threonine phosphatase, negatively regulates many of the downstream factors of KRAS. It is a heterotrimeric complex with the A (scaffolding), B (regulatory), and C (catalytic) subunits. The B subunit is highly variable and contributes to the phosphatase's substrate specificity. The B56α subunit has been shown to have strong tumor suppressive capabilities, so PP2A-B56α likely has an important role in preventing oncogenesis. Further, PP2A-B56α is not often mutated in PDAC, and the endogenous cancerous inhibitor of PP2A (CIP2A) is expressed at higher levels in PDAC compared to healthy tissue. CIP2A inhibits PP2A by sequestering its regulatory subunit, preventing the subunit from associating with the complex.Together, this data suggests that PP2A-B56α activity is suppressed in PDAC through CIP2A, but reactivation remains possible. This increases our interest in PP2A reactivation through CIP2A suppression as a potential therapeutic strategy. To understand both the mechanism through which KRAS upregulates CIP2A and CIP2A's role in oncogenesis via PP2A suppression, we have measured rate of protein production and degradation, protein localization changes, and differences inKRAS signaling and oncogenic phenotypes with the loss of CIP2A. Our results suggest a novel mechanism of KRAS activation increasing the rate of CIP2A translation. Additionally, the findings indicate that the loss of CIP2A is sufficient to prevent oncogenic signaling and phenotypes, suggesting an increase in PP2A-B56α tumor suppressive activity. This further implicates PP2A-B56α reactivation as a potential therapeutic strategy in PDAC.

Basic Science ***Undergraduate Student***

POSTER #65

THE ROLE OF METHYLATION IN REGULATING THE CELLULAR STRESS RESPONSE OF HSP31

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The purpose of this study was to understand the role of methylation in regulating the cellular stress response of Hsp31 in Saccharomyces cerevisiae yeast cells. Hsp31 is known to be methylated by the N-terminal methyl transferase Tae1. Changing the methylation site can affect the methylation status of Hsp31, which may play a role in the protective activity of Hsp31 against cellular stress.

Glo1 is a gene in yeast involved in catalyzing the detoxification of methylglyoxal (MGO), which is a byproduct of glycolysis. We established that Saccharomyces cerevisiae in the *Glo1*Δ and background is sensitive to cellular stress by MGO. Mutant strains in the *Glo1*Δ background will simulate methylation levels, which can be used to determine if methylation increases or decreases the protective activity of Hsp31 under cellular stress. Hsp31 overexpression successfully rescues mutants in the *Glo1*Δ background when treated with MGO. In a high throughput screen using CRISPR-based technology from INSCRIPTA, a biotechnology company, we aim to replicate the results we observed in the *Glo1*Δ background and potentially uncover new phenotypes in a series of experiments that will investigate the role of methylation in cellular processes in addition to oxidative stress, including heat stress and protein synthesis. The INSCRIPTA library will allow us to collect data from approximately 1000 mutants simultaneously rather than completing the experiment for each mutant individually. These results will provide much greater insight as to how methylation globally affects the cellular processes involved in the protective activity of Hsp31 and other substrates in Saccharomyces cerevisiae.

Basic Science ***Undergraduate Student***

POSTER #66

WANT TO UNDERSTAND RADIATION RESPONSE: KNOCK OUT AND SEE!

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P53 plays a key role in maintaining cell viability especially after induction of DNA damage such as by X-rays. MDM2 and MDMX alters the functions of the P53 protein. We investigated whether the knockout of specific gene combinations of P53, MDM2, and MDMX affected the X-ray sensitivity and repair capacity of mouse embryo fibroblasts (MEFs). The three combinations of gene knockouts were as follows: P53-/- , P53-/- & MDM2-/-, and P53-/- & MDM2-/- & MDMX -/-. We irradiated these cells with single doses of 0 Gy or 5 Gy to assess cell viability / clonogenic capacity (0 Gy) and radiation sensitivity (5 Gy). We also irradiated the cells with split doses (2.5 Gy + 4 hours + 2.5 Gy) of X-rays to assess repair capacity. We show that magnitude of cell clonogenic capacity is reduced for the p53-/- & MDM2-/- and lowest for the P53-/- & MDM2-/- & MDMX-/- cells versus the P53-/- cells. By contrast, single dose 5 Gy survival appears highest for the P53-/- & MDM2-/- & MDMX-/- cells. The split dose experiments indicate all three cell types exhibit repair and increase cell survival after split dose versus single dose but only the p53-/- cells achieve statistical significance. Overall our data indicate that in p53-/- cells, MDM2 and MDMX knock out affect cell viability and radiation sensitivity differently. Further investigation will be required to understand these differences and mechanisms involved.

Basic Science ***Undergraduate student***

POSTER #67

PHARMACOLOGICAL ACTIVATION OF PP2A CAUSES NON APOPTOTIC CELL DEATH IN PANCREATIC DUCTAL ADENOCARCINOMA

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Pancreatic cancer is the fourth leading cause of cancer-related deaths in the United States. KRAS is mutated in 90% of pancreatic cancer patients. Oncogenic KRAS leads to aberrant cell proliferation and survival. Drugs targeting KRAS have been developed but resistance mechanisms against them have already been identified. This highlights the importance of identifying alternative strategies to target this pathway.

Pancreatic tumour microenvironment evolves with cancer cell progression leading to vascular remodelling and blood vessel collapse. This makes tumours nutrient deplete compared to surrounding normal tissues. To circumvent this, cells employ KRAS dependent macropinocytosis- an actin driven nutrient scavenging pathway. These macropinosomes then fuse with lysosomes and are degraded to recycle nutrients. Serine/Threonine kinases regulate the uptake and recycling kinetics of macropinosomes but the role of phosphatases is not well understood. Protein phosphatase 2A (PP2A) is a heterotrimeric Serine/Threonine phosphatase which inhibits downstream effectors of KRAS pathway and regulates KRAS dependent macropinocytosis. However, the mechanism of regulation is not understood.

PP2A is made up of three subunits A, B, and C. The B subunit of PP2A is known to provide substrate specificity to the enzyme. DT061 is a small molecular activator of PP2A that preferentially incorporates the regulatory subunit B56α into the PP2A complex. We observe that pharmacological activation of PP2A-B56α with DT061causes aberrant vesicle formation in cells that do not fuse with lysosomes. These vesicles build up in the cells eventually leading to cell deathconsistent with methuosis, a cell death pathway triggered by macropinosome accumulation. Since there are no methuosis markers we can confirm that the cell death is due to methuosis by ruling out other forms of cell death like apoptosis. When we inhibited apoptosis using Z-VAD, we found that the cell death by PP2A activator-DT061 is not prevented. These findings suggest that the cell death that occurs by activating PP2A could be methuosis, hence highlighting the novel role of PP2A in regulation of methuosis.

Basic Science ***Undergraduate student***

POSTER #68

THE PHENOTYPIC CONSEQUENCE OF CRISPR-CAS9 MEDIATED DELETION OF VAT1 IN ANGIOSARCOMA

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Angiosarcoma (AS) is an aggressive vascular cancer in which the genetic drivers of the disease are largely unclear, and the prognosis of patients is very poor with only a 30 percent 5-year survival rate. Previously, our lab found that endothelial-specific Dicer1 knockout drives the development of AS in mice. Dicer1 is vital in microRNA (miRNA) biogenesis, therefore our finding indicates that miRNAs play a role in the development of AS. Previous work has identified microRNA-497-5p (miR-497) to have a tumor-suppressive role in AS and our lab confirmed that miR-497 suppresses cell viability more significantly than several other tested miRNAs. Vat1 has been validated by our lab as a direct target of miR-497 and is upregulated in AS tumors. Vat1 is also upregulated and promotes cell migration in glioblastoma, while also shown as a mitofusion-binding protein, meaning Vat1 directs mitochondrial fusion. Given these previous findings, we aim to investigate the function of Vat1 in AS cell migration and mitochondrial fusion. To do this, we have generated an AS cell line with CRISPR-Cas9 mediated deletion of Vat1. We will determine the consequence of this deletion on cell viability and cell migration, and in the future, we will determine the consequence on clonogenic colony formation and mitochondrial fusion. The completion of this work will further define the potential tumor-promoting functions of Vat1 in AS.

Basic Science ***Undergraduate student***

POSTER #70

CHARACTERIZING THE ANTI-PROLIFERATIVE AND ANTI-MIGRATORY EFFECTS OF NEOCARZILIN A AND ITS DERIVATIVES IN ANGIOSARCOMA.

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Angiosarcoma (AS) is a rare and aggressive endothelial cell cancer with a 5-year survival rate of 30-40%. Due to rising cases of AS and limited knowledge of its spontaneous drivers, research is necessary to uncover its mechanisms.The conditional deletion of Dicer1 has shownAS in endothelial cells and DICER1 is crucial for canonical microRNA (miRNA) biogenesis, indicating that miRNAs may have a role in AS progression. In previous studies, miR-497 was significantly down-regulated in a variety of tumors compared to normal tissue, including AS.Therefore, we aim todetermine thekey target genes that miR-497 may regulate in AS. Our lab has validated that miR-497 targets Vat1, which is also upregulated in patient tumors. The Vat1 gene codes for a vesicle amine transport protein that aids cell migration and has been shown as a pathogenic factor in many tumors. Another study reported that Neocarzilin A (NCA), an anticancer natural product, irreversibly binds to VAT1, thereby inhibiting its function. NCA was shown to inhibit cell viability and migration in a panel of cancer cell lines, however, its effect in AS cell lines was unknown. Therefore, we hypothesized that NCA would inhibit cell viability and migration in AS cell lines. We have found that AS cell lines are more sensitive to NCA than normal endothelial cell lines, and that NCA inhibits cell migration in AS cell lines. Future studies will focus on testing other derivatives of NCA, which may be more potent than the parental NCA compound.

Basic Science ***Undergraduate student***

POSTER #71

PREVALENCE OF CERVICAL CANCER SCREENING UNDER THE AGE OF 21 YEARS BEFORE AND AFTER THE 2012 GUIDELINES IN THE UNITED STATES: ANALYSIS OF THE NHIS 2005-2018

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Background: Cervical cancer (CC) is nearly fully preventable, but its screening's uptake among women, as recommended in the United States, is still under targeted rates. The American Cancer Society (ACS) 2012 guidelines recommend women aged under age 21 years not to screen for CC, as this results in a net harm. Expert consensus found positive screens rarely useful in prevention in teenagers, but a burden in the healthcare system, with negative effects on fertility. Despite recommendations, screening continues in this population, and the extent to which this occurs, and the associated factors are not well studied. The purpose of this study was to assess the extent to which women aged 18 to 20 years received CC screening before and after the ACS 2012 guidelines, and describe associated factors, using a nationally representative survey.

Methods: We analyzed data compiled by IPUMS from six National Health Information Surveys (2005, 2008, 2010, 2013, 2015, and 2018), during which participants were asked the question "Ever had Pap smear test". Our independent variable was "After versus Before the 2012 Guidelines" (before the 2012 guidelines, after the 2012 guidelines), and our outcome was "Ever had Pap smear test" (no, yes). We added relevant demographic, healthcare-related, and reproductive covariates. Applying survey weights, we conducted bivariate analyses comparing participants aged 18-20 years who ever had and had not had a Pap smear test before and after the 2012 guidelines, then multivariable logistic regression to identify factors associated with Pap test under 21 years before and after the 2012 guidelines.

Results: 2759 participants aged 18-20 years were included in the analysis, with 51% being in the before-2012-guidelines group. 2023 (76.3%) were White, 2073 (80.2%) were non-Hispanic, and 990 (35.3%) lived in the South region. Of the 525 without heath coverage, 62% were in the after-2012 group; 2184 (81%) had a usual place to go for healthcare, with 50.7% in the before-2012 group. 812 (26.8%) reported recommendation by a doctor to screen in the last 12 months, 29.3% after the 2012 guidelines. 597 (21.6%) reported having received at least one dose of HPV vaccine (63.7% after the 2012 guidelines). 249 (6.1%) reported having given birth to a living infant, with 21.4% after the 2012 guidelines. Overall, 1358 (46.3%) received Pap smear, corresponding to 58.4% before and 35% after the 2012 guidelines, showing a significant decrease in proportion ($p<.0001$). Participants were 62% less likely to receive Pap Smear after the 2012 guidelines, OR (95% CI) = 0.38 (0.32, 0,46) in the unadjusted association, but 32% less likely in the multivariable association [0.68 (0.54, 0.87)]. Pap test was associated with race, region of residence, smoking status, alcohol drinking status, history of childbirth, and taking birth control pills at the time of survey, but disproportionately with doctor's recommendation to receive pap test in the last 12 months.

Conclusion: Women under the age of 21 in the United States were still receiving cervical cancer screening after the ACS 2012 guidelines despite not being recommended to, leaving out the main motivation for the current guidelines saying not to screen. There has been a significant decrease in the proportion of the uptake of screening tests in this population since the ACS 2012 guidelines, but the current uptake is still high, despite the demonstrated lack of benefit in prevention and the psychological harm that goes with these tests at this age. Among other factors, doctor's recommendation to test plays a key role in the persistent screening uptake in this young population. Further study including more recent NHIS data is needed.

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

POSTER #72

CLOSING THE CARE GAP: AN ACCEPTANCE AND COMMITMENT THERAPY MOBILE HEALTH APPLICATION FOR BREAST CANCER SURVIVORS

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Background: Up to 50% of the 3.8 million breast cancer survivors (BCS) in the United States experience stress, anxiety or depressive symptoms. Many BCS lack access to in-person mental health support due to clinician shortages, financial constraints, and limited time to attend therapy. Mobile health (mHealth) applications (apps) show promise in supporting mental health care of BCS, especially when created through the process of user-centered design (UCD). Oncologists and oncology mental health providers have called for the development of UCD mHealth apps to fill the gap of unmet needs, however no mHealth app exists to assist BCS in managing stress, anxiety, or depressive symptoms. To address this gap, we used UCD to build an mHealth app aimed at reducing stress, anxiety and/or depressive symptoms in BCS with acceptance and commitment therapy (ACT). ACT is a modern form of cognitive behavioral therapy that has been used effectively in mHealth and shown promise in improving the mental health of BCS.

Objective: To engage BCS, ACT clinicians and design experts in co-designing an mHealth app to reduce stress, anxiety, and/or depressive symptoms for BCS.

Method: Five UCD sessions were conducted from September to November 2022, and included five BCS, three ACT clinicians and two user design experts. Sessions were iteratively planned by our research team and included a three-stage structure: priming, a design activity and debriefing. Sessions were audio-recorded, transcribed and qualitatively analyzed.

Results: The BCS co-designers ranged in age from 45 to 64 years old, average age 54 years, with a diagnosis of stage I-III breast cancer. Each of the BCS had completed cancer treatment ≤ 5 years ago (ongoing endocrine therapy was allowed) and previously participated in an ACT intervention group. Thematic analysis revealed stressor themes, preferred ACT exercises, and desired app characteristics. Three stress-based themes emerged from the data: self (e.g., fear of recurrence), relationships (e.g., impact of cancer on children or other loved ones) and work/financial (e.g., financial stability). Co-designers recommended ACT exercises such as mindfulness practices and values-based committed action planning to address these stressors. Six design characteristics were preferred by co-designers: simple app entry, manageable number of intervention choices, social support, highly visual content, engagement reminders, and skill-building exercises.

Conclusion: An mHealth app to reduce stress, anxiety and/or depressive symptoms in BCS was developed by BCS and ACT clinicians using UCD. The app format and sample pages are included in the presentation.

Behavioral ***Graduate Student***

POSTER #73

BEYOND THE BREAST CANCER DIAGNOSIS: A QUALITATIVE STUDY

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Introduction/Background:

Breast cancer is the most common cancer among women and the second most commonly occurring newly-diagnosed cancer. Over the years, there have been astonishing advancements in breast cancer therapy which in turn have streamlined the process from diagnosis to remission and have led to improved treatment outcomes. Nevertheless, the impact of breast cancer on a patient's psychosocial experience has been oftentimes forgotten, resulting in psychosocial and practical support needs being unmet. Few studies have explored the breast cancer patient experience beyond the therapeutic realm. While current medical education has incorporated aspects of patient advocacy, studies show there remains a need for providers to offer holistic care to patients.

Study objective/hypothesis:

This study seeks to outline the breast cancer journey and identify patient pain-points within the breast cancer treatment algorithm.

Methods:

This is a qualitative study in which researchers conducted semi-structured interviews with providers specialized in treating breast cancer, non-profit breast cancer organizations, and breast cancer survivors. The interviews were audio-recorded and transcribed. Data were processed via thematic analysis. Online breast cancer patient blogs were assessed for additional thematic saturation.

Results:

Researchers interviewed 10 key stakeholders involved in the breast cancer diagnosis and plan of care. 4 online patient blogs were reviewed. Five stages were identified that correspond to the different medical processes: detection/diagnosis, initial treatment plan, follow-up care, remission/survivorship, and advanced treatment options. Interviews revealed the role of non-profit organizations in addressing additional patient needs. Thematic analyses of the interview transcripts and blogs revealed five broad themes regarding patient pain-points and highlighted the impact of the breast cancer culture of "survivorship" and "the fight" on patients.

Conclusion:

This qualitative study offered new insights on how to improve breast cancer care through identifying unmet patient needs. The outline of the breast cancer patient journey throughout the healthcare system revealed several gaps in care and can be used to implement an evidence-based, patient-centered approach to improve cancer outcomes and quality of life. The results obtained from this study can provide invaluable perspective on the breast cancer patient experience and can translate to other medical diagnoses. Moreover, this study highlights the need for an enhancement of medical education that further considers the patient's psychosocial experience. Future research is needed to change the culture surrounding breast cancer treatment.

Behavioral ***Medical Student***

POSTER #74

PATTERNS OF TOXIC STRESS AND MEDICAL TRAUMA FOR PEDIATRIC PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

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Background. Medical trauma is a common occurrence, especially for intensive and invasive treatments like Hematopoietic Stem Cell Transplant (HSTC). For children, the impact of such medical trauma can have a life-long psychological and physical impact. Psychosocial care professionals in pediatric hospitals are present and equipped to address medical trauma both during and in the immediate aftermath of the traumatic event but limited research is available to understand how to proactively intervene. This research study was completed to better understand patterns of trauma and stress within the pediatric HSCT experience so that proactive psychosocial interventions can be designed to potentially mitigate the impact of medical trauma. A model for practice was created.

Method. This study utilized a multi-case design in two phases and employeed time-series analysis and template analysis as the primary analytic methods. This study utilized data from the Electronic Medical Record at a Cincinnati Children's Hospital Medical Center (CCHMC). This study was approved by the CCHMC IRB (2019-0528). Phase one of this study utilized a common case selection strategy for case selection (3 cases). This allowed the construction of a "common experience" initial template for a practice model. Phase two utilized a theoretical replication model for case selection (11 cases). The phase two cases were divided into four cohorts based on the gaps in information from the phase one cases. Utilizing template analysis procedures, the initial template was systematically subjected to rigorous challenge with the phase two cases data.

Results. The resulting practice model delineates the pediatric HSCT experience into three distinct phases, each with their own specific focus for intervention. Phase 1 (admission to Day -4) focuses on multidisciplinary assessment, relationship building, and preparation. Phase 2 (Day -3 to Day +10) focuses on changing the subjective experience of this phase. Phase 3 (Day +11 through discharge) focuses on managing the instability of this phase. Across phases, there is an emphasis on fortifying supportive/buffering relationships.

Conclusions and Implications. This research and resulting model have implications for psychosocial care providers working with pediatric HSCT patients. This model visually depicts the patterns of potential stress and trauma with corresponding foci, based in the data and relevant theory, so that interventions may be proactively utilized to offer the right intervention at the right time to mitigate the impact of medical trauma.

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

POSTER #75

ASSOCIATIONS OF NEUROPSYCHOLOGICAL SYMPTOMS BURDEN WITH PHYSICAL FUNCTION AMONG PATIENTS WITH STAGE IV CANCER FROM HEALTH PRECISION GENOMICS PROGRAM

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Background: Stage IV cancer patients experience extreme psychoneurological symptoms, including anxiety, depression, fatigue, pain interference, and reduced physical function, which deteriorate patients' quality of life. Although all of these symptoms are clustered as psychoneurological, impaired physical function warrants greater attention in assessment and intervention development, especially since it impacts Activities of Daily Living (ADLs), limits physical activity, and may increase the risk of fall in older adults. This study aimed to identify the associations between physical function severity and domains of neuropsychological symptoms among patients with stage IV cancer.

Method: Patients with advanced cancer from the Indiana University Health Precision Genomics (PG) Program who participated in genomic testing completed a survey comprised of patients' perception of the purpose of genomic testing, patients' instrumental attitude toward genomic testing, and physical function, pain interference, depression, anxiety, sleep disturbance, and fatigue as six domains of PROMIS-29. Groups were identified based on low severity (LS-PF: scores $\geq$ 46) and high severity (HS-PF: scores $\leq$ 45) of physical function. Multivariate analysis of variance (MANOVA) was conducted to determine whether there were significant differences between LS-PF vs. HS-PF groups in domains of fatigue, pain, anxiety, depression, and sleep disturbance.

Results: From June 2022-March 2023, 218 participants with a mean age of 58.43 ($\pm$ 17.63) years old completed the survey. A total of 134 patients (61.4%) were categorized in the HS-PF group versus 84 patients (38.6%) with LS-PF. The results of MANOVA showed that there was significant difference across all symptoms scores based on physical function severity groups, with overall significant F-statistic: $F(5, 212) = 14.724$, $p = <.001$; $\lambda = 0.742$, $\eta_p^2 = .26$. Univariate tests revealed a significant association of physical function severity with fatigue score, $F(1, 216) = 56.72$; depression $F(1, 216) = 13.36$; anxiety $F(1, 216) = 10.64$; sleep disturbance $F(1, 216) = 13.44$; Pain $F(1, 216) = 487.80$; all variables were significant at $p < .001$. Anxiety ($M = 54.1$, $SD = 8.8$) and pain ($M = 53.1$, $SD = 10.1$) were found to have the highest average scores out of the six domains. However, the domains with the highest percentage of moderate and severe prevalence were physical function (59.0%), followed by pain interference (27.5%), and sleep disturbance (25.3%). The medical team identified pain interference (n = 58; 26.0%), anxiety (n = 48; 22.0%), and depression 38 (n = 38; 17.2%) to be high priority areas that require attention.

Conclusion and future direction: Patients with lower physical function may experience a higher symptom burden. Offering interventions that improve physical function, such as exercise rehabilitation programs, could be beneficial to enhance physical function and reduce symptom burden among advanced cancer patients. Precision medicine programs should consider assessment of patient-reported outcomes to enhance personalized, supportive cancer care.

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

POSTER #76

HIGH-RISK HUMAN PAPILLOMAVIRUS (HR-HPV) TESTING COMPARED TO VISUAL INSPECTION WITH ACETIC ACID (VIA) IN CERVICAL CANCER SCREENING OF KENYAN AND UGANDAN WOMEN LIVING WITH HIV

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Introduction: Women living in sub-Saharan Africa have a high incidence of cervical cancer; women living with HIV/AIDS (WLWH) have the highest incidence of this malignancy. The current model of centralized screening by VIA has not led to a reduction in cervical cancer. Although HR-HPV testing is available for cervical cancer screening in wealthier countries, this method has not been adequately studied in WLWH in sub-Saharan Africa. A four-year longitudinal study is being conducted in Kenya and Uganda to assess cervical cancer screening strategies for WLWH; results of enrollment data are presented here.

Methods: Women aged 21 to 60 years provided self-collected vaginal swabs for HR-HPV testing (Roche Cobas Assay, 14 HR-HPV types detected); all women then underwent VIA.All WLWH underwent cervical biopsy (two specimens); among women not living with HIV (WNLWH), only those with an abnormal VIA examination underwent biopsy.For WLWH, sensitivity/specificity and positive/negative predictive values were estimated for HR-HPV and VIA for the detection of biopsy-proven CIN2/3 and CIN3.A second analysis was done for WLWH with a positive HR-HPV test to determine if VIA would be a useful second step in detecting CIN3.

Results for all women:121 WLWH (median age 38.2 years) and 119 WNLWH (median age 33.2 years) were enrolled.All women provided self-collected vaginal swabs; all swabs were adequate for HPV testing.HR-HPV was detected in 49 (40.5%) WLWH and 35 (29.4%) WNLWH (Pearson Chi-square test p=0.054).VIA was abnormal in 17 (14.0%) WLWH and 4 (3.4%) WNLWH (Pearson Chi-square test p=0.003).

Results specifically for WLWH: All WLWH were receiving ART; median CD4 cell count (IQR) was 773 (544, 971) cells/μL.Biopsy results for 120 WLWH (one woman did not undergo biopsy): CIN2/3 among 14 (11.7%) women and CIN3 among 6 (5.0%) women. Sensitivity of HR-HPV testing was excellent for detection of CIN3, but poor for VIA (detailed results will be shown).

Conclusions: HR-HPV was detected in a large percentage of both WLWH and WNLWH. VIA was more likely to be abnormal in WLWH than in WNLWH. or WLWH. HR-HPV testing was sensitive but not specific for detection of CIN2/3 and CIN3. Sensitivity of VIA was poor for detection of CIN2/3 and CIN3 in WLWH, but specificity of VIA was good. VIA performed poorly as a second step for CIN3 detection among 49 WLWH with a positive HR-HPV test. Future screening strategies for WLWH could utilize HR-HPV testing of self-collected swabs for CIN3 detection; costs will be higher than programs using VIA, but fewer cases will be missed.

Population Science/Epidemiology ***Faculty***

POSTER #77

STATINS AND MORTALITY IN VETERANS WITH COLORECTAL CANCER

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Background: While statins may have a modest protective effect on risk for colorectal cancer (CRC), the role of statins in CRC prognosis is less well defined. We sought to delineate statin use in patients with CRC and its association with both all-cause mortality (ACM) and CRC-specific mortality (CSM).

Methods: The current study was conducted within the framework of a Veterans Affairs-funded study on prevalence, risk factors, and outcomes of post-colonoscopy CRCs, which was a retrospective cohort study of Veterans aged 50 to 85 years diagnosed with incident CRC between 2003 and 2013 and followed until death or censored on 12/31/2018. Demographic and clinical data were collected at CRC diagnosis along with treatment for CRC. Pre-CRC use of statins, aspirin, and NSAIDs was defined as ≥ 30 days of use in each of the 2 years prior to CRC diagnosis. Post-diagnosis use was categorized in several ways including at least 30 days of use within a calendar year. Cox proportional hazards was used to model univariate and multivariate effects of statins on ACM and CSM with adjustment for demographics, comorbidity, smoking status, family history of CRC, aspirin and NSAID use, CRC stage, and CRC treatments (surgery, chemotherapy, radiation, and immunotherapy). Three models were proposed that differed in which statin-use variables were entered as predictors: (1) pre-CRC statin use only, (2) post CRC statin use only, and (3) both pre- and post-CRC statin use as two separate variables in the same model. Post-CRC statin use was measured as a time-varying predictor.

Results: We identified 29,877 Veterans with CRC (mean [SD] age 67.7 [9.0] years, 98% male, 80% White, 18% Black, 2% other), 10,780 (36%) of whom were statin users at CRC diagnosis. As of 12/31/2018, 19,620 (65.7%) were deceased due to all causes and 9,915 (33.2%) deceased due to CRC. Five years post-CRC diagnosis, there were 13,966 (46.7%) decedents due to all causes and 8,622 (28.9%) decedents from CRC. The effect of statins on ACM was inconsnstent; however, their effect on CSM was very consistent, with adjusted hazard ratios ranging from 0.84 to 0.92, indicating an 8%-to-16% decrease in CSM.

Conclusion: In this cohort of Veterans with incident CRC diagnosed between 2003 and 2013, statin use was associated with a modest but consistent decrease in death from CRC. Statins may be a useful adjunctive therapy for CRC. Further study is needed to validate these findings in a more current patient cohort and to identify statin effect-modifying factors.

A Table and Figure will be included in presentation showing detailed results.

Population Science/Epidemiology ***Faculty***

POSTER #78

THE MOTHER-DAUGHTER PROJECT: A COMMUNITY-BASED APPROACH TO CERVICAL CANCER PREVENTION IN WESTERN KENYA

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Introduction. Rural women have the highest incidence of cervical cancer in Kenya. A community-based pilot study known as the Mother-Daughter Project (MDP) was initiated in 2019. Major aims of MDP are to 1) screen rural Kenyan women for cervical cancer and determine if a triage strategy using HPV testing followed by VIA can be developed, 2) vaccinate their daughters against HPV, and 3) determine factors associated with screening and immune response to HPV vaccination.

Methods. A community entry strategy was utilized to introduce the MDP and invite participants. At community meetings, women are educated about cervical cancer, then provided self-collected vaginal swabs for oncogenic HPV testing (Roche Cobas Assay). All women then travel to the local clinic for VIA. In all three 2-year cycles of the MDP (MDP-1/2/3), women with abnormal VIA undergo cervical biopsy. In MDP-3, 10% of women with normal VIA will undergo biopsy, and biopsy of suspected anogenital warts will be performed. Daughters (ages 9 through 14) of women in MDP-2/3 are offered 9X-HPV vaccination. Additionally, benefits of the MDP and obstacles to participation will be evaluated.

Results. Five hundred women were enrolled in MDP-1/MDP-2 (mean age 36.0 years); 83.4% HIV-uninfected and 16.6% HIV-infected; all women preformed vaginal swabs; HR-HPV test results were available for all women, positive in 144 women (28.8%). VIA was performed in 491 women, abnormal in 20 (4.0%); cervical biopsies were performed in 19 women; CIN2/3+ detected in 6 of the 19 women. The distribution of HR-HPV and VIA results by HIV status were calculated. A total of 2091 girls received their first HPV vaccine dose at community meetings, second vaccine dose in progress.

Conclusions. The MDP has built trust in the community. Kenyan women are willing to attend meetings, learn about cervical cancer, provide swabs for HPV testing, travel to the clinic for VIA. Comparison of HR-HPV and VIA for detection of CIN2/3+ will be performed in MDP-3. Kenya women are willing to have their daughters vaccinated against HPV. As a result of this pilot study, this community-based strategy to prevent cervical cancer will be continued.

Population Science/Epidemiology ***Faculty***

POSTER #79

ASSOCIATION OF PLASMA AFLATOXIN WITH PERSISTENT DETECTION OF ONCOGENIC HUMAN PAPILLOMAVIRUSES IN CERVICAL SAMPLES FROM KENYAN WOMEN ENROLLED IN A LONGITUDINAL STUDY

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Background. Cervical cancer is common among Kenyan women and is caused by oncogenic human papillomaviruses (HR-HPV). Identification of factors that increase HR-HPV persistence is critically important. Kenyan women exposed to aflatoxin have an increased risk of cervical HR-HPV detection. This analysis was performed to examine associations between aflatoxin and HR-HPV persistence.

Methods. Kenyan women were enrolled in a prospective study. The analytical cohort for this analysis included 67 HIV-uninfected women (mean age 34 years) who completed at least two of three annual study visits and had an available blood sample. Plasma aflatoxin was detected using ultra-high pressure liquid chromatography (UHPLC)-isotope dilution mass spectrometry. Annual cervical swabs were tested for HPV (Roche Linear Array). Ordinal logistic regression models were fitted to examine associations of aflatoxin and HPV persistence.

Results. Aflatoxin was detected in 59.7% of women and was associated with higher risk of persistent detection of any HPV type (OR = 3.03, 95%CI = 1.08–8.55, P = 0.036), HR-HPV types (OR = 3.63, 95%CI = 1.30-10.13, P=0.014), and HR-HPV types not included in the 9-valent HPV vaccine (OR = 4.46, 95%CI = 1.13–17.58, P=0.032).

Conclusions. Aflatoxin detection was associated with increased risk of HR-HPV persistence in Kenyan women. Further studies are needed to determine if aflatoxin synergistically interacts with HR-HPV to increase cervical cancer risk.

Population Science/Epidemiology ***Faculty***

POSTER #80

CAROTENOID AND TOCOPHEROL INTAKE IN RELATION TO PANCREATIC CANCER RISK IN A POPULATION-BASED CASE-CONTROL STUDY

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Pancreatic cancer is the third leading cause of cancer-related death in the U.S. and has a 5-year relative survival rate of only 11.5%. The etiology of pancreatic cancer is not well understood, with cigarette smoking, type 2 diabetes, and chronic pancreatitis as the only well-established risk factors. Therefore, it is critical to identify modifiable risk factors for primary prevention. Persistent oxidative stress and resultant oxidative damage can lead to accumulated mutations in oncogenes and tumor suppressor genes and subsequent carcinogenesis of the pancreas. Carotenoids are abundant in fruits and vegetables, while tocopherols are rich in oilseeds and nuts. Both carotenoids and tocopherols have antioxidant functions. Thus, it is possible that high intake of these bioactive compounds can reduce risk of pancreatic cancer. The present study sought to investigate this question in a case-control study conducted during 1994-1998 in Minnesota. Cases (n=150), aged 20 years or older, were ascertained from all hospitals in the metropolitan area of the Twin Cities and the Mayo Clinic; from the latter, only cases residing in the Upper Midwest of the US were recruited. Controls (n=459) were randomly selected from the general population and frequency matched to cases by age (within 5 years) and sex. Intake of carotenoids and tocopherols from diet and supplements was estimated from a validated food frequency questionnaire. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated using unconditional logistic regression. As lutein and zeaxanthin are isomers that have identical chemical formulas, they are combined into a single variable in the analysis. All dietary variables were adjusted for energy intake with the residual method prior to data analysis. After adjustment for age, sex, race, education, physical activity, cigarette smoking, and alcohol consumption, intake of lutein and zeaxanthin was associated with a significantly reduced risk of pancreatic cancer [OR (95% CI) for quartile 4 (median: 4972 μg/day) vs. quartile 1 (median: 976 μg/day): 0.39 (0.19-0.79); p-trend: 0.06]. This inverse association became slightly weaker but remained statistically significant after additional adjustment for intake of energy, fat, and fiber. No significant associations were observed for intake of α-carotene, β-carotene, lycopene, β-cryptoxanthin, α-tocopherol, β-tocopherol, and δ-tocopherol, although a higher intake of all these nutrients except α-tocopherol and δ-tocopherol tended to confer a lower risk. Our study shows that there is a significant, beneficial effect of lutein and zeaxanthin intake on pancreatic cancer risk, although it may be a chance finding due to multiple comparisons. More epidemiologic studies are warranted to further elucidate the roles of carotenoids and tocopherols in the etiology and prevention of pancreatic cancer.

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

POSTER #81

FUNCTIONAL CHARACTERIZATION OF GENETIC LOCI IN BASAL CELL CARCINOMA

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Background

Genome-wide association studies (GWAS) have identified over 30 loci associated with basal cell carcinoma (BCC) susceptibility. However, the causal variants and mechanisms at these loci remain unknown. We performed statistical fine mapping and functional enrichment to identify credible causal variants at 31 established BCC risk loci.

Methods

We performed statistical fine mapping by applying the sum of single effects (SuSiE) method to a previously published GWAS from 12,945 BCC cases and 274,252 controls. To identify genomic annotations enriched for BCC-associated variants, we compared high Posterior Inclusion Probability (PIP) variants from SuSIE to low PIP variants: we performed multivariable logistic regression of high-PIP status on 17 variant annotations from the Functional Annotation of Variants Online Resources (FAVOR) database, including sequence-contextual and functional annotations.

Results

We identified 6 variants with PIP>0.99: rs1126809 (*TYR*), rs1805007 (*MC1R*), rs78378222 (*TP53*), rs12203592 (*IRF4*), rs35407(*SLC45A2* a.k.a. *MATP*), rs12916300 (*HERC2*). Five of these six variants are in pigmentation genes; the sixth is in the tumor suppressor *TP53*. At a seventh region, the 95% credible set contained two variants in or upstream of *RALY*, which has previously been shown to be associated with pigmentation traits and multiple non-skin cancers. Another four regions had fewer than 10 variants in the credible set; the median size of the credible set was 11 (range: 1-76).

After adjusting for sequence context (including location relative to exons, introns, UTRs; GC content; local mutation density, and nucleotide diversity) variants in active chromatin regions (as determined from multi-tissue methylation marks), variants in transcription factor binding sites, and skin eQTLs were significantly more likely to be high-PIP variants.

Conclusions

Our results identify candidate variants, genes, and mechanisms for further study in BCC carcinogenesis.

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

POSTER #82

ASSOCIATION OF SUN-SEEKING BEHAVIORS WITH INDOOR TANNING HABIT IN US FEMALES

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Importance: Frequent exposure to ultraviolet light in early life has more detrimental and long-term effects on skin than in adulthood. Teenagers with strong sun-seeking behaviors may be more likely to use an indoor tanning bed than those who seek less sun, probably due to addictiveness of ultraviolet exposure.
Objective: To examine associations between sun exposure behaviors and average annual indoor tanning usage frequency during high school/college in US females.
Design: Cross-sectional study.
Setting: We used data from The Nurses' Health Study II, a large prospective cohort of US female nurses.
Participants: We included a total of 81,746 white females who answered the average annual frequency of indoor tanning during high school/college.
Exposure: Average weekly time spent outdoors in a swimsuit and average percentage of time of wearing sunscreen at the pool or beach during their teenage years, average weekly hours spent outdoors in direct sunlight in daytime during high school/college, and the number of severe sunburns which blistered between ages 15-20.
Main Outcomes and Measures: Average annual frequency of indoor tanning bed usage during high school/college.
Results: In multivariable-adjusted logistic regression, we demonstrated positive associations between the sun exposure behaviors and the indoor tanning habit. Specifically, teenagers who spent daily outdoors in a swimsuit (adjusted odds ratio [aOR], 95% confidence interval [CI] for daily vs. <1/week: 2.68, 1.76-4.09) or who had ≥10 sunburns that blistered (aOR, 95% CI for ≥10 vs. never: 2.18, 1.53-3.10) were more likely to use indoor tanning beds ≥12 times/year. Also, teenagers/undergraduates who spent ≥5hours/week outdoors in direct sunlight during daytime used indoor tanning ≥12 times/year (aOR, 95% CI: 2.18, 1.39-3.44) than those who spent <1/week. However, there was not a significant association between average uses of sunscreen at the pool/beach and indoor tanning bed. Multivariable-adjusted linear regression models also showed similar results.
Conclusions and Relevance: Teenagers who spent more time outdoors or got more sunburns tended to use indoor tanning more frequently. These findings provide evidence that teenagers with strong sun-seeking behaviors may have excessive exposure to artificial ultraviolet radiation as well.

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

POSTER #83

UNDERSTANDING THE ROLE OF PHOSPHATIDYLINOSITOL PHOSPHORYLATION IN RACIAL DISPARITIES IN TRIPLE NEGATIVE BREAST CANCERS.

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The incidence of breast cancer is similar between Non-Hispanic White women and Non-Hispanic Black women, however Black women in the United States are 40% more likely to die from this cancer than their White counterparts. Black women tend to incur breast cancer with earlier onset, higher grade, and lacks the expression of estrogen receptor (ER), progesterone receptor (PR), and Human Epidermal Receptor 2 (HER2) therefore being known as triple negative breast cancers (TNBC). TNBC has a higher rate of metastasis and poorer survival rate, contributing to the disparity. Due to the lack of specific targets, TNBC is treated with generalized chemotherapy and surgery with ~80% incomplete response, further contributing to worse outcomes. Studies have suggested that type 2 diabetes (T2D) is associated with a higher incidence of breast cancer and related mortality rates, combined with a higher incidence of T2D in the black community. Women with T2D and breast cancer have a 50% increase in mortality compared to breast cancer patients without diabetes. Therefore, we hypothesized that T2D related hyperglycemia reprograms the mammary cells to initiate tumorgenesis and more aggressive cancer phenotypes, thereby leading to the known health disparity. To that end, our laboratory has focused on characterizing plasma membrane lipids, specifically phosphatidylinositol (PI) and its phosphorylated derivatives, as a selective TNBC biomarker for potential aggressiveness. Our previous studies suggest that hyperglycemia led to increased di- and tri-phosphorylated PI concentrations due to turnover of PI3P in triple negative breast cancer (Devanathan 2018). This work presented here will build on this study as we use fluorescence spectroscopy to analyze plasma membrane relative lipid concentration, cell proliferation, and cell migration to characterize this hyperglycemic reprogramming effect in primary TNBC cells derived from White vs Black patients. We expect that the racially related enzymatic diversity will cause Black cells to respond to T2D like conditions with more metastatic phenotypes than White cells. If there are no changes in the lipidome of these conditions, then there may be other T2D related enzymatic that can be considered by using IUSCCC T2D patient derived cells (4 Black T2D and 3 White T2D) for hyperglycemic immortalized, thereby preserving native microenvironment for differential PI enzyme analysis. The results will give significant insight into how the T2D may direct racial disparities in triple negative breast cancer aggressiveness and determine specific lipids that can be targeted for selective treatments.

Basic Science ***Undergraduate Student***

POSTER #84

SHP2 INHIBITION ENHANCES ANTITUMOR EFFECT OF MIRDAMETINIB IN A PEDIATRIC BRAIN TUMOR MODEL BEARING CDC42SE2-BRAF FUSION BY REWIRING THE PROTEOME AND PHOSPHOPROTEOME LANDSCAPE

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Background: Pediatric gliomasare the most common type of pediatric brain tumors representing wide range of molecularly and clinically heterogenous subtypes. The hyperactivity of mitogen-activated protein kinases (MAPK) pathway has been identified in the majority of pediatric glioma suggesting its therapeutic potential. However, pharmacologic targeting of one of MAPK pathway's component is limited due to the development of drug resistance and differential response associated with tumor molecular landscape. Therefore, effective combination strategy in the framework of precision medicine is needed. Here we report combination benefit and molecular underpinning synergy of brain penetrant MEK inhibitor (mirdametinib) and SHP2 inhibitor (SHP099) in a pediatric patient-derived xenograft (PDX) and xenoline developed at our institution.

Methods and Results: Our model was derived from a pediatric patient who was diagnosed with rare high-grade subtype of glioma, anaplastic pleomorphic xanthoastrocytoma, and did not respond to MEK inhibitor, trametinib. Integrative multi-omics revealed molecular fidelity between our model and its patient tumor counterpart including the presence of 7q35 fusion, *CDC42SE2-BRAF*, *CDKN2A/B* loss, and MAPK pathway hyperactivation. *In vitro* studies using our xenoline IU-X128 demonstrated synergy between SHP099 and mirdametinib to curtail cell proliferation ($p<0.05$). Moreover, this combination was well tolerated in our PDX, IU-RHT128, and potentiated anti-tumor effect of single agent alone within clinically achievable doses. Reverse Phase Proteome Array (RPPA) identified MAPK reactivation via Mushasi RNA binding protein-PI3K-AKT crosstalk as a potential innate resistance mechanism to single agent MEK inhibitor in the PDX tumor. Further, tandem mass tags (TMT)-LC-MS/MS profiling on tumor treated with single agent SHP099 or mirdametinib and their combination revealed that combination therapy does not only revert certain proteome and phosphoproteome reprogramming from single agent treatment but also created a novel landscape which can be associated with anti-tumor effect. In this case, kinase network reprograming leading to MAPK reactivation was identified in mirdametinib treated tumor which was attenuated in the combination treatment.

Conclusions: In summary, our results demonstrated that combination SHP099 and mirdametinib is superior to single agent alone in the pediatric A-PXA brain tumor model with proteome and phosphoproteome reprogramming of multiple networks as potential molecular mechanisms underlying therapeutic benefit of combination therapy. Ultimately, clinical translation of this finding will potentially benefit patient of this malignant rare pediatric glioma subset which currently does not have standard therapy.

Translational/Clinical Research ***Faculty***

POSTER #85

IDENTIFICATION OF HIGH-RISK PHF19 EXPRESSING CELLS IN MYELOMA SINGLE CELL MULTIOMICS

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Myeloma is a plasma cell malignancy in which a high-risk set of patients can be defined by genomic markers including cytogenetic groups such as loss of 1p, gain or amplification (gain/amp) 1q, and *TP53* abnormalities. Increasingly, other markers are also being identified that are associated with progression such as *PHF19*. Bone marrow aspirates were collected from Smoldering Multiple Myeloma (SMM; n=10), Newly Diagnosed Multiple Myeloma (NDMM; n=22), and Relapsed/Refractory Multiple Myeloma (RRMM; n=17) patients after treatment with Immunomodulatory, proteasome inhibitors, and immunotherapies. All these samples underwent $CD138^{+}$ sorting and single cell multiomic sequencing (RNA-seq and ATAC-seq; 10X Genomics).

Samples initially had an average of 9484 cells, removal of low-quality cells and non-plasma cell removal resulted in 6819 cells per sample for our downstream analyses. Based on our analysis pipeline to integrate all of our patient samples, 325,025 high quality myeloma cells were retained for further study. These patients could be stratified into major translocation groups based on marker gene expression**.** Cells were integrated and formed 25 distinct clusters which represented myeloma cells with unique transcriptome profiles that frequently correlated with genomic events. Using the defined clusters, we determined if any were associated with disease stage, or cytogenetic subgroup, and determined that cluster 11 was associated with later stages of myeloma progression and increased in proportion from SMM to NDMM (P=0.025), and NDMM to RRMM (P<0.001). Due to their greater enrichment in RRMM patients in comparison to earlier stages of myeloma, we denote cluster 11 as relapse/refractory plasma cells (RRPCs).

Aside from a clear association of these cells to myeloma progression, there were also associations between the proportions of these cells and some high-risk genomic events. RRPCs also had significantly greater expression of *PHF19* compared to other clusters (Log2FC=2.43, P<0.001), reduced chromatin accessibility of *CDKN1C*(Log2FC=-0.54, P<0.001) and reduced expression of *CDKN1C* (Log2FC=-1.07, P<0.001). As a result, RRPCs had a proliferative signature indicated by the increase expression of *MKI67* (Log2FC=3.77, P<0.001). The proportion of RRPCs in each patient sample was associated with amplification of 1q such that samples with a normal copy number of 1q had significantly lower proportion of RRPCs than amplification of 1q (0.09 vs. 0.03, P=0.003). In addition, samples with a *TP53* mutation had a significantly greater proportions of RRPCs than samples without a *TP53*mutation (0.08 vs. 0.03, P=0.004). Samples with normal copy number of 1q had a higher proportion of RRPCs if they also had a TP53 mutation (0.07 vs. 0.03, P=0.038). A similar pattern was seen in the 1q gain or amplification samples but was only significant at. a=0.10.

Based on these analyses we find a progression associated cluster of plasma cells, RRPCs, which are associated with 1q amplification and *PHF19* overexpression.

Translational/Clinical Research ***Faculty***

POSTER #86

TARGETING POISON EXONS THAT ARE HETEROGENEOUSLY SPLICED IN MULTIPLE MYELOMA

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Background:

Alternative Splicing (AS) plays a key role in regulating numerous cellular processes in both normal and malignant cells. A poison exon (PE) is a special type of AS that widely exists in the human genome. PE exclusion in oncogenes and inclusion in tumor suppressors is critical for tumorigenesis and proliferation. PEs can be heterogeneous among cancers, however, neither their function nor targetability has been systematically characterized in multiple myeloma (MM).

Method:

RNA-seq data from 754 newly diagnosed MM (NDMM) patients and 98 relapsed and refractory MM (RRMM) samples from the MMRF CoMMpass study were utilized and compared against 8 normal bone marrow plasma cells. A transcriptome PE landscape was established for 18 subgroups, covering all major subtypes of MM such as 5 translocation types, hyperdiploidy and other high-risk groups such as *DIS3*, *TP53*, *TENT5C*, *KRAS*, *NRAS*, *BRAF, SF3B1* mutated as well as 1q gain and amplifications. Significantly differentially spliced PEs among subtypes and/or between disease stages were identified. Their prognostic value was evaluated by survival analysis. Moreover, transcriptome PE splicing profiles from 28 MM cell-lines in DepMap were established and their essentiality measured by the paired CRISPR screening results on the corresponding genes.

Results:

6,598 and 1,305 significantly differentially spliced PEs were identified in newly NDMM and RRMM compared to normal BMPCs, respectively, including 15 oncogenes (e.g. *EIF4A2*) and 28 tumor suppressors (e.g. *NF2*) documented in 'ONCOKB' database. 172 PEs were consistently aberrantly spliced across four primary subtypes in NDMM while >20 uniquely spliced PEs were found in each subtype. 53 PEs were differentially spliced between NDMM and RRMM. This indicated the consistency and heterogeneity of PEs splicing among subtypes and stages. For instance, a PE on *EIF4A2* was less frequently used in t(11;14) samples. When t(11;14) samples were divided into those with high or low PE usage, their gene expression showed no significant difference (p=0.53) while the high subgroup was associated with significantly worse progression-free survival (p=0.01), indicating a potential role associated with outcome. Among all identified PEs in NDMM, 370 PEs reflected significant associations with poor prognosis. 75 were further identified as independent predictors besides other well known risk factors such as 1q amplification or biallelic *TP53* inactivation. 103 PEs were prioritized due to their high essentiality in cell-lines (e.g. *ARIH1*). Inclusion/Exclusion of such PEs led to significant cell viability loss (chronos score<-0.5, CRISPR KO experiment). Moreover, 7 gene pairs with such PEs were found to have synthetic lethality when they were targeted together in *in vitro* CRISPR experiment in SynthlethDB database (e.g. *ARIH1* and *UBA3*).

Conclusion:

We demonstrated the heterogeneous usage of PEs among subtypes of MM and connected such usage with survival, which might shed light on further investigation on targeting abnormal usage of PEs.

Translational/Clinical Research ***Faculty***

POSTER #87

CHARACTERISTICS OF THE IMMUNE LANDSCAPE IN THE BONE MARROW MICROENVIRONMENT AND ASSOCIATION WITH RESPONSE TO HIGH DOSE MELPHALAN AND AUTOLOGOUS STEM CELL TRANSPLANT (ASCT) IN MULTIPLE MYELOMA

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Introduction: High dose melphalan and autologous stem cell transplant (ASCT) remain a cornerstone of therapy for eligible newly diagnosed MM patients. Some patients have long-term remissions but for others relapse may occur within a few months after ASCT. The bone marrow microenvironment may affect the response of MM cells. Cell-type specific gene expression and prevalence changes in the bone marrow microenvironment are associated with high dose melphalan resistance. We use paired pre- and post-ASCT samples from patients who had extremes of response to ASCT and were enrolled in Indiana Myeloma Registry to investigate the role of the microenvironment in MM resistance to high dose melphalan using scRNA and TCR sequencing.

Methods: Paired bone marrow (BM) aspirates were collected from MM patients (n=40) after induction therapy but before ASCT and after ASCT but prior to maintenance therapy. All patients had measurable disease after induction therapy. After ASCT, patients were evaluated based on their response to ASCT and divided into those who achieved a complete response (n=17) and were measurable residual disease (MRD) negative at 10^{-6} by flow cytometry (responders), or those who achieved a partial response or less (n=23) (non-responders). BM mononuclear cells were isolated by Ficoll and CD138 magnetic bead selection into CD138+ve and CD138–ve subsets. Paired pre- and post-ASCT CD138-ve samples underwent scRNA and TCR sequencing (10X Genomics), resulting in 488,479 high quality cells. Samples were independently normalized, cells integrated, and clusters annotated for cell type.

Results: Significant differences in the proportions of different immune cells in pre- vs. post-ASCT samples were identified with increases in B cells (2.6% vs 19.6%; P<.001) and B-cell progenitors (1.5% vs 8.9%; P<0.001) and decreases in CD4+T cells (24.8% vs 10.2%; P<0.001) and monocytes/myeloid cells (27.8% vs 21.0%; P=0.005) in post-ASCT samples. The overall proportions of CD8+T cells remained stable (27.1% vs 25.1%; P=0.2), but there were significant differences in the proportion of cells within the CD8+T cell clusters. When comparing responder and non-responder pre-ASCT samples, we found a higher percentage of Th2 CD4+T cell subset (10.0% vs 7.6%; P=0.04) in responders, which expressed AP-1 transcription factor family of genes. There was also a higher proportion of myeloid dendritic cells (1.47% vs 1.08%; P=0.05) and granulocyte progenitors (0.98% vs 0.62%; P=0.04) in responders in post-ASCT samples. TCR clonotypes revealed naïve CD4+T cells are higher in non-responders both pre- and post-ASCT. Also, we observed CD16+ve monocytes were enriched in post-ASCT non-responders (10.43% vs 5.37%; P=0.03).

Conclusions: We comprehensively describe the immune microenvironment in the BM of MM patients around ASCT and identify a pre-existing CD4+T cell subset that is associated with depth of response to ASCT. Prospective follow up of patients is ongoing to assess which clusters are associated with progression-free and overall survival.

Translational/Clinical Research ***Faculty***

POSTER #88

A HUMAN SKELETAL MUSCLE STEM/MYOTUBE MODEL REVEALS MULTIPLE SIGNALING TARGETS OF CANCER SECRETOME IN SKELETAL MUSCLE

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Skeletal muscle dysfunction is observed in multiple cancer types and extreme dysfunction is manifested as cachexia. Major preclinical studies on cancer-associated muscle defects utilized mouse models or C2C12 mouse myoblasts for in vitro studies. Due to species specificity of certain cytokines/chemokines in the secretome, a human model system is required to fully comprehend the effects of cancer secretome on skeletal muscle. Here, we report a simple method to establish skeletal muscle stem cell line (hMuSC), which can be differentiated into myotubes. Using single nuclei ATAC-seq (snATAC-seq) and RNA-seq (snRNA-seq), we document chromatin accessibility and transcriptomic changes associated with hMuSCs to myotube transition. Cancer cell line derived factors accelerated stem to myotube differentiation with accompanying changes including an increase in PAX7+/MyoD+ myogenic progenitor cells. Among the pathways activated by cancer-derived factors include inflammatory pathway involving CXCL8 (also called IL-8), glucocorticoid receptor (GR) pathway, and wound healing pathway. Furthermore, cancer-derived factors significantly altered splicing machinery in hMuSCs. Additionally, AKT and p53 pathways that function in metabolic/survival pathways of the skeletal muscle were adversely affected when hMuSCs were exposed to cancer cell-derived factors. Cancer-derived factors increased the expression levels of previously known cachexia-associated genes such as MT-2, ZIP14, and PDK4. Thus, the model system not only recapitulates results of previous mouse studies but also provides much needed human model system that can easily be adapted for large scale studies to explore the epigenomic changes during hMuSC differentiation and to screen for drugs that restore skeletal muscle function in various diseases.

Translational/Clinical Research ***Faculty***

POSTER #89

NOVEL INTERACTION BETWEEN HSF1 AND ERRα AND ITS POTENTIAL ROLE IN ONCOGENESIS.

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Ovarian cancer is among the most common cancers in women, with a 5-year relative survival of just around 50% due to a lack of early detection strategies and aggressive disease progression in advanced stages.
ERRα is an orphan nuclear receptor that regulates metabolic gene expression and has been linked to both ovarian and breast cancer. Inhibiting ERRα has been identified as a potential therapeutic strategy but the underlying mechanism is not fully understood. HSF1, a master transcription regulator involved in heat shock response and protein homeostasis, has been implicated in oncogenesis and promotes cell proliferation and invasion and any interaction or cooperation between ERRα and HSF1 has not been investigated. Analyses of ChIP Seq from four cancer cell lines revealed a large number of overlapping binding peaks and shared target genes between HSF1 and ERRα. Gene ontology enrichment revealed these shared target genes are enriched for pathways frequently dysregulated in cancer.
To further understand any interaction between ERRα and HSF1, we used an ERRα inhibitor (XCT790) and an HSF1 inhibitor (DTHIB) on ovarian cancer cells (OVCAR8) and observed a reduction in HSF1 protein after treatment with XCT790 and a reduction in ERRα protein after treatment with DTHIB, further suggesting some biological interaction. Our preliminary data suggests a possible transcriptional complex with HSF1 and ERRα that stabilizes both proteins and binds pro-cancer genes.
Future directions will be looking at the role of this potential interaction on oncogenesis and progression of cancer and probable therapeutic strategies exploiting this interaction, leading to better outcomes in patients.

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

POSTER #90

ORTHOGONAL CLICK CHEMISTRY TO GENERATE PHYSICOCHEMICAL GRADIENTS IN GRANULAR HYDROGELS

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Granular hydrogels or packed microgels have gained increasing interest in recent years owing to their high tunability compared with “bulk hydrogels”. Although granular hydrogels have been utilized for the assessment of cancer cell invasion, the heterogeneous stiffness increase in the tumor microenvironment (TME) due to the accumulation of extracellular matrix and how the mechanical properties of TME and accumulation of extracellular matrix impact cell fate has not been well studied. We have previously synthesized norbornene and carbohydrazide dually modified gelatin (i.e., GelNB-CH) for dynamic tuning gel stiffness. In this study, we sought to create granular hydrogels using GelNB-CH and sequentially use orthogonal click chemistry to form hyaluronic acid and stiffness gradients through diffusion and hydrazone/tetrazine click reactions.GelNB-CH microgel was created through water-in-oil emulsification and crosslinked with PEG-tetra-thiol (PEG4SH) through thiol-ene reaction. The GelNB-CH microgels were packed, transferred to a cylindrical mold, and either deposited without annealing or annealed with PEG-tetrazine through tetrazine-norbornene click chemistry. As a comparison, GelNB-CH bulk gel was made using the same gel precursor in the same cylindrical mold, but without the additional step of emulsification. Aldehyde-bearing oxidized HA (oHA) solution was labeled with green fluorescent dye and added on top of the microgels to enable diffusion and additional hydrazone bonds for immobilization of HA. GelNB-CH microgels were successfully crosslinked through UV light induced polymerization (size: ~100 to ~300 μm). oHA gradient was successfully created through diffusion and hydrazide/hydrazone click reaction between the aldehyde on oHA and the carbohydrazide on GelNB-CH. Specifically, the gradient distance is the shortest (~1 mm) in bulk gel, whereas gradient distance was increased in annealed microgels, and reaches the longest distance in non-annealed microgels (~4 mm), due to the most permeable interstitial space for diffusion. Future work will be focused on different physicochemical gradient and cell response to the HA gradient.

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

POSTER #91

INVESTIGATING THE ROLE OF CDX2 IN REGULATING TUMOR CELL DIFFERENTIATION IN BRAF MUTANT COLON

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BRAF activating mutations occur in approximately 10% of colorectal cancers (CRCs) and are associated with a poor prognosis due to an inferior response to standard chemotherapy. Therefore, *there is an urgent and unmet need for effective therapeutic targets for BRAF mutant CRC.* Studying the molecular mechanisms that contribute to mutant *BRAF* CRC progression will lead to the identification of such targets. The differentiation of enterocytes and secretory cells from intestinal stem cells in the normal colon epithelium is regulated by lineage-specific transcription factors. During tumorigenesis, lineage-specific transcription factor expression can alter, resulting in altered differentiation patterns that promote tumorigenic phenotypes. Poorly differentiated tumors have an increase in stem cell-like cells, which are associated with CRC development and progression. My overall goal is to determine the molecular mechanism that causes defects in colon epithelial cell differentiation and results in an increase in the stem cell population during *BRAF* mutant colon tumorigenesis. CDX2 (caudal type homeobox 2) is a transcription factor that induces intestinal differentiation and development. *BRAF* mutant CRC progression is usually associated with *CDX2* loss of expression which leads to poor prognosis in *BRAF* mutant CRC patients. Loss of CDX2 in combination with *BRAF* mutation is associated with poorly differentiated *BRAF* mutant CRC. However, how CDX2 regulates *BRAF* mutant colon tumor cell differentiation is still unknown. Our single-cell RNA-sequencing data showed that murine *BRAF* mutant colon tumors had increased expression of *Cdx2* relative to *BRAF* wild-type tumors. Furthermore, *BRAF* mutant tumors contained more differentiated enterocytes and secretory cells than *BRAF* wild-type colon tumors. Interestingly, RNA velocity analysis demonstrated that the decision of cell commitment to secretory and enterocyte differentiation occurred in *Cdx2*-expressing trans-amplifying (TA) cells suggesting that CDX2 induces differentiating cells to commit to the secretory and enterocyte lineage. *Cdx2* knockdown (KD) in reduced secretory marker gene expression and increased TA markers in organoids derived from *BRAF* mutant colon tumors. Based on these findings, we *hypothesize that* CDX2 maintains tumor cell differentiation by promoting TA cell differentiation in *BRAF* mutant colon tumors and loss of CDX2 will alter cell fate resulting in increases in the stem cell-like population, which promotes *BRAF* mutant CRC progression. An increase in the stem cell population due to the loss of CDX2 will cause an imbalance between undifferentiated and differentiated cells, which is a known driver for CRC progression.Therefore, elucidating how CDX2 regulates *BRAF* mutant colon tumor differentiation is key to understanding how the loss of CDX2 promotes *BRAF* mutant CRC development and progression.

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

POSTER #92

PROTEIN AGGREGATION PROMOTES HSF1 ACTIVITY ENHANCING CELL SURVIVAL DURING METASTATIC BREAST CANCER COLONIZATION

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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. We have previously shown that heat shock factor 1 (HSF1) promoted epithelial-to-mesenchymal transition (EMT) and the breast cancer stem-like population, potentially linking HSF1 to metastasis. 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. The physiological function of HSF1 is the master regulator of the heat shock response wherein it upregulates chaperone proteins under stress conditions. 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. To test this, we subjected human breast cancer MDA- MB-231 cells with or without HSF1 knockdown to intracardiac injections in 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. Mice receiving cells with knockdown of 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 observed increased protein amyloid aggregates that correlate with an increase in HSF1 activity during mammosphere formation, suggesting that colonization induces aggregation leading to HSF1 activation that promotes a cell survival response. Overall, my results indicate that HSF1 activity increased during breast cancer metastasis and HSF1 is necessary for colonization. Going forward, I want to understand what controls HSF1 activation during metastasis, which I hypothesize that protein aggregation is increased leading to HSF1 activation.

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

POSTER #93

HSF1 EXCLUDES CD8+ T CELLS FROM BREAST TUMORS VIA SUPPRESSION OF CCL5

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Breast cancer is the leading cause of cancer related deaths in women. The presence of cytotoxic immune cells, specifically CD8+ T cells, in breast tumors is associated with better patient outcomes. Our goal is to understand mechanisms that regulate the infiltration of CD8+ T cells. This will in turn, improve the treatment and enhance patient overall survival. Utilizing a novel HSF1 activity gene signature, we observed that HSF1 activity was negatively associated with the presence of CD8+ T cells in breast cancer patients. Both CIBERSORT analysis of TCGA data as well as direct assessment of patient primary specimens demonstrated that patient tumors with high HSF1 activity had lower numbers of CD8+ T cells. To functionally test this relationship, HSF1 was knocked down in 4T1 breast cancer cells and grown orthotopically in BALB/c mice. Tumors with HSF1 knockdown showed lower tumor volumes and increased CD8+ T cell infiltration. We subjected the control and knockdown tumors to single- cell RNA sequencing analysis and found increased immune cell presence specifically CD8+ T cell presence in knockdown tumors. We further tested the effect of CD8+ T cells on the growth of HSF1 knockdown tumors by depleting CD8+ T cells in BALB/c mice. HSF1 knockdown tumors were significantly larger with CD8+ T cell depletion suggesting a functional role for HSF1 to inhibit CD8+ T cell infiltration and protect the tumor from immune-mediated killing. To investigate the mechanism by which HSF1 affects CD8+ T cells, we investigated whether HSF1 affects chemotactic cytokines. We found that loss of HSF1 significantly increased secretion of CCL5, a known chemoattractant for CD8+ T cells. Loss of HSF1 also increased mRNA levels of CCL5, suggesting a transcriptional effect on CCL5. To test the importance of CCL5 in the phenotype of HSF1 knockdown, a transwell migration assay was performed wherein T cells from BALB/c mice were placed in the upper chamber of a transwell chamber and the lower chamber contained conditioned medium from 4T1 cells with knockdown of HSF1 with or without knockdown of CCL5. The lower chamber was subjected to flow cytometry for CD3/CD8 to identify CD8+ T cells and we observed that loss of HSF1 increased migration of CD8+ T cells and this was ablated with additional loss of CCL5. These results suggest CCL5 is a significant mediator for the recruitment of CD8+ T cells when HSF1 is lost in cancer cells. We propose the model whereby high HSF1 activity in breast cancer cells suppresses expression and secretion of the chemo-attractant cytokine CCL5 that ultimately leads to a decrease in CD8+ T cells in breast tumors microenvironment.

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

POSTER #94

INVESTIGATING CANCER STEM CELL PLASTICITY IN OVARIAN CANCER

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Most women diagnosed with late-stage high grade serous ovarian cancer (HGSOC) develop recurrent, platinum-resistant tumors. Ovarian cancer stem cells (OCSCs) are hypothesized to contribute to the emergence of these resistant tumors. CSCs have been postulated to reside in a plastic state, which can allow for the conversion of non-CSC to CSC. This process of dedifferentiation continues during tumor development and chemotherapeutic agents like platinum can exaggerate CSC plasticity. We have previously demonstrated that acute platinum treatment enriched for OCSCs. However, whether platinum transforms non-OCSCs into OCSCs to contribute to this subpopulation of cells remains unclear, and the underlying mechanism remains incompletely understood. To examine OCSC plasticity, aldehyde dehydrogenase (ALDH; functional marker) and fluorescence activated cell sorting were used to isolate OCSCs (ALDH+) and non-OCSCs (ALDH-) from HGSOC cell lines, OVCAR5 and OVCAR3. To determine the stability of the non-OCSC phenotype, ALDH- cells were cultured for 3 and 5 days and ALDH activity was measured using flow cytometry. At both timepoints examined, ALDH- cells remained ALDH-, with approximately less than 1% being ALDH+ ($p<0.05$). To determine if platinum can induce conversion of non-OCSC to OCSC, ALDH- cells were treated with cisplatin (12μM for 16h), and the percent of ALDH+ cells was measured using flow cytometry. Treatment of ALDH- cells with cisplatin resulted in conversion of approximately 4% and 10% of ALDH- cells into ALDH+ cells ($p<0.05$) in OVCAr5 and OVCAR3 cells, respectively. Furthermore, increased expression ($p<0.05$) of stemness genes *BMI1, NANOG, OCT4,* and *SOX2*, analyzed using qRT-PCR, was observed in converted OCSCs compared to parental ALDH+ and whole cell populations treated with cisplatin (12μM for 16h), suggesting that platinum induced the observed differences in the stemness phenotype. With the goal of targeting key genes and pathways to inhibit platinum-induced OCSC conversion, RNA-sequencing was performed using converted OCSCs. Current investigation of the genes and pathways driving platinum-induced conversion on non-OCSCs to OCSCs will be used to understand the mechanisms involved in OCSC plasticity, which is critical to developing targeted therapies to block the persistence of OCSCs and ultimately reduce mortality in patients.

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

POSTER #95

INHIBITION OF THE SMALL GTPASE ARF6 IN TUMOR CELLS ENHANCES ANTI-TUMOR IMMUNE RESPONSE THROUGH BLOCKADE OF MHC-I ENDOCYTOSIS IN TRIPLE NEGATIVE BREAST CANCER

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Immune checkpoint blockade (ICB) therapies have shown promise in treating Triple Negative Breast Cancer (TNBC) patients and has recently been added as a neoadjuvant therapy in combination with chemotherapy for advanced TNBC patients. However, response rates have only slightly increased, mainly due to the lack of immune cell infiltration into the tumor, which is a result of reduced antigen-MHC-I (major histocompatibility complex-I) molecules on the tumor cell surface. We hypothesized that inhibition of MHC-I endocytosis will result in increased surface MHC-I levels leading to enhanced cytotoxic T cell infiltration, which would enhance efficacy of ICB therapy. The small GTPase ARF6 has been implicated in the trafficking of MHC-I complexes and thus, we sought to analyze its expression levels from TCGA data of breast invasive carcinoma. Interestingly, Arf6 expression was significantly higher in breast tumor tissue compared to normal breast tissue. To investigate the effect of ARF6 on antigen presentation, we generated Arf6 lentiviral shRNA knockdown (KD) murine and human TNBC cell lines and measured their surface MHC-I levels by flow cytometry. We observed a significant increase in MHC-I levels upon Arf6 KD. To investigate the effect of Arf6 KD on tumor cell killing, we performed a coculture of EO771 OVA cells (overexpressing the foreign chicken protein) and autologous T cells. Interestingly, an enhanced tumor cell killing was observed upon Arf6 KD compared to control cells. Furthermore, to test the effect of Arf6 KD on tumor burden, we inoculated murine 4T1 TNBC cells orthotopically into the 4th left mammary fat pad of BALB/c mice and measured their tumor growth. 4T1 Arf6 KD tumors showed reduced growth compared to 4T1 control tumors and upon immune cell profiling of the harvested tumors, it was revealed that Arf6 KD tumors had increased CD8 T cell infiltration compared to control tumors. Next, to investigate if Arf6 KD causes inhibition of MHC-I endocytosis, we performed single particle tracking of the antigen OVA-Alexa Fluor 647 loaded onto MHC-I molecules of EO771 cells. We were able to visualize two types of motion on the plasma membrane of tumor cells: confined motion indicating active endocytosis and directed motion indicating endocytosis inhibition. Interestingly, after capturing the 2.5-minute movies, tracking particles and analysis of their biophysical parameters, we found a marked increase in mean squared displacement (measure of how far the particle has traveled on the membrane) upon Arf6 KD with a corresponding increase in length of constraint (how confined the particle is). Collectively, these observations suggest that the inhibition of ARF6 reduces the endocytosis of antigen-MHC-I complexes, thus enhancing infiltration of CD8 T cells into the tumor microenvironment and subsequent tumor cell killing. These findings would suggest the potential combination of ARF6 inhibition with ICB to enhance patient prognosis.

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

POSTER #96

THE HISTONE DEACETYLASE INHIBITOR ENTINOSTAT AS A TREATMENT FOR HSF1-MYC COAMPLIFIED OVARIAN CANCER

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Ovarian cancer is a disease with a 5-year relative survival rate of only 49%. This low survival is in part because 80% of patients have recurrence after standard platinum-based chemotherapy. This high recurrence rate shows the need for finding better treatments for this disease. An ideal cancer treatment would require finding a way to target a unique biological aspect of the cancer. Our lab has found that 36% of ovarian cancer patients have copy-number amplifications of the two oncogenes Heat shock factor 1 (HSF1) and Cellular myelocytomatosis (MYC) which encode for transcription factors of the same name. These transcription factors are known to drive cancer through multiple mechanisms including an increase in stemness, metabolism, and survival. Since transcription factors are notoriously hard to target with a drug, we aimed to inhibit the activity of these transcription factors with an inhibitor of a chromatin modifier. We performed a drug screen to find chromatin modifier inhibitors that selectively killed ovarian cancer cells with amplifications of the HSF1 and MYC genes. In the drug screen we found that ovarian cancers that carry the HSF1-MYC coamplification are more sensitive to the class I (HDAC) inhibitor Entinostat than ovarian cancers without this coamplification. Our preliminary results show Entinostat decreases HSF1 and MYC protein levels as well as decreases the mRNA expression of their direct downstream targets. Our lab will further investigate the effects of Entinostat on HSF1 and MYC as well as its efficacy for therapeutic use.

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

POSTER #97

PHARMACOGENOMICS GENOTYPING FROM CLINICAL SOMATIC WHOLE EXOME SEQUENCING: ALDY, A COMPUTATIONAL TOOL

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Pharmacogenomics Genotyping from Clinical Somatic Whole Exome Sequencing: Aldy, A computational Tool

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Background

Pharmacogenomics (PGx) testing can reduce toxicities and improve efficacy of several drugs used to treat cancer and associated symptoms. PGx results can be determined from germline whole-exome sequencing (WES), but somatic mutations may cause discordance between tumor and germline DNA. Since clinical diagnostic sequencing in oncology frequently only includes tumor DNA, there would be clinical value in calling germline PGx genotypes from tumor DNA. Thus, the purpose of this study was to assess the feasibility of using somatic WES data to call germline PGx genotypes.

Methods

Germline and somatic WES data were obtained as part of the clinical workflow for 64 patients treated at the solid molecular tumor board clinic at Indiana University. Aldy v3.3 was implemented in LifeOmic's Precision Health Cloud™ to call PGx genotypes from somatic WES.Somatic Aldy calls were compared with previously validated Aldy germline calls for 8 genes: *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A4*, *CYP3A5*, *CYP4F2*, *DPYD*, and *TPMT*. Somatic read depth was >100x, except for the intronic *CYP3A4*22* variant, which was >30x.

Results

Somatic and germline Aldy calls was compared for a total of 512 genotypes and 56 (11%) calls were discordant. Discordant calls were most common for *CYP2B6* (23.4%) followed by *CYP2D6* (14.1%), *CYP2C19* (10.9%), *CYP2C8* (6.3%), and *DPYD* (6.3%). In contrast, all Aldy calls were concordant for *CYP3A5* and *TPMT*. 38 out of 64 subjects (59%) had discordant calls for at least one gene. The most common first cancer diagnoses in our cohort were colorectal (9.3%), breast (7.8%), and pancreatic (7.8%), and the rates of discordant Aldy calls did not differ by cancer type ($p>0.05$ for all cancer types). Based on our analyses of discordant calls, we anticipate that adjusting Aldy's thresholds for variant calling may allow Aldy to determine genotypes from somatic WES data.

Conclusion

In most cases, genotype calls of drug metabolism genes from tumor DNA reflected the germline genotypes; however, additional work needs to be done to determine if the remaining discordant calls can be corrected by modifying the informatics tools or if they are due to somatic mutations.

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

POSTER #98

THERAPEUTIC TARGETING OF BET BROMODOMAIN PROTEINS INCREASES DNA DAMAGE AND POTENTIATES SALVAGE THERAPY IN OSTEOSARCOMA XENOGRAFTS DERIVED FROM PATIENTS WITH REPLICATION STRESS SIGNATURES

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Osteosarcoma (OS) is an aggressive cancer of the bone with high metastatic potential in pediatric as well as adolescent and young adults. The survival rate for metastatic and relapsed OS patients is <30% and there is currently no effective standardized salvage therapy. Lack of efficacy is attributed to genetic complexity present in OS that is partly due to moderate levels of replication stress (RS). While high levels of RS can induce cell death, moderate RS levels may cause genomic instability that contributes to OS progression. Therefore, induction of RS to high levels that cause cell death could be a promising therapeutic strategy. Bromodomain and extra-terminal domain [BET proteins (BRD2,3, and 4)] are a family of epigenetic readers that not only regulate gene expression networks, but also regulate DNA replication and RS. Thus, we tested the hypothesis that BET inhibition will potentiate the efficacy of salvage therapy through exacerbation of RS in xenograft models of aggressive OS. The effect of the bivalent BET inhibitor (BETi), AZD5153, as a single agent and in combination with cytotoxic agents such as topotecan and ifosfamide was evaluated. Combination index and Bliss independence analyses demonstrated additive to synergistic cell growth inhibition in OS cell lines upon treatment with clinically relevant concentrations of AZD5153+ topotecan/ifosfamide. Treatment with PROTAC ARV825 that degrades BET proteins, resulted in similar growth inhibitory effects. Significant increase in PARP cleavage was observed following AZD5153+topotecan treatment compared to single agent, indicating enhancement of apoptosis. In addition, western blot and comet assays showed that BETi+topotecan induces its effect, at least partly, through increased DNA damage and RS *in vitro*. *In vivo* efficacy and safety studies focused on patient-derived xenografts (PDXs) of naive and pre-treated OS that harbor RS signatures. AZD5153 as a single agent significantly suppressed tumor growth in both naïve (PDX96) and pretreated (TT2) OS PDX models compared to vehicle ($p<0.05$, Two-way ANOVA; Holm-Sidak). Anti-tumor effect correlated with increased γ-H2AX following AZD5153 exposure in PDX, indicative of increased RS. Moreover, RNA-seq analysis integrated with kinome profiling data from BETi-treated PDX exhibited deregulation of factors involved in RS. Combination treatments of BETi+topotecan/ifosfamide indicated that AZD5153 potentiated the anti-cancer effect of salvage therapy in TT2 OS PDX, was well tolerated, and increased the probability of survival in mice. Efficacy in an OS RS+ metastatic lesion model is in progress. These data collectively suggest that BET inhibition as a single agent and in combination with low-dose salvage therapy holds promise as novel treatment strategies for inducing RS-mediated cell death in aggressive OS.

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

POSTER #99

THE HDAC INHIBITOR ROMIDEPSIN BLOCKS PROLIFERATION AND CAUSES CELL DEATH AT NANOMOLAR CONCENTRATIONS IN HIGHLY METASTATIC OSTEOSARCOMA SARCOSPHERES

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Osteosarcoma is the most common primary malignant bone tumor and affects adolescents, young adults, and canines. The five-year survival rate of human patients with detectable pulmonary metastases is only 30% and most dogs succumb to metastatic disease within a year, indicating that standard therapy (MAP: methotrexate, adriamycin, cisplatin) is not effective in these patient groups. It is therefore necessary to identify novel therapies for osteosarcoma that target the progression of pulmonary metastases. Our lab previously screened 114 FDA-approved anti-cancer drugs to identify agents that decrease the growth of 3D spheroids (sarcospheres) generated from 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 histone deacetylase inhibitors (HDACi's) that were tested. In follow-up experiments with and without MAP, romidepsin was the most potent and safest of the five FDA-approved HDACi's and seven that rare in clinical trials. Our goal was therefore to further evaluate romidepsin as a potential therapy for metastatic osteosarcoma. Romidepsin decreased viability of sarcospheres generated from metastatic osteosarcoma cell lines with IC50s of 2-6nM and was additive to synergistic with MAP. In highly proliferative sarcospheres (derived from 143B and MG63.3 cells), romidepsin blocks growth at all doses (0.1nM-10uM) and increases cytotoxicity at 10uM over the course of 48 hours. Flow cytometry cell cycle analysis of 143B sarcosphere following 24 hours of treatment with 10nM romidepsin shows a cell cycle block at G2/M and an increase in Sub-G1 cells, indicating a block in proliferation and an increase in cell death. In less proliferative LM7 sarcospheres, romidepsin has no effect on growth over 48 hours no cell cycle arrest following 24 hours of treatment with 10nM. However, there is an increase in Sub-G1 cells and a dose dependent increase in cytotoxicity at 0.1uM, 1uM and 10uM, suggesting that romidepsin primarily increases cell death in less proliferative sarcospheres. To directly treat osteosarcoma lung metastases, we used a murine tail vein injection model with 143B cells. The highest two doses of romidepsin (2.25mg/kg and 0.75mg/kg) prevented cancer-induced morbidity during the experiment. Quantitation of metastatic burden at the endpoint of the study was done using both histological analysis and species-specific qPCR with genomic DNA. In mice treated with 2.25mg/kg romidepsin, histology showed a decrease in number of lung metastases and trend toward reduced metastatic area, and qPCR showed decrease in human DNA. To increase translatability of our work, we treated canine and human patient-derived sarcospheres with romidepsin and found that 3 out of five canine samples and 4 out of 5 human samples responded to romidepsin at nanomolar concentrations. These results suggest that sarcosphere screening may be useful to identify patients that will respond to romidepsin.

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

POSTER #100

THE ROLE OF MICROENVIRONMENTAL FACTORS BFGF AND IGFBP6 IN OVARIAN CANCER METASTATIC COLONIZATION THROUGH ETS1 INDUCTION

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Most ovarian cancer patients present with disseminated disease at the time of diagnosis and extensive metastasis is responsible for the poor patient prognosis. Metastatic colonization is the penultimate and rate limiting step of metastasis. It requires productive reciprocal interactions with the metastatic microenvironment that help the cancer cells adapt to the new microenvironment and establish the metastases. The underlying mechanisms regulating the crosstalk and adaptation remains the least understood aspect of the disease. Using a 3D organotypic culture model mimicking early metastatic colonization, we have previously demonstrated that the reciprocal interactions of cancer cells with the microenvironment of the metastatic site induces the transcription factor ETS1 in the cancer cells. We showed that ETS1 induction promoted metastatic colonization of ovarian cancer both in vitro and in vivo, though its transcriptional targets PTK2, EHD1, and BCL2L12. Thereafter, we proceeded to study the mechanism of induction of ETS1 by the metastatic microenvironment. ETS1 was regulated by p44/42 MAP kinase signaling activated in the ovarian cancer cells when they interacted with mesothelial cells at the site of metastasis. Using heterotypic co-culture models, conditioned medium experiments, and secretome analysis, we identified basic FGF (bFGF) and Insulin like growth factor binding protein 6 (IGFBP6) as the key mesothelial cell factors that were responsible for the regulation of ETS1 in cancer cells (OVCAR4, OVCAR8 and Kuramochi) in a paracrine manner. This was further confirmed by analysis of single cell RNA-seq data from ovarian cancer patient metastases. We further evaluated the role of these factors in the regulation of ETS1 and metastatic colonization by blocking α5β1-integrin, the putative receptor of IGFBP6, using a neutralizing antibody (Volociximab) and inhibiting FGFR using AZD4547. Both Volociximab and FGFR inhibitor reduced p44/42 MAP kinase phosphorylation, ETS1 expression, and cancer cell motility. Taken together, our results indicate that FGF and IGFBP6 are essential microenvironmental factors mediating the crosstalk with the ovarian cancer cells that induces ETS1 and enables metastatic colonization. Collectively, this study demonstrates the potential of targeting bFGF and IGFBP6 to prevent productive interactions of the cancer cells with the microenvironment and treat ovarian cancer metastasis.

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

POSTER #101

MCAK INHIBITORS INDUCE ANEUPLOIDY IN TRIPLE NEGATIVE BREAST CANCER MODELS

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The standard of care for triple negative breast cancer (TNBC), the most lethal breast cancer subtype, is chemotherapy using a combination of microtubule poisons and DNA damaging agents. Microtubule poisons, like paclitaxel, are proposed to induce lethal levels of aneuploidy in tumor cells. While these drugs are initially effective in treating cancer, dose-limiting peripheral neuropathies are common, and patients often relapse with drug resistant tumors. Developing agents against targets that limit aneuploidy may be a valuable approach for therapeutic development. One potential target is the microtubule depolymerizing kinesin, MCAK, which limits aneuploidy by regulating microtubule dynamics during mitosis. Analysis of MCAK expression levels in the TCGA and GSE47651 breast tumor databases showed MCAK is upregulated across all breast cancer subtypes, with the highest levels in TNBC. Additionally, high MCAK expression was associated with reduced overall survival and distant metastasis-free survival, indicating that MCAK may be able to serve as a biomarker of disease severity. Knockdown of MCAK in tumor-derived cell lines caused a 2 to 5-fold reduction in the IC_{50} for paclitaxel, but there was no change in normal diploid lines, indicating MCAK loss may have cancer-specific effects. Treatment of cells with paclitaxel or knockdown of MCAK both caused an increase in aneuploidy, but combination treatments did not have an additive effect, suggesting another mechanism is responsible for the increase in taxane sensitivity. Interestingly, MCAK knockdown also induced aneuploidy in a taxane resistant breast cancer line. To identify potential MCAK therapeutics, we developed two screens using FRET and image-based assays and identified three candidate inhibitors. All three inhibitors potently inhibit MCAK in an in vitro microtubule depolymerization assay. Similar to our knockdown studies, these drugs increased aneuploidy in breast cancer cells, regardless of taxane-resistance. These inhibitors also caused a potent reduction in colony formation assays in both taxane-sensitive and resistant cells. The most potent inhibitor, C4, also caused an approximate 2.3-fold reduction in the IC_{50} of paclitaxel in a TNBC cell line. 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 #102

ELUCIDATING THE ROLES OF INTERFERONS AND INTERFERON RESPONSES IN PLATINUM RESISTANCE IN OVARIAN CANCER

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High-grade serous ovarian cancer (HGSOC) is the most common subtype of ovarian cancer (OC). The current standard of care for OC patients is surgery debulking, followed by a combination of platinum- and taxane-based chemotherapies. Despite the high initial response rate to chemotherapies, most patients experience relapse, and the recurrent tumors no longer respond to platinum and other therapies. Relapse and chemoresistance contribute to a low five-year survival rate of less than 30%, making OC the most fatal gynecological cancer. A population of OC cells termed ovarian cancer stem cells (OCSCs) are enriched in recurrent tumors. It is well-established that OCSCs contribute to platinum resistance in OC. In addition to OCSCs, recurrent OC tumors also have aberrant promoter DNA hypermethylation resulting in the silencing of genes involved in tumor suppression and DNA repair, thus allowing OC cells to survive therapeutic assaults. Our lab has shown that pre-treatment of OC cells with a DNA methyltransferase inhibitor (DNMTi) blocked platinum-induced OCSC enrichment. My project aims to understand the mechanism of action behind this observation so that we can ultimately develop strategies to prevent recurrent OC following platinum therapy. Interestingly, it has been reported that both platinum and DNMTi can trigger interferon (IFN) responses, even though they have opposite effects on the OCSC population. IFNs and IFN responses are part of the body's first line of defense against pathogens, called the innate immune system. Besides their roles in fighting infections, IFNs have been suggested to be either pro- or anti-stemness in cancer cells, depending on the type of cancer and context. In the preliminary data, I found that platinum, DNMTi, or a combination of both treatments induce distinct IFN responses in the HGSOC cell lines OVCAR5 and OVCAR3. I also found that single treatment of platinum and DNMTi induced expression of different type I IFNs, IFNa and IFNb, respectively. The combination of platinum and DNMTi increased the expression of both IFNa and IFNb. Based on my preliminary data, I hypothesize that platinum and DNMTi induce distinct IFN responses resulting in contrasting outcomes in the OCSC population. To address this hypothesis, I will determine mechanically how platinum and DNMTi induce IFN production and downstream signals and investigate the role of different type I IFNs in platinum-induced OCSC enrichment. The outcomes from this study will lead to the development of new therapeutic strategies to reduce platinum-induced enrichment of OCSCs and thus prevent recurrent and platinum-resistant OC. Additionally, the type I IFNs, IFNa and IFNb, are rarely studied separately, so this work will provide insights into the distinct roles of different type I IFNs.

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

POSTER #104

CANCER ASSOCIATED FIBROBLASTS SERVE AS A CANCER STEM CELL NICHE AND IMPART CHEMORESISTANCE IN OVARIAN CANCER

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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 we have shown a subpopulation of CAFs secrete WNT5A to regulate cancer cells and enrich cancer stem cell population. My analysis of 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 revealed that the percentage of CAFs in the TME increases with the cancer stage. Deconvolution of the Australian Ovarian Cancer Study dataset showed significant CAF enrichment in chemo-resistant tumors. TUNEL and immunofluorescent staining in frozen sections of chemo-naïve and post-chemo patient tumors. While chemo-naïve 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 and increases the dedifferentiation of bulk ovarian cancer cells. We applied a single-cell RNA sequencing approach to identify and characterize the subpopulations of CAFs that are capable of inducing stemness and chemoresistance, and the cancer subpopulations that are responsive to these signals. Heterotypic 3D cocultures of patient-derived ovarian cancer cells and CAFs were thus analyzed. Published datasets further allowed us to validate our findings in patient samples. We characterized pathways activated in CAFs with high WNT5A expression and found upstream targets of WNT5A expression and secretion. By blocking the upstream signaling of WNT5A, we are looking forward to attenuating WNT5A expression in CAFs and thus preventing ovarian cancer relapse.

We also found cancer cell subpopulations, that respond to CAF signals and become cancer stem cells, using trajectory inference and ligand-receptor network analysis. Our research uncovered the heterogeneity of CAFs in ovarian cancer TME and demonstrated the role of CAF subpopulations that can serve as a cancer stem cell niche, giving rise to disease relapse. We also uncovered how OC cells modulate CAFs in TME by secreting ligands and increasing $WNT5A^{high}$ CAFs. Targeting this communication to prevent ovarian cancer recurrence will help improve patient outcomes.

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

POSTER #105

TARGETING METHYL DONOR SYNTHESIS TO PREVENT OVARIAN CANCER STEM CELL ENRICHMENT

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Ovarian cancer (OC) is the leading cause of gynecologic cancer death. Platinum (Pt)-based chemotherapy can reprogram cancer cells to ovarian cancer stem cells (OCSCs) and contribute to disease relapse and death. Pt-induced alterations in DNA methylation have been shown to enhance CSCs malignant properties. In the current study, we demonstrated that Pt drugs can enrich for OCSCs. In high grade serous ovarian cancer (HGSOC) cell lines treated with cisplatin (IC50), the percentage of cells with high aldehyde dehydrogenase (ALDH+) activity, a known OCSCs marker, increased ($p<0.01$). In addition, cisplatin treatment altered the methylation pattern in OCSCs, indicated by increased percentage of 5-methylcytosine. Furthermore, cisplatin treatment altered methionine (Met) metabolism in OC, demonstrated by a decrease ($p<0.01$) in the level of S-adenosyl-methionine (SAM) metabolite and an increase ($p<0.01$) expression of methionine adenosyltransferase 2A (MAT2A) in short time treatment, both of which presumably contributed to the cisplatin-induced changes in DNA methylation pattern. In many cancers, CSCs have been shown to have a high-flux metabolic state and be dependent on Met metabolism. In support of this, MAT2A expression was higher ($p<0.01$) in ALDH+ cells compared to the whole cell population. To demonstrate that Met metabolism plays a crucial role in OCSCs enrichment, Met was depleted from the media (Met-), combined with cisplatin or vehicle. Met- blocked ($p<0.01$) the expected Pt-induced increase in %ALDH+ cells and spheroid formation. MAT2A is the rate limiting enzyme of Met utilization in the metabolism. MAT2A activity inhibitor FIDAS-5 combined with cisplatin inhibited Pt-induced %ALDH+ cells ($p<0.01$) and spheroid formation. FIDAS-5 treatment also arrested cells in G1/S phase ($p<0.05$) may through down-regulating mTOR activities and G1 regulators, but the underlying mechanism remains to be established. Collectively, these results demonstrate that blocking Met metabolism inhibited Pt-induced OCSCs reprogram *in vitro*. To further investigate the mechanism underlying Met metabolism inhibition, global changes in differential methylation are being examined after FIDAS-5 and cisplatin, as well as effects of altering the cell cycle on OCSCs enrichment. MAT2A has been recognized as a therapeutic target for the treatment of cancers, MAT2A inhibition could represent a therapeutic approach for inhibiting CSC in ovarian cancer.

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

POSTER #106

TARGETING CDK4/6 AND BET PROTEINS FOR THE TREATMENT OF RB+ AND RB- OSTEOSARCOMA

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Osteosarcoma (OS) is the third most common cancer in children, adolescents, and young adults. At initial presentation, ~20% of OS patients already have metastatic disease resulting in a poor prognosis of 25% long-term survival compared to 65% survival for patients with localized lesions. To improve clinical outcomes, there is a critical need to identify novel efficacious therapies for OS. Data from the precision genomics initiative at Riley Hospital for Children has provided insight into actionable therapeutic biomarkers such as MYC and CDK4/6 hyperactivation in a subset of OS patients. However, patient stratification for targeted therapy for pediatric cancers is still in its infancy and requires further validation of biomarker linkage with therapeutic outcomes. These actionable signatures can be targeted using therapies such as CDK4/6 inhibitors (CDK4/6i), but monotherapy often fails due to cytostatic response and loss of the Retinoblastoma (RB) tumor suppressor. In addition, 70% of OS patients have decreased function or loss of RB. In the clinical setting RB proficiency (RB+) is typically used as a biomarker of therapy effectiveness to CDK4/6i. Studies are emerging that indicate RB-deficient (RB-) tumors can respond to CDK4/6i due to the presence of RB-related proteins such as p105, p130 and FOXM1. Therefore, identifying strategies to optimize CDK4/6i in patients regardless of RB status is vital.

Based on a nonbiased screening approach in OS human and murine cell lines, bromodomain and extraterminal domain inhibitors (BETi) have been prioritized for study in combination with CDK4/6 inhibition. Previous data has shown this combination induced synergistic cell growth inhibition in a wide range of inhibitor ratios in both human and murine OS RB+ cell lines. BETi are a novel class of drugs that bind acetylated lysines on histones and modify transcription of genes such as MYC, BCL2, FOXM1, involved in limiting cell growth and inducing apoptosis. To date, no study has compared CDK4/6i, Abemaciclib versus Palbociclib, in combination with a BETi AZD5153 in OS. Additionally, to what extent RB- patients can benefit from this combination therapy still needs to be elucidated. To this end, CRISPR mediated RB-knockout OS human and murine clones were developed and exhibited synergistic growth inhibition at low doses of CDK4/6 and BET inhibitors indicating utility of this approach in RB- and RB+ patients. Based on these data, we hypothesize that dual inhibition of CDK4/6 and BET pathways will be more efficacious than single agent therapy in RB+ and RB- in-vivo models of pediatric and AYA OS. Data obtained in this ongoing project will provide new clinically relevant information on therapeutic response and stratification of OS patients based on RB status.

Translational/Clinical Research ***MD/PhD Student***

POSTER #108

DECIPHERING THE IMMUNE MICROENVIRONMENT OF NF1-ASSOCIATED PERIPHERAL NERVE SHEATH TUMORS: IDENTIFYING EARLY BIOMARKERS OF DISEASE PROGRESSION AND MALIGNANT TRANSFORMATION

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Neurofibromatosis type 1 (NF1) is a multisystem, autosomal dominant disorder that affects approximately 1/3000 newborns. Plexiform neurofibromas (PN) are present in about half of cases and frequently cause pain, disfigurement and motor dysfunction in affected individuals. PN can transform (lifetime risk of 8-13%) into malignant peripheral nerve sheath tumor (MPNST), a highly aggressive and metastatic form of soft tissue sarcoma with poor survival. However, there are no reliable biomarkers to identify PN at high risk of undergoing malignant transformation. Our research has revealed that a subset of benign appearing PN and premalignant atypical neurofibromas exhibit deregulated signatures of immune surveillance and T cell infiltration that precede malignant transformation. In this study, we will analyze the tumor microenvironment and immune landscape of archival plexiform and atypical neurofibroma specimens, either associated or not associated with malignant transformation, to identify and validate potential biomarkers of disease progression. De-identified clinical data including treatments (chemotherapy/radiation), presence of metastatic disease at diagnosis, and imaging features will also be analyzed. This study represents a critical first step to developing molecular diagnostic tools to guide risk adapted care in patients with NF1-associated PN. Moreover, the results of this study will provide valuable insights that further inform hypothesis-driven research in preclinical models of NF1-tumorigenesis to validate these findings and identify novel treatment approaches for individuals affected by these rare but devastating tumors.

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

POSTER #109

IDENTIFYING NOVEL OPPORTUNITIES TO IMPROVE CANCER SCREENING ATTENDANCE AMONG EMERGENCY DEPARTMENT OBSERVATION UNIT PATIENTS

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Background and Objectives: Millions of patients in need of recommended cancer screenings are cared for in emergency departments (EDs), many of whom experience barriers to accessing care and in other healthcare settings. A critical unmet need exists in improving participation in cancer screening programs, especially for medically underserved populations. The ED is increasingly being used as a location for novel public health interventions to reach populations that otherwise would not have access to care. This study aims to describe the rate of missed opportunities for addressing cancer screening for breast, colorectal, lung, and cervical cancers among ED observation unit (EDOU) patients at a safety-net health care system.

Methods: We performed a retrospective cohort study of 100 patients admitted to the ED observation unit from 5/1/2022-5/31/2022. Patients ³ 18 years old with accessible electronic health records (EHR) were included. Demographics, length of stay (LOS), smoking histories, and whether EDOU patients were overdue for the most common cancer screenings (breast, colorectal, lung, and cervical) were collected. Further, we assessed the accuracy of the electronic health record (EHR) for identifying EDOU patients that were overdue for cancer screenings.

Results: Among 100 EDOU patients, 46% were female, 54% were non-white, median age was 55.5 (IQR 32.2-78.8) years, and average length of stay in the EDOU was 19.5 hours. Thirty-seven percent of females were eligible for breast cancer screening, meanwhile 71% of these females were overdue for screening. Thirty-one percent of patients were eligible for colorectal cancer screening, meanwhile 74% of eligible patients were overdue. Thirty-four percent of patients were eligible for lung cancer screening, and 94% of them were overdue. Lastly, 31% of patients were eligible for cervical cancer screening, with 74% being overdue. Overall, the EHR correctly identified 75%, 85%, 74%, and 80% of eligible screening patients for breast, colorectal, lung, and cervical cancers, respectively.

Conclusion: Our EDOU is a target-rich environment for patients who are overdue for recommended cancer screenings. While the EDOU was designed to resolve acute problems, the reality is our EDOU may serve as the only access point to medical care for many patients. Our EDOU visit represents a large, missed opportunity to address recommended cancer screenings for a medically underserved population.

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

POSTER #111

MOLECULAR AND CLINICAL EFFECTS OF AROMATASE INHIBITOR THERAPY ON SKELETAL MUSCLE FUNCTION IN EARLY STAGE BREAST CANCER

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Introduction: Aromatase inhibition (AI) promotes osteoclastic bone resorption and bone loss, predisposing to well-established skeletal complications. In contrast, little is known about muscle impairments associated with AIs. Preclinical data have established that estrogen deprivation instigates bone turnover and release of TGF-β, in turn potentiating muscle dysfunction through oxidation of the skeletal muscle ryanodine receptor RyR1, loss of stabilizing protein calstabin1, and causing calcium leak. We aim to explore this in a clinical population, investigating the impact of AI-induced bone turnover on post-translational changes in RyR1 and contractile muscle properties.

Methods: Fifteen postmenopausal women with stage I-III breast cancer planning to initiate AI enrolled. Baseline visits prior to AI and follow-up visits after six months of AI consisted of a muscle biopsy and isokinetic dynamometry. Muscle tissue was obtained from the quadriceps femoris using the modified Bergström technique. Relative levels of oxidized RyR1 and RyR1-calstabin1 complexes pre- and post-AI exposure were coimmunoprecipitated and immunoblots quantified. Muscle contractile properties were assessed with a Biodex 4 isokinetic dynamometer. Patients performed 3-4 maximal knee extensions at angular velocities (0, 1.57, 3.14, 4.71, 6.28 rad/s). Peak power was determined at each velocity and the resulting power-velocity curve was fit with a parabolic function to determine maximal torque, speed, and power. Subsequently, an “all out” 50 contraction fatigue test was performed to evaluate fatigue resistance. Recovery of muscle function was measured by restoration of torque during contractions performed periodically over 10 minutes. Values were compared using paired t-tests, with p value of <0.05 considered significant.

Results: 14/15 participants completed all assessments. Relative oxidized RyR1 levels significantly increased (0.23 ± 0.34 to 0.88 ± 0.78, $p<0.05$) and bound RyR1-calstabin1 significantly decreased post AI-exposure (1.68 ± 1.48 to 0.74 ± 0.82, $p<0.01$). Analysis of muscle contractile properties pre- and post-AI exposure demonstrated no significant difference in maximal torque (1.54 ± 0.31 vs 1.53 ± 0.34; $p=0.9417$), speed (12.2 ± 2.4 vs 11.8 ± 1.1; $p=0.5770$), or power (3.45 ± 1.07 vs 3.61 ± 0.79; $p=0.4879$). Fatigue-recovery testing also showed no significant change in percent reduction in power from start to end of the “all out” contract test (58 ± 10 vs 62 ± 10; $p=0.2475$) or rate of recovery half-life (72 ± 38 vs 62 ± 31; $p=0.4739$).

Conclusion: Supporting benchwork at the “bedside”, estrogen deprivation therapy in early stage breast cancer was associated with biochemical changes in muscle associated with calcium leak (oxidized RyR1 and loss of bound calstabin1). No change in clinical muscle contractile properties was seen. Next steps will include correlation with serum measures of bone turnover and patient-reported functional measures. In addition, it is possible that the biochemical changes seen may be early the in treatment course and could impact function with a longer duration or larger patient population. Evaluating the impact of endocrine therapy in breast cancer on muscle function is novel and needed as a comprehensive focus on MUSCULOskeletal health.

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

POSTER #112

DOSE-ADJUSTED EPOCH VERSUS CHOP IN HIGH RISK DIFFUSE LARGE B-CELL LYMPHOMA

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Double hit lymphoma (DHL), defined as high grade B-cell lymphoma harboring MYC rearrangement occurring with either B-cell CLL/lymphoma 2 (BCL2) and/or B-cell CLL/lymphoma 6 (BCL6) and triple hit lymphoma (THL), defined as harboring all three rearrangements, represents around 10% of diffuse large B-cell lymphomas (DLBCL). Multiple retrospective series suggest that patients with DHL and THL face poor prognoses when treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP), with a median overall survival of 12 months or less. Due to this poor survival, chemotherapy intensification has been explored as a strategy to improve outcomes. The current national comprehensive cancer network (NCCN) guidelines recommend the use of dose-adjusted EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin) for treatment of DHL. At Indiana University Health, patients diagnosed with DHL or THL are receiving DA-EPOCH preferentially over CHOP.

The purpose of this study is to add to assess outcomes and toxicities of patients treated with DA-EPOCH and CHOP at Indiana University Health.

This study is a retrospective chart review of patients with DHL or THL who completed 6 cycles of doseadjusted EPOCH/R-EPOCH or CHOP/R-CHOP at Indiana University Health between January 1, 2015, and July 31, 2022. Patients were excluded if they were less than 18 years old diagnosed with Burkitt lymphoma, or less than 3 months after completion of front line therapy. Patients receiving DA-EPOCH were also excluded if they did not complete at least 5 cycles of chemotherapy. The primary endpoint is progression free survival (PFS). Secondary endpoints include median overall survival (OS) and incidence of toxicities. Categorical data was be compared using the chi-square test, while continuous data was compared using the paired t-test or Wilcoxon-signed rank test

Results: There were a total of 11 patients included in this study, 9 patients who received DA-EPOCH and 2 patients who received CHOP. The mean age of the DA-EPOCH group was 58.3 years compared to 77.0 years with the CHOP group (p=0.399). A total of 6 patients progressed following therapy, 4 patients with DA-EPOCH and both CHOP patients. Mean PFS in the DA-EPOCH group was 44.17 months compared to 1.35 months with CHOP (p=0.006) but due to a small sample size, these results are disputable. Median OS in DA-EPOCH was 5.06 months compared to 4.6 months with CHOP (p=0.695). Neutropenia occurred at least once in 22 of the 49 cycles of DA-EPOCH and 1 of the 12 cycles of CHOP with a median duration of 4.0 days and 2.5 days (p=0.399) respectively. The most common toxicities reported were neuropathy, fatigue, and constipation.

Conclusion: Based on the results of this retrospective review the choice of DA-EPOCH and CHOP for high risk diffuse large B-cell lymphomas remains unclear. Additional studies should be further conducted.

Translational/Clinical Research ***PGY2 Oncology Pharmacy Resident***

POSTER #113

COMPARISON OF FULL VERSUS REDUCED INITIAL PAZOPANIB DOSING IN ADVANCED, RECURRENT, OR METASTATIC SARCOMA: A RETROSPECTIVE REVIEW

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The objectives of this study are as the followings: Part 1) Describe real-world efficacy and toxicity of pazopanib in patients with advanced, recurrent, or metastatic STS at Indiana University Health Simon Comprehensive Cancer Center (IUSCCC); Part 2) Compare the efficacy and toxicity outcomes for patients treated with different initial dosing (pazopanib 800 mg daily vs. 200 mg daily) between the cohorts at IUSCCC and University of North Carolina Medical Center.

An Institutional Review Board approved, multicenter, retrospective study was conducted in patients ages 18 years or older who received at least one dose of pazopanib. Patients were treated with pazopanib between May 2015 and October 2022 at IUSCCC. Data collected included patient demographics, histology subtype, tumor grade, pazopanib's line of therapy, and prior lines of therapy. The primary outcome was time on pazopanib therapy. The secondary outcomes were progression-free survival according to RECIST (Version 1.1) by imaging, overall survival (OS), frequency and types of adverse events, reason for discontinuation of therapy, and frequency and reason for dose reduction.

A total of 67 patients met the inclusion criteria.The median age was 57 years, and pazopanib was started as third or later line of therapy for 72% of patients. The median time on pazopanib therapy was 5 months (range 3.2-7.1). At the time of the data cutoff on 10/1/2022, 18% of patients remained on pazopanib. The most common reason for discontinuation of therapy was due to disease progression followed by death and toxicities. The mean starting dose of pazopanib was 371.6 mg with 200 mg (48%) being the most common starting dose. The median OS was 29 months (95% CI, 21.8 to not estimable). Most of the patients (87%) experienced 0 or 1 dose reductions due to toxicities. Treatment interruptions occurred in 45% of patients mostly due to toxicities and surgery. The three most commontoxicities found were consistent with what would be expected for pazopanib including fatigue, hypertension, and nausea/vomiting.

The median time on pazopanib and overall survival found in this study were longer than those have been reported in the literature, potentially due to 28% of patients being started on pazopanib as the first or second line of therapy. The incidences of dose reductions and interruptions were similar to those reported in the literature even though the mean starting dose of pazopanib was lower.Progression-free survival and part 2 data collection and analysis are in process. Results from part 2 will allow the clinicians make treatment decisions when starting, increasing/decreasing, and holding pazopanib in their practice.

Translational/Clinical Research ***PGY2 Oncology Pharmacy resident***

POSTER #114

EVALUATION OF POTENTIAL THERAPEUTIC IMMUNOHISTOCHEMICAL TARGETS WITH EXPERIMENTAL OR FDA-APPROVED THERAPIES IN THYMIC EPITHELIAL TUMOR MICROARRAYS

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Background:

Thymus epithelial tumors (TETs) are rare malignancies of the anterior mediastinum. The current standard of care for metastatic TETs is a combination of platinum-based chemotherapy. Here, we have evaluated the experimental and FDA-approved makers in a large TET tissue array with the hope of identifying a potential therapeutic option.

Methods:

A tissue microarray (TMA) containing ninety malignant thymic tumors and seven normal thymus was assembled. The protein expression of GLUT1, TROP2, PSMA, ROS1, ALK, HER2 and PD-L1 was tested with immunohistochemical assays. Expression was quantified using a "staining score (SS)" which is a 0-3 numerical score that results from the product of the intensity of expression: 0= negative, 1=weak, 2=moderate, 3=strong and the area of expression in fractions of a percent (0=no expression, 1=100% area). Expression of HER2 and PD-L1 was quantified according to existing guidelines (HER2 score and Combined Positive Score (CPS)).

Results:

The TET tissue array showed no significant expression of ALK, ROS1 or HER2. GLUT1 was highly expressed in thymic carcinomas (81.8%, SS: 2.1±1.1). PSMA expression was limited to intratumoral vessels of thymic carcinoma. PDL1 expression was expressed in all tissues: normal thymus:50%, CPS 3.8; thymomas A/AB/B1/B2: 63%, CPS: 44.7, thymoma B3: 69%, CPS 84, thymic carcinoma: 50%, CPS 57. Interestingly, expression of TROP2 was observed in all thymic tissues: normal thymus: 100%, SS 1.4, thymoma types A/AB/B1/B2: 87.5%, SS 1.2, Type B3: 57%, SS 1.1, Carcinoma: 100%, SS 2.7.

Conclusion:

Consistent high expression of Trop-2 protein in thymoma and thymic carcinoma indicate the potential therapeutic values for antibody-drug conjugate (ADC) such as Sacituzumab-govitecan in metastatic or recurrent thymic tumors.

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

POSTER #115

GENOMIC DETERMINANTS OF PIK3CA MUTATION-DRIVEN BREAST CANCER INITIATION

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PIK3CA is the second most commonly mutated gene in breast cancer. Two mutations in the helical domain, *E542K* and *E545K*, and one mutation in the kinase domain, *H1047R*, are the most frequent mutations. PIK3CA-specific inhibitor Alpelisib is an FDA approved treatment for breast cancer. Toxicity and rapid development of resistance limit its clinical utility. Therefore, additional therapeutic targets for *PIK3CA*-mutated breast cancer need to be identified. Previous studies in our lab have demonstrated that immortalized breast epithelial cells derived from *BRCA1/2* mutation carriers are susceptible to transformation by the $PIK3CA^{H1047R}$ mutant, whereas transformation of immortalized breast epithelial cells from healthy donors required combinations of $PIK3CA^{H1047R}$ +SV40-T/t antigens. These results suggest that transformation of breast epithelial cells by *PIK3CA* mutants requires additional genomic aberrations similar to those induced by SV40-T/t antigens or *BRCA1/2* mutations. To decipher genomic aberrations required for *PIK3CA* mutant driven breast epithelial transformation, we developed two model systems. The first is the isogenic human immortalized mammary epithelial cell (HMEC) system with genome edited $PIK3CA^{E545K}$ or $PIK3CA^{H1047R}$ mutation to characterize signaling pathways specifically activated by these mutants. Through immunoprecipitation using AKT substrate motif antibodies followed by mass-spectrometry, we observed that these two mutants target distinct transcriptional machinery. For example, while $PIK3CA^{E545K}$ mutant harboring cells displayed higher phosphorylation of YAP1, $PIK3CA^{H1047R}$ targeted several histone deacetylases including HDAC5, HDAC7 and HDAC9. Since transformed HMECs generate tumors that do not represent human breast cancers, we created the second model system of immortalized breast luminal epithelial cell lines from healthy donors that overexpress $PIK3CA^{E542K}$, $PIK3CA^{E545K}$ or $PIK3CA^{H1047R}$ mutants. Because our single cell analysis of breast tissues from healthy donors and BRCA1/2 mutation carriers has revealed specific upregulation of NF-kB signaling network in BRCA1/2 mutant cells compared to cells from healthy donors, and 30% of breast cancers with *PIK3CA* mutations also carry amplification of *IKBKB* or *IKBKE*, two endogenous activators of NF-kB, we are now generating PIK3CA mutant expressing cells that additionally overexpress constitutively active p65 subunit of NF-kB, IKBKB or IKBKE and testing these cells for transformed phenotype. Results from these studies should reveal signaling pathways required for *PIK3CA* mutation-driven breast initiation/progression and new therapeutic targets for such tumors.

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

POSTER #116

IDENTIFYING HIGH-RISK TUMOR REGIONS IN TRIPLE NEGATIVE BREAST CANCER BIOPSIES USING DEEP TRANSFER LEARNING AND SPATIAL ANALYSIS

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Triple negative breast cancer (TNBC) is a severe subtype of breast cancer with poor prognosis, high metastatic potential and low (77%) 5-year survival rate. Traditional chemotherapy is not effective due to lack of expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2). Although immunotherapy drugs have been approved for TNBC treatment, only 10-20% of patients respond. Therefore, there is a need for effective precision medicine for TNBC. Previously our lab has developed a deep-transfer learning-based technique called Diagnostic Evidence GAuge of Single cells (DEGAS) which has helped identify high-risk components in TNBC biopsies using TNBC single cell RNA sequencing data. The aim of the current research is to develop an effective methodology for identifying high-risk regions of tumors using TNBC spatial transcriptomics (ST) data. The specialized data-structure of ST data is realized in the form of a multidimensional array of spatial coordinates and the gene expression, which is then used to learn tissue-level information. Transfer learning techniques are subsequently implemented to identify various gene markers that are highly associated with the disease. By visualizing the gene expression patterns across spatially defined regions of a tissue sample, potential therapeutic targets can be identified. Future work may include the inclusion of patient-level covariates, such as age, gender, disease stages, treatment information, etc., using reduced-rank regression-based techniques to modify the patient-level input of the DEGAS methodology. This will allow us to identify factors related to patient survival and disease progression. In addition, the topological image features such as cell size and shape, nucleus area, and cell density in specific tissue regions can be extracted from the ST data images of TNBC patients. By comparing these features with those previously identified for the TCGA-BRCA cohort, potential image markers associated with high-risk regions from TNBC biopsies can be identified.

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

POSTER #117

TARGETING OVARIAN CANCER METASTASIS INITIATING CELLS INDUCED BY THE MICROENVIRONMENT THROUGH ERK/EZH2/DNMT1 MEDIATED MIR-193B DOWNREGULATION

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Extensive metastasis and frequent relapse are major 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 crosstalk 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 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 chemo resistant 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) 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 hypermethylation 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 chemo resistant OC PDX with miR-193b significantly reduced metastases, indicating that miR-193b replacement therapy could be a promising approach to treat OC patients.

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

POSTER #118

PRIMARY MEDIASTINAL SEMINOMA: TREATMENT AND SURVIVAL OUTCOMES

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Background: Primary mediastinal pure seminoma is a rare entity and there is no clear consensus for optimal management. We report treatment and survival outcomes of patients with primary mediastinal seminoma evaluated at our institution.

Methods: The Indiana University (IU) testicular cancer database was queried for patients with primary mediastinal seminoma. 21 patients evaluated at IU between 1994-2022 were included in this analysis. Kaplan-Meier methods were used for progression-free (PFS) and overall survival (OS) analysis.

Results: Median age at diagnosis was 40.7 (range, 19.4-54.2). 18 (85.7%) patients were IGCCCG good risk and 3 (14.3%) were intermediate risk. Median pre-chemotherapy hCG was 10.3 (range, 0.6-1500). 12 (57.1%) patients had metastatic disease. Metastatic sites included mediastinal/supraclavicular lymph nodes in 9 patients (42.9%), lungs in 3 (14.3%), retroperitoneal lymph nodes in 3 (14.3%), brain in 1 (4.8%), liver in 1 (4.8%), adrenal gland in 1 (4.8%), kidney in 1 (4.8%) and bone in 1 (4.8%). First-line chemotherapy was BEPx3 in 9 (42.9%) patients, EPx4 in 4 (19.0%), BEPx4 in 3 (14.3%), BEPx3 + EPx1 in 2 (9.5%), VIPx4 in 2 (9.5%), and VIPx1 + BEPx3 in 1 (4.8%). With a median follow-up of 4.65 years from first-line chemotherapy start date, 3 (14.3%) patients progressed after first-line chemotherapy. Among patients who progressed, salvage 2nd line therapy was high-dose chemotherapy in 1 patient. 1 patient received more than 2 lines of therapy. 1 patient underwent surgical resection of residual mediastinal mass due to concern for local progression, with pathology revealing necrosis only. The 5-year progression-free survival (PFS) from first-line chemotherapy was 82.4% (95% CI 54.7-93.9). The 5-year overall survival (OS) was 94.1% (95% CI 65.0-99.1). At last follow-up, 20 (95.2%) patients had no evidence of disease, and 1 (4.8%) was dead of disease.

Conclusions: Patients with primary mediastinal pure seminoma have high cure rates with risk adapted cisplatin-based combination chemotherapy. Despite residual masses, further surgical resection or radiation is not required in the vast majority of these patients and these residual masses can be safely monitored with surveillance.

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

POSTER #119

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL TO ASSESS THE DRUG-DRUG-GENE INTERACTION POTENTIAL OF BELZUTIFAN IN COMBINATION WITH CYCLIN-DEPENDENT KINASE 4/6 INHIBITORS IN HEALTHY VOLUNTEERS

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Background: Belzutifan is a novel treatment for several von Hippel-Lindau-associated cancers and is being evaluated in clinical trials for the treatment of renal cell carcinoma in combination with the cyclin-dependent kinase 4/6 inhibitors abemaciclib and palbociclib. Belzutifan is metabolized via UGT2B17 and CYP2C19 and is a weak inducer of CYP3A4, with greater induction predicted in dual UGT2B17/CYP2C19 poor metabolizers (PMs). Our objective was to assess the drug-drug-gene interaction potential of belzutifan when co-administered with the CYP3A4 substrates abemaciclib and palbociclib in individuals with varying UGT2B17/CYP2C19 phenotypes.

Methods: Physiologically based pharmacokinetic (PBPK) models for belzutifan, abemaciclib, and palbociclib were constructed in Simcyp V21 and confirmed using data from FDA reviews and prior publications. Virtual trials evaluating the $AUC_{0\text{-inf}}$ of single dose palbociclib (125 mg) or abemaciclib (200 mg) alone and following 7 days of belzutifan (120 mg once daily) were simulated in healthy volunteers with differing UGT2B17/CYP2C19 phenotypes.

Results: Compared to dual UGT2B17/CYP2C19 extensive metabolizers (EMs), belzutifan exposure increased approximately 4.4-fold in dual PMs. Belzutifan decreased the $AUC_{0\text{-inf}}$ of abemaciclib by 24.3% and 52.2% in dual EMs and dual PMs, respectively, with the relative potency-adjusted unbound AUC decreasing by 23.5% and 47.9%. Similarly, belzutifan decreased the $AUC_{0\text{-inf}}$ of palbociclib by 13.2% and 36.8% in dual EMs and dual PMs, respectively.

Conclusion: Belzutifan has the potential to decrease the exposure of combination therapies metabolized via CYP3A4, with the interaction dependent on UGT2B17/CYP2C19 phenotypes.

[Encore Presentation: ASCPT 2023 Annual Meeting]

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

POSTER #120

PROTEIN ARGININE METHYLTRANSFERASES 1 (PRMT1) AS A THERAPEUTIC POTENTIAL TARGET FOR TREATMENT OF MULTIPLE MYELOMA

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Arginine methylation catalyzed by protein arginine methyltransferases (PRMTs) is one of the most abundant post-translational modifications of protein that plays a crucial role in multiple cellular processes. PRMT1 is a Type I PRMT enzyme responsible for most of the asymmetric di-methylation in the cells. PRMT1 is an essential regulator of transcription, RNA splicing, signal transduction, metabolism, and protein homeostasis. Aberrant expression of PRMT1 is strongly correlated with poor survival of both solid cancers and hematological malignancies. However, how PRMT1 regulates multiple myeloma is virtually unknown.

Multiple myeloma (MM) is a plasma cell malignancy characterized by the extensive expansion of monoclonal aberrant plasma cells in the bone marrow. MM is a severe disease associated with typical symptoms: hypercalcemia, renal failure, anemia, and bone lesions. Combination of therapeutic drugs significantly improve patient survival, relapsed/refractory patients are incurable. Thus, there is an unmet clinical need of identifying novel targets for the treatment of MM.

Exploiting publicly available transcriptomic data, we found that, among PRMTs, PRMT1 is the highest expressed gene in both MM cell lines and primary cells from MM patients. The expression of PRMT1 in relapsed patients is higher than that of newly diagnosed ones. Intriguingly, patients strongly expressing PRMT1 are significantly associated with poor outcomes. Thus, we hypothesize that PRMT1 might play an indispensable role in MM. Indeed, we found that genetically deletion of PRMT1 in MM cell lines by the CRISPR/Cas9 system strikingly suppressed cell growth and induced cell death. Enzymatic suppression of PRMT1 by a specific type I PRMT inhibitor (MS023) inhibited MM cell growth, arrested cell cycles, and enhanced cell death. We also found that primary cells isolated from both newly diagnosed and relapsed patients are highly sensitive to the MS023 in a dose-dependent manner. Of note, using the established xenograft model, we demonstrated that suppression of PRMT1 in vivo by either genetic or pharmaceutical approaches robustly inhibits MM growth and mitigates the tumor burden. Collectively, our data convincedly showed that PRMT1 is indispensable to MM cells. Targeting PRMT1 potentially provides a novel therapeutic approach to treat both newly diagnosed and relapsed MM. We are currently investigating the underlying mechanisms of how PRMT1 regulates MM development and assessing the therapeutic efficacy of targeting PRMT1 alone or in combination with standard-of-care drugs for MM treatment.

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

POSTER #121

SINGLE- INSTITUTION RETROSPECTIVE STUDY IN THE PEDIATRIC POPULATION AT INDIANA UNIVERSITY: CLINICAL AND MOLECULAR CHARACTERIZATION OF PLEOMORPHIC XANTHOASTROCYTOMA WITH AND WITHOUT ANAPLASTIC FEATURES.

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Objective: Several limitations associated with the diagnosis and treatment for pleomorphic xanthoastrocytoma (PXA), and its anaplastic form have not been analyzed in detail. Thus, in this study the authors explore the clinical and molecular landscape of PXA and APXA to improve its management.

Methods: The authors retrospectively evaluated clinical and molecular characteristics in children and adolescents (0-21 years) who underwent primary tumor resection for PXA at Riley Children's Hospital, Indiana University from 1992-2021. Clinical, immunohistological, and molecular data were assembled based on historical records. Molecular data including tumor's DNA and RNA sequencing were retrieved through IU Precision Health Initiative (IU-PHI) and assessed with LifeOmic software (Regenstrief Institute) to identify genomic short variants, structural rearrangements, copy number variations and transcript expression. Transcriptomic data were further subjected to unsupervised hierarchical clustering, principal component, and pathway analysis.

Results: Out of the 29 PXA patients, 4 patients were initially diagnosed with anaplastic features, 1 patient progressed to WHO grade III and 2 patients developed WHO grade IV. Most of the tumors (n=29) were identified in the temporal (41%) and occipital lobes (21%). All tumor samples were positive for GFAP staining (n=29), and a portion of samples were positive for S100 (17.24%), reticulin (58.62%) and synaptophysin (44.83%). All patients with anaplastic features as well as those with progression were positive for reticulin (n=7). BRAF mutations were identified in 41% of patients (BRAFV600E= 11, CDC42SE2BRAF=1) and in all patients with anaplastic features (BRAFV600E=3, CDC42SE2BRAF=1) but none in the progression group. Copy number variation with homozygous 9p21.3 loss of CDKN*2A* was associated with poorer survival in the PXA with anaplastic feature or progression compared to those with complete copy of CDKN2A (median survival 2.3 years VS undefined). There were significantly higher tumor somatic mutations in the PXA tumors with anaplastic features and progression compared to PXA (median= 32.5 VS 3, $P<0.05$). Increased tumor mutational burden (TMB) was also observed during tumor progression in a patient with anaplastic features. Unsupervised hierarchical clustering and principal component analysis on tumor transcript expression correctly classified patient tumors (n=7) into PXA and its anaplastic counterpart based on available RNA-seq data. Further, in a patient with anaplastic PXA, tumor progression with leptomeningeal spread from occipital lobe to scalp was reflected in the differential transcriptomic landscape at each stage of the disease. Pathway analyses identified MAPK, RAS, neurodegeneration, and PI3KAKT/mTOR as major pathways associated with anaplastic progression. In patients with anaplastic features (n=4), 2 patients receiving early adjuvant combination therapy are still alive. The other 2 patients received monotherapy later in their disease progression and are deceased. Our results warrant further study to evaluate the timing and modality of adjuvant therapy during patient management.

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

POSTER #122

DISPARITIES IN PEDIATRIC BONE SARCOMA

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Osteosarcoma, the most common primary bone cancer, is the 3rd most common cause of cancer-related deaths in adolescents and young adults. Conventional treatment of preoperative chemotherapy followed by surgical resection and postoperative chemotherapy has led to a 5-year survival of approximately 70%. A poor histologic response to preoperative chemotherapy is associated with worse event-free survival and overall survival. We recently published our findings that a cutoff of 90% tumor necrosis provides the best prognostic value for patients with osteosarcoma undergoing chemotherapy. Importantly, we found that sociodemographic factors were associated with histological response less than 90%. In this present study, we hypothesize that sociodemographic factors are associated with delays in care for patients with osteosarcoma.

A retrospective review of pediatric sarcoma patients diagnosed from 2010 to 2012 at a single institution identified 86 patients diagnosed with osteosarcoma. Patients without documentation of initial presentation and biopsy, incomplete chemotherapy, or those lost to follow up during initial treatment were excluded. 62 patients with high-grade osteosarcoma were included. The duration of symptoms prior to lesion discovery on imaging, time to biopsy, treatment initiation, and treatment duration were collected from the medical record. Patient demographic data of age, sex, patient reported race/ethnicity, insurance status, and home address were collected. A socioeconomic score was determined using the patient's domicile address and the Center for Disease Control's Social Vulnerability Index (SVI) Socioeconomic Status subtheme. Univariate analysis was performed with Mann-Whitney tests and Spearman's correlation for continuous variables.

Patient's socioeconomic status as determined by the SVI was significantly correlated with duration of symptoms prior to evaluation by specialist ($r = 0.27$, $n = 61$, $p < .036$), the total duration of chemotherapy ($r = 0.46$, $n = 38$, $p < .004$), and patient BMI percentile at presentation ($r = 0.28$, $n = 54$, $p < .043$). The histological response to chemotherapy was significantly lower in patients with abnormal BMI compared to normal BMI (median 75%, IQR: 45 to 93 vs. median of 94%, IQR: 76 to 99; $p<0.004$). There was no difference in histological response, duration of symptoms prior to evaluation by a specialist, or length of treatment between insurance types ($p > 0.05$).

We found that residence in communities with high social vulnerability is associated with increased time to diagnosis of pediatric osteosarcoma and increased treatment duration. Disruptions to planned treatment schedules may account for the increased duration of chemotherapy. Further study is needed to determine whether medical or social causes lead to disruptions in care. Finally, we also demonstrate the novel finding that abnormal BMI at presentation is associated with worse response to chemotherapy and may contribute to worse response to chemotherapy seen in patients with low socioeconomic status.

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

POSTER #123

UQCR11 GENOMIC LOSS INDUCES SYNTHETIC LETHALITY, A PROMISING THERAPEUTIC TARGET IN TRIPLE-NEGATIVE BREAST CANCER

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Although recurrent loss-of-function deletions are common in tumors, they can sometimes lead to conditional therapeutic vulnerabilities. Our prior investigation used the TCGA-HGSOC dataset to determine that the 19p13.3 locus was the most frequent deletion in high-grade serous ovarian carcinoma (HGSOC). Using a machine learning integrated-precision oncology platform, we found that the loss of UQCR11, a gene located within the 19p13.3 locus, produced a therapeutic vulnerability that could be exploited through targeting the MTHFD2 gene. The machine learning integrated-precision oncology platform subsequently identified a therapeutic collateral target, the MTHFD2 gene, that resulted from the loss of UQCR11 located within the 19p13.3 locus. In our in vivo mouse model, complete tumor remission was achieved in UQCR11-deleted cells through pharmacological inhibition of MTHFD2 (Nature metabolism, 2022). Building on this promising result, we expanded our investigation to other tumor types and analyzed TGCA datasets, revealing UQCR11 deletion to be a prevalent genomic alteration in the most common solid tumors, particularly in pan breast and lung cancer. Within the pan breast cancer dataset, we found that over 50% of patients with triple-negative breast cancer (TNBC), the most difficult-to-treat breast cancer subtype in women, displayed either heterozygous or homozygous deletions in the UQCR11 gene. Moreover, the TGCA-TNBC dataset analysis revealed that the genomic copy loss of UQCR11 positively correlates with the deceased mRNA expression of the gene, bringing our interest to validate the collateral lethal targets in this breast cancer subtype. We first analyzed The Cancer Cell Line Encyclopedia (CCLE) database and further explored the correlation between the copy number alteration and mRNA expression of UQCR11 in several breast cancer cell lines. Then, we selected seven human TNBC cells with copy number neutral or deletion UQCR11 and validated the copy-number and gene expression of UQCR11 and MTHFD2 using qPCR and western blot. We next treated these cells with a selective MTHFD2 inhibitor, DS18561882, and found that UQCR11-deleted cells showed a more favorable response than the UQCR11-intact cells. Finally, we generated isogenic mouse breast cancer cell lines using shRNA-mediated UQCR11 knockdown to test the therapeutic potential of targeting MTHFD2-null tumors in vivo. Our findings showed that MTHFD2 expression was upregulated upon UQCR11 knockdown, indicating a compensatory function in UQCR11-deleted cells. Additionally, the UQCR11 knocked-down cells responded better to MTHFD2 inhibitor treatment than the parental control cells. Therefore, our study provides evidence that targeting MTHFD2 holds strong therapeutic potential in more than 50% of TNBC patients with UQCR11 deletion, highlighting the broad efficacy of targeting MTHFD2 for personalized therapeutic strategies in different tumor types co-occurring with UQCR11 deletion.

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

POSTER #124

A PHASE 2 BASKET TRIAL OF AN ERK1/2 INHIBITOR (LY3214996) IN COMBINATION WITH ABEMACICLIB FOR PATIENTS WHOSE TUMORS HARBOR PATHOGENIC ALTERATIONS IN BRAF, RAF1, MAP2K1/2 ERK1/2, AND NF1.

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Background:LY3214996 (L) is a potent inhibitor of ERK1/2 kinases that can potentially obviate resistance mechanisms in cancers that harbor *BRAF*, *RAS*, *NF1*, *MAP2K1/2*, and other MAPK alterations. Abemaciclib (A) is a CDK 4/6 inhibitor. Studies have shown that concomitant inhibition of the MAPK pathway exemplified by ERK inhibitors may augment the antitumor activity of CDK4/6 inhibitors. In this biomarker-informed phase 2 study, we assess the clinical efficacy of L in combination with A.

Methods: The study enrolled patients with advanced unresectable or metastatic cancer with no other standard of care options available. The malignancy must harbor pathogenic alterations in *BRAF*, *RAF1*, *MAP2K1/2*, *ERK1/2,* or *NF1,* in whom standard treatments failed to provide clinical benefit. L was administered at a dose of 200 mg orally QD in combination with A at 150 mg BID hours on Days 1 through 28 of a 28-day cycle until progression, toxicity, or withdrawal. The primary endpoint is the objective response rate. Secondary outcomes include evaluation of safety and tolerability, progression-free survival (PFS), and duration of the overall response. Additionally, tumor samples were collected for organoid development.

Results: Between September 1, 2020, and February 1, 2023, 12 patients were enrolled. Primary malignancies included colorectal (6), lung (1), breast (2), pancreas (1), uterus (1), and thyroid (1). The pathogenic alterations included *NF1* (4), *BRAF* (6), and *MAP2K1/2* (2). There were no CR or PR observed. Stable disease was achieved in 4 patients (33%) with a median disease control rate of 6.5 months. Pathogenic alterations in patients with stable disease included *BRAF*, *MAP2K1/2*, and *NF1.* Most common treatment-related adverse events of any grade included fatigue (41%), rash (41%), nausea (33%), vomiting (33%), anemia (25%), anorexia (25%), and increased creatinine (25%). There were no grade 4 or 5 adverse events.

Conclusions: The combination of L+A is safe and promising in patients whose tumors harbor pathogenic alterations in *BRAF*, *MAP2K1/2*, and *NF1*. Enrollment is ongoing, and efficacy and toxicity outcomes continue to be assessed. (NCT04534283).

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

POSTER #125

MIRROR-IMAGE THREE-WAY-JUNCTION NANOPARTICLE FOR TRIPLE-NEGATIVE BREAST CANCER TREATMENT

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RNA molecules hold immense promise in treating diverse malignancies and viral infections. However, their clinical utility is frequently impeded by their inherent instability and cytotoxicity, casting a shadow over their therapeutic potential. Here we unveil that by transforming the chirality of the sugar backbone of oligonucleotides to L-ribose, the L-RNA molecules present striking stability in physiological conditions and reduced immunogenicity. Meanwhile, their inherent capability to form versatile three-dimensional geometries is retained. Therefore, we harness the functions of L-RNA molecules to engineer an advanced mirror-image RNA three-way-junction (3WJ) nanoparticle. With the aid of this remarkable L-3WJ, we load diverse therapies to combat the aggressive triple-negative breast cancer (TNBC). The conjugated RNA aptamer targets the TNBC stem cell biomarker CD133 receptor, enabling precision drug delivery that infiltrates TNBC cells through aptamer-mediated endocytosis. The co-encapsulated chemotherapy, Doxorubicin, can effectively target TNBC cells with optimal accuracy and get released inside cells, ultimately culminating in apoptosis. As a result, the unintended side effects on healthy cells are avoided, allowing for a more targeted and efficient treatment strategy. Our study revealed that the synthetic L-RNA 3WJ nanoparticle has great potential to administer cancer therapies with remarkable stability and precision, as well as minimal toxicity and immunogenicity.

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

POSTER #126

ONCOLOGIC ANTHROPOLOGY: IMPACT OF DUFFY ADAPTIVE TRAIT ON BREAST EPITHELIAL AND TUMOR CELL BIOLOGY

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Adaptation to diverse environments during human evolution including those conditions that favor certain infectious diseases to become endemic in a region has resulted in natural selection of genetic variants. These variants remain fixed in current day populations despite changes in the environment. At least 20 such variants, also called adaptive traits, have been described to date and Duffy phenotype is one among them. Individuals in sub-Saharan Africa carry an inactivating mutation in a gene called atypical cytokine receptor 1 (ACKR1 or DARC) and this phenotype is called Duffy-phenotype. ACKR1 is a decoy receptor for CCL2 and IL-8 (CXCL8) and limits the activity of these cytokines. The Duffy-null phenotype confers protection against malarial infection. However, recent studies suggest that breast cancers in Duffy-null women display a distinct immune profile and have worse outcomes, possibly due to altered signaling by cytokines/chemokines. Using our institutional resource of breast tissues from >5000 healthy women, we have initiated studies to examine the impact of adaptive traits on normal breast and breast cancer biology. Towards this goal, we have created breast epithelial cell lines from Duffy wild type and Duffy-null/heterozygous carriers, all of African ancestry. ACKR1 is expressed in breast epithelial cells of Duffy-wild type but not in Duffy-null carriers. Breast epithelial cell lines as well as their transformed variants from Duffy-null/heterozygous carriers contained elevated levels of phosphorylated cMET(Y1349) and pERK(T202/Y204) compared to cell lines from Duffy-wild type carriers. Transformed Duffy-wild type and Duffy-heterozygous cells generated adenocarcinomas in NSG mice, which indicates that our model system closely recapitulates breast cancers in human. Cell lines created from Duffy-heterozygous tumors compared to Duffy-wild tumors displayed elevated pERK levels. Duffy phenotype also affects parainflammatory signals from transformed cells as tumor-derived cells from Duffy-null/heterozygous carriers expressed higher levels of IGFBP2 compared to tumor-derived cells from non-carriers. Additional studies are underway to determine whether Duffy phenotype alters sensitivity to chemo and targeted therapies. Collectively, these results suggest that adaptive traits alter epithelial cell intrinsic signaling pathways, which may affect tumor growth patterns, tumor microenvironment, and potentially sensitivity to specific therapies.

Translational/Clinical Research ***Research Associate***

POSTER #127

OPTIMIZATION OF A HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED SENSORY NEURON MODEL FOR THE ASSESSMENT OF DOCETAXEL-INDUCED NEUROTOXICITY

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Human induced pluripotent stem cells (iPSCs) have the ability to differentiate into sensory neurons (iPSC-dSNs) and are a valuable resource for the evaluation of drug neurotoxicity. This *ex vivo* model has several advantages over often poorly translatable animal models and immortalized cell lines, as well as provides an alternative solution to the limited availability of viable human nerve tissue for study. However, iPSC-dSNs are known to be significantly variable and lack reproducibility, frequently due to a failure to optimize important experimental parameters in the model design. In this study, we identified and evaluated significant sources of variability in an iPSC-dSN model for the evaluation of neurotoxicity caused by docetaxel, a commonly used chemotherapeutic that can cause debilitating peripheral neuropathy.

Four iPSC lines were generated from the whole-blood samples of four patient donors and differentiated into iPSC-dSNs. During the fourth week of maturation, iPSC-dSNs were re-seeded into 96-well plates at 10,000-50,000 cells/well on three consecutive days. On the fourth day, cells were treated with docetaxel (0.01-1000 nM) or a matched vehicle (0.004%-1% DMSO). Three replicates were included for each condition. Cell viability was assessed after either 24 or 48 hours of treatment and relative viability was calculated based on the matched controls. Dose-response curves were generated and IC_{50} was calculated using a four-parameter logistic regression model. We then evaluated the effect of cell line, drug dose, treatment duration, cell seeding density, and seeding-treatment interval on cell viability using one-way ANOVA corrected for multiple comparisons.

In comparison to the 24-hour treatment group, the 48-hour group consistently demonstrated a standard sigmoidal dose-response and was markedly less variable across replicates. Calculated IC_{50} values were also substantially less variable between replicates in the 48-hour (4.66 ± 4.70) group compared to the 24-hour (49.8 ± 81.4 nM) group. Cell line and drug dose were significant sources of variation in both the 24- and 48-hour treatment groups. Cell seeding density only affected viability in the 48-hour group. In the 24-hour group, there were no significant decreases in cell viability as compared to vehicle control at any dose, supporting a lack of a dose-dependent response in this group. In the 48-hour group, significant cell death occurred at docetaxel concentrations ranging from 1 to 1000 nM, while no significant decrease in viability was detected at 0.01 to 0.1 nM.

These results identify important parameters that require optimization before aniPSC-dSN model can be utilized to reliably evaluate neurotoxicity *ex vivo* and also reflect its suitability for capturing and assessing cell line genetic differences. Future studies evaluating the effects of docetaxel on other iPSC-dSN phenotypes, such as neurite outgrowth, would allow for further optimization and reproducibility.

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

POSTER #128

INTEGRATIVE ANALYSIS OF MULTI-OMICS DATA ON PEDIATRIC AND AYA SOLID TUMOR PATIENT-DERIVED XENOGRAFTS UNVEILS BIOMARKERS OF THERAPEUTIC RESPONSE

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Solid tumors account for ~60% of pediatric, as well as adolescent, and young adult (AYA) cancers. Notably, prognosis remains quite poor for those patients with progressive disease, highlighting the need for developing safe and efficacious novel therapeutic approaches. Precision genomics has emerged as a valuable tool for revealing potential disease mechanisms and treatment options. A multi-OMICs approach that identifies and cross-validates oncogenic signaling networks in aggressive cancers is an attractive method to help prioritize therapeutic vulnerabilities that can be tested in preclinical models and ultimately in clinical trials.

Establishment of patient-derived xenograft (PDX) models provides an opportunity to expand tumor specimens from patients so that genomically-guided therapies can be tested. An integrated multi-OMICspipeline was developed to identify therapeutic targets and understand the stability or evolution of molecular signatures in a panel of PDXs following serial passaging in immunodeficient mice.

PDX models derived from naïve and pre-treated sarcomas [osteosarcoma (OS) and rhabdomyosarcoma (RMS)] or Wilms tumor patients, which differ in genomic complexity, were established via direct implantation of tumor specimens into NOD/SCID/γnull mice. The original tumor and corresponding PDX passages were evaluated by a multi-OMICs approach including whole genome sequencing, RNA-seq, reverse phase protein array, and pathway enrichment analyses. Major tumorigenic gene and pathway alterations were validated, and potential therapeutic targets identified by integrative pathway analysis. Overall, cancer-associated genes and oncogenic pathways were retained in the majority of the PDXs analyzed following serial passaging.

CDK4/6 pathway hyperactivation was prioritized as a high-risk signature for validation in an OS PDX derived from a patient with metastatic disease. CDK4/6 inhibitor (CDK4/6i) Palbociclib decreased OS tumor growth, providing validation of a targeted anti-tumor response but also highlighted the need for combination therapy.

Similarly, activated CDK4/6 pathway has also been a molecular signature of interest in the highly aggressive embryonal RMS (ERMS) models we have developed. In ERMS PDX HT174, we employed a small molecule inhibitor screen based on actionable components of the molecular signature (RNA-seq and protein validation) which included inhibition of CDK4/6 pathway. While the tumor was sensitive to a CDK4/6i, the highest sensitivity was to a bromodomain and extra-terminal domain proteins inhibitor (BETi)/AZD5153. Notably, BETi resulted in a profound decrease in tumor growth during the initial phase of dosing, but the tumors eventually developed AZD5153 resistance and grew, again highlighting the need for combination therapies. The mechanism-of-resistance is under investigation, and data obtained will provide new avenues to maximize the benefit of BETi therapy in ERMS.

As such, through multi-OMICsanalyses, we were able to use PDXs that retained the original oncogenic molecular signatures as a way to identify actionable pathways and help prioritize targets for development of efficacious and safe therapies that can ultimately be translated to the clinic.

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

POSTER #129

AQUEOUS VEGF-A AS A BIOMARKER TO TRACK THE TREATMENT RESPONSE OF RETINOBLASTOMA VITREOUS SEEDS

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Purpose: In the last decade, intravitreal chemotherapy enabled safe and effective treatment of retinoblastoma vitreous seeds. Treatment decisions are based on patient clinical evaluations, but even the best examinations may miss seed relapse leading to delays in vision and life saving treatment. Conversely, if seed regression is not yet clinically evident, a patient may be subjected to unnecessary chemotherapy. We previously identified VEGF-A as a biomarker that tracks with vitreous seed growth and response to treatment in the aqueous humor of rabbit eyes with human retinoblastoma vitreous seed xenografts. Here, we investigated how aqueous VEGF-A levels correlate with clinical observations during the treatment of vitreous seeds in human patient samples.

Methods: *VEGFA* mRNA expression in vitreous seeds was verified by RNAscope in sections from enucleated retinoblastoma patient eyes. Patient aqueous humor was collected at CHLA pro forma prior to each intravitreal melphalan treatment of retinoblastoma with vitreous seeding. VEGF-A levels were measured in 5 µL aqueous with an ELISA for human VEGF-A and compared to levels in aqueous humor from patients with pediatric cataracts (low) or neovascular glaucoma (high). Patient charts were consulted for clinical annotation at each treatment visit to compare to VEGF-A data.

Results: *VEGFA* mRNA was robustly expressed in retinoblastoma vitreous seeds in sections of eyes from two patients. Retinoblastoma patients' aqueous VEGF-A protein levels were consistent with those in another known high-VEGF state (neovascular glaucoma). VEGF-A levels tracked with clinical annotation of disease burden at subsequent treatment visits in samples taken from five patients. Some showed aqueous VEGF-A decrease as disease burden decreased, while some showed VEGF-A increase when disease burden increased, and then resumed a downward trend as disease came under better clinical control with successive intravitreal melphalan injections.

Conclusions: VEGF-A is expressed by retinoblastoma vitreous seeds and can be reliably detected in small volumes of aqueous humor. VEGF-A levels in the aqueous tended to track clinical changes in disease statusand these biomarker levels sometimes preceded subsequent clinically observed changes in disease burden. Tracking VEGF-A levels can be another powerful tool to help determine treatment decisions for these young patients.

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

POSTER #130

DEVELOPMENT OF A SMART THERANOSTIC AGENT THAT ELICITS TUMOR-SELECTIVE DESTRUCTION OF RECALCITRANT CANCERS.

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Cancer remains the second leading cause of all deaths in the United States. Every hour, an estimated 5,200 new cases will be diagnosed and about 1,670 cancer patients will succumb to this disease this year. Pancreatic ductal adenocarcinoma (PDAC) and nonsmall cell lung cancer (NSCLC) represent some of the deadliest that are largely considered refractory to conventional chemotherapy that are often non-tumor selective causing debilitating side effects triggered by dose-limiting toxicities and acquired drug resistance. Indeed, there is an urgent need to develop an arsenal of tumor-selective anti-cancer agents and innovative treatment strategies with predictive biomarkers to selectively eradicate malignant tumors while sparing the normal tissues for precision medicine. One approach to reduce host toxicity is to develop new molecules that are nontoxic to normal cells but can be activated only under tumor-specific conditions, which we coined as SMART (Selective Method of Activation for Response in Tumors) therapeutic agents. Furthermore, development of a SMART anti-cancer agent with both therapeutic and diagnostic (theranostic) capabilities will have a major impact in precision medicine to monitor response and adapt subsequent treatment to maximize tumoricidal effect, enabling clinicians to personalize management of cancer by maximizing treatment efficacy for a specific cancer patient. Aberrantly high amount of reactive oxygen species (ROS), particularly stable hydrogen peroxide (H_2O_2), is an exclusive feature in recalcitrant cancers that can be a therapeutic advantage for tumor selectivity. Normal cells have inherently high expression of Catalase (CAT), which efficiently neutralizes (H_2O_2) to protect them from H_2O_2-induced DNA damage. Thus, we hypothesize that the higher level of ROS-H_2O_2 in tumors can be exploited for the development of SMART H_2O_2-activatable theranostic DNA cross-linking agent for tumor-selective “kiss of death”. Using medicinal chemistry and *in vitro* biochemical techniques, we developed a SMART DNA crosslinking agent, eRxLinker, that was activated by H_2O_2 and detected via “click” chemistry. Using immunohistochemistry, we noted the presence of higher ROS in PDAC/NSCLC compared to corresponding normal pancreas/lung from patients. Bioinformatics and Western blot showed significantly higher CAT expression in normal versus PDAC/NSCLC. Using 2D long-term DNA survival assays and 3D spheroid co-culture models, we found that eRxLinker selectively killed cancer cells and spared normal cells. Our animal studies have also demonstrated that our SMART agents are highly effective and less toxic compared to cisplatin, which is a non-tumor-selective DNA crosslinking agent commonly used as a standard of care for the treatment of certain NSCLC or PDAC. Overall, the results of pre-clinical studies with our SMART theranostic agent should lead to the development of innovative treatment strategies to improve the overall quality of life for patients with recalcitrant cancers in the clinic.

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

POSTER #131

SURVIVAL BENEFIT OF NEOADJUVANT CHEMOTHERAPY IN RESECTABLE PANCREATIC CANCER DEPENDENT ON ADJUVANT TREATMENT

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Introduction: The advantages of neoadjuvant chemotherapy (NAC) over upfront surgery in patients with resectable pancreatic adenocarcinoma (PDAC) remain under debate. In this study, we aim to evaluate the impact of NAC and its dependence on receiving adjuvant therapy on the oncologic and survival outcomes of patients with resectable PDAC.

Methods: All patients with resectable PDAC (defined by AHPBA/SSO/SSAT consensus guidelines) who underwent oncologic resection at a single, high-volume institution between Jan 2017 and Feb 2020 were retrospectively reviewed. Groups were compared using chi-squared or Mann-Whitney U-test. Kaplan-Meier and Cox proportional-hazards regression were used for survival analysis.

Results: Out of 228 patients with resectable PDAC, 93 (40.8%) had neoadjuvant chemotherapy and 135 (59.2%) underwent upfront surgery (US). Patients who received NAC were younger (NAC vs US, med[IQR]: 67.5[12.7] vs. 80.0[13.7] yrs). Groups were similar in comorbidities except for COPD (3.2% vs 10.4%, $p=0.04$). Patients with NAC had larger tumors at diagnosis (T2 disease: 58.7% vs 39.0%, T3 disease: 16.3% vs 11.4%, $p<0.01$), but similar clinical nodal staging ($p=0.89$). The med[IQR] duration of NAC was 2.30[0.96] mos. Surgery type, approach, duration, and EBL were similar between groups. NAC was associated with more node negative disease on pathology (56.7% vs 43.3%, $p<0.01$) and lower 30-d readmission rates (5.4% vs 13.3%, $p=0.05$). Groups were comparable in postoperative complications such as pancreatic fistula, DGE, and organ space infection, as well as 90-d mortality (4.3% vs 9.6%, $p=0.13$), and similar proportions went on to reach adjuvant chemotherapy (77.5% vs 75.0%, $p=0.70$). NAC was associated with higher rates of one-year survival (88.5% vs 58.3%, $p<0.01$) and better overall survival (med (95% CI): 31.7 (24.2 – 39.3) vs 15.3 (11.5 – 19.0) mos, $p<0.01$). When considering patients with adequate records on the course of systemic therapy ($n=174$), the survival benefit of NAC is lost in patients who do not receive adjuvant chemotherapy (Figure, $p<0.01$). This difference remained significant after adjusting for differences between groups, clinical stage, and other factors (NAC w/ vs w/o adjuvant, HR (95% CI): 0.36 (0.15 – 0.86), $p=0.02$).

Conclusion: In resectable PDAC, neoadjuvant chemotherapy was associated with improved overall survival and more node negative disease after surgery. However, the survival benefit is lost if patients do not receive adjuvant chemotherapy. This supports further investigating the potential role of total neoadjuvant chemotherapy in resectable pancreatic cancer.

Translational/Clinical Research ***Resident***

POSTER #132

A ROADMAP TO THE DEVELOPMENT OF THE OSTEOSARCOMA PATIENT DECISION AID FOR SURGERY IN THE LOWER LIMB: FROM INCEPTION TO IMPLEMENTATION

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Background: Osteosarcoma is a rare bone cancer with about 1,000 cases annually in the United States. Most tumors present in around the knee joint; surgical options include amputation, rotationplasty, and limb salvage surgery. Families and patients often struggle to find clear information to understand risk, benefits, and long-term outcomes of the options. The International Patient Decision Aid Standards guided patients, parents, and providers in the development of the first online decision aid for future families.

Methods: A roadmap of IRB-approved studies from 2019-2023 will be detailed, from inception to implementation. These engaged patients, families, and providers in needs assessments for a decision aid. Studies included a content analysis of Facebook posts to identify existing knowledge gaps and needs, and to inform interview guides for personal interviews with patients, survivors, and families (n=29), surveys with providers (n=29), alpha testing of the first decision aid with stakeholders (n=33), and pilot testing/implementation in 2023. The Osteosarcoma Decision Aid Medical Advisory Team (O-DAMAT) consisted of patient and professional stakeholders to advise the early framework for the decision aid.

Alpha Test Results: Participants (n=33) were survivors (15), parents (11), providers (5), and researchers (2). Content analysis was performed by all three coders who identified four focus areas in their comments: content, structure, visuals, and accessibility. Participants felt the content was clear, thorough, and relatable. They liked the testimonials, support links, and long-term outcomes, but disliked a noted bias: they felt it needed more evidence and citations. Visually they liked the images, PDFs, videos, and the layout of the site, but disliked the lack of diversity and age range of patients featured across the tool. Participants also wanted more images to break up the text-heavy page layout. They felt the structure was generally well organized and liked the bullet points, checklists, and color blocking, but felt it could be even more clearly laid out. For accessibility, it was well-written and easy to both read and understand, but some felt it could be overwhelming and was very text heavy with inconsistent use of text. Recommendations include more visuals, specific content additions, larger font, links to scientific studies, a glossary, increased accessibility (e.g., captions for videos), and a balance in the overall tone of the site between offering realistic expectations and hope.

Conclusions: Revisions to the final decision aid were made in consultation with healthcare providers. The final version will be freely available in April 2023 to surgeons and patients, with distribution in patient support groups for this population. Continued testing plans include options for patients and families to participate in a usability study and a longitudinal study.

Translational/Clinical Research ***Staff***

POSTER #133

EVALUATION OF REPLICATION PROTEIN A INHIBITOR, NERX329 IN COMBINATION WITH EGFR MUTANT TARGETED THERAPY

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Lung cancer is the leading cause of cancer related deaths worldwide with the majority (~80%) of patients diagnosed with non-small lung cancer (NSCLC). Targeted therapies directed against tumor initiating growth factor receptors are effective treatment options for driver mutation positive NSCLC. Osimertinib is a third-generation EGFR-Tyrosine Kinase inhibitor (TKI) that has activity against EGFR exon 19, exon 21, and T790M mutations. Unfortunately, Osimertinib treated patients acquire resistance and median progression free survival is 18 months. Moreover, Osimertinib does not cure stage IV EGFR mutant NSCLC. The acquisition of secondary mutations leading to activation of bypass pathways, MET amplification, EMT, compensatory pathway activation and histological transformation to small cell phenotype are major causes of acquiring resistance to EGFR TKIs. Proliferating cancer cells regularly experience a low level of replication stress. Additionally, receptor tyrosine kinases such as EGFR are known to interact with DNA repair proteins and impact DNA damage repair following chemotherapy, radiation therapy, and EGFR TKI treatment. However, the role of DNA repair pathways in EGFR-TKI resistance is unknown and the interaction between EGFR pathway, DNA damage, and repair pathways has not been fully elucidated. At low-level replicative stress promotes genomic instability but at a high level through mitotic catastrophe it causes cell death. Replication protein A is a critical sensor of the DNA damage response detecting replication stress. The small molecule inhibitor of RPA, **NERx 329**, sequesters active RPA and induces replication catastrophe and cell death. Here we demonstrate that NERx329 in combination with Osimertinib enhanced Osimertinib mediated cell death. CCk-8 assays performed after 48h of **NERx329** and Osimertinib combination treatment result in a robust decrease in IC_{50} compared to single agent therapy. Dissection of signaling pathways leading to this death-promoting effect of **NERx329** showed a combination of bypass pathway marker (AXL) and compensatory pathway marker (STAT) inhibition in AXL-high expressing EGFR mutant lung cancer cells. Whereas inhibition of STAT pathway was responsible for the death-promoting effect of **NERx329** in AXL-low expressing EGFR mutant lung cancer cells. AXL and STAT inhibition are associated with induced DNA damage and impaired DNA repair. From these data, we infer that DNA damage repair pathways could be involved in TKI resistance and **NERx329** could be a promising drug candidate for combination targeted therapy.

Translational/Clinical Research ***Associate Scientist in Medicine***

POSTER #134

OSTEOSARCOMA PATIENT-DERIVED XENOGRAFTS DERIVED FROM NAÏVE AND PRETREATED METASTATIC PATIENTS WITH HIGH-RISK CDK4/6 HYPERACTIVATION SIGNATURES ARE SENSITIVE TO DUAL INHIBITION OF CDK4/6 and PI3K/MTOR

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Precision genomics studies have demonstrated hyperactivation of cyclin-dependent kinases 4 and 6 (CDK4/6) as a top actionable marker in children, as well as adolescents and young adults (AYA) with osteosarcoma (OS). CDK4/6 binds to cyclin D resulting in a complex that mediates RB phosphorylation leading to cell cycle progression. Preclinical modeling approaches are critical for identification of tumor adaptive responses to CDK4/6 inhibitors (CDK4/6i) as well as validation of alternative or combination therapies. Although CDK4/6i are clinically well-validated, cytostatic effects make combination treatments essential. Moreover, concomitant dysregulation of CDK4/6 and PI3K/mTOR pathways are observed in aggressive OS. Multiple positive feedback loops between these pathways exacerbates the hyperactivation of CDK4/6 and PI3K/mTOR signaling. Thus, we hypothesize that dual inhibition of CDK4/6 and PI3K/mTOR will be efficacious and safe in RB+ OS PDXs. In this study, OS PDX models (TT2-77 derived from pretreated patient) and HT96 (treatment-naïve patient) with molecular signatures indicative of therapeutic sensitivity to palbociclib (RB+, CDKN2A null, CCND3 amplified) were treated long-term with CDK4/6i (palbociclib) (50 mg/kg), PI3K/mTOR inhibitor (PI3K/mTORi; voxtalisib) (50 mg/kg) or combination palbociclib+voxtalisib. In both PDXs, growth was significantly reduced in single-agent and combination groups compared to vehicle (p<0.05, two-way ANOVA). Importantly, combination palbociclib + voxtalisib was more efficacious than single-agents following prolonged treatment and well tolerated based on histological analyses. Kinome profiling analysis of long-term treated HT96 PDX demonstrated that compared to single agents, dual inhibition of CDK4/6 & PI3K/mTOR significantly decreased PI3K pathway activity, including downregulation of Pik3ca , mTOR, and the G2 to M transition regulator CDK1 (-log10[p] ≥1.3). OS metastatic lesion 143B model indicated increased survival based on body scoring criteria in combo versus single agent. In RB+ OS cell lines and TT2-77 xenoline, palbociclib+voxtalisib caused additive-to-synergistic cell growth inhibition, G1 arrest, and minimal apoptosis at clinically relevant doses. Senescence biomarker beta-galactosidase indicated that dual inhibition of CDK4/6 and PI3K/mTOR induced senescence that was mediated by palbociclib. Mechanistic siRNA RB studies indicated CDK4/6i effect was partially dependent on RB status. These data provide evidence that combination palbociclib+voxtalisib therapy is safe, efficacious, and increases CDK4/6i efficiency in both pretreated and naive PDX models of OS. These studies provide rationale for earlier therapeutic intervention in pediatric and AYA OS patients with CDK4/6 hyperactivation signatures.

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

POSTER #135

B-Lapachone Treatment Promotes Tumor Associated Neutrophils (Tans) Polarized Towards Anti-Tumor (N1) Phenotype

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NAD(P)H:quinone oxidoreductase1 (NQO1) is a two-electron oxidoreductase overexpressed (>20-100 fold) in most solid cancers and has emerged as a promising target for tumor selective killing in recent past. β-lapachone (β-lap) is one of such prominent NQO1 bioactivatable drug, which has been shown significant antitumor effects on $NQO1^+$ human solid cancer cell lines. Also, this targeted prodrug reported to induce immunogenic cell death in tumor microenvironment (TME) to trigger innate sensing and synergizes with anti-PD-L1 immunotherapy to overcome adaptive resistance. However, it remains unclear whether β-lap-mediated anti-tumor immune response by primary mediators of innate sensing, neutrophils. In this present study, we found that β-lap treatment selectively killed $NQO1^+$ murine tumor cell lines [MC38 (colon), TC-1 (lung), 4T1 and E0771 (breast)], and NQO1 specific inhibitor, dicoumarol (DIC) spared the lethality. Exposure to sub-lethal (2 μM) and lethal (4 μM) doses of β-lap for 3h induced significant increase in intracellular ROS formation. Additionally, immunofluorescence analysis displayed increased number of □H2AX foci/nuclei following β-lap treatment, supporting ROS induced DNA damage in the form of DNA double strand breaks. Further, flow cytometric analysis of immune cells in TME of subcutaneous tumor models indicated that there is a significant infiltration of neutrophils ($CD11b^+$ $Gr1^{high}$ cells) or Tumor Associated Neutrophils (TANs) following β-lap treatment. Depletion of neutrophils by co-addition of anti-Ly6G antibody abolished β-lap-induced anti-tumor efficacy in immune competent mice. Neutrophil mediated tumor cell killing determined *ex vivo* using bone marrow derived neutrophils and it was observed that in the presence of β-lap induced antigen, neutrophils were killing murine tumor cells. Further, exposure of these neutrophils to β-lap induced antigen medium induce antitumor N1-like properties, include increase in certain surface markers such as CD95 (Fas) and CD54 (ICAM-1) as well as increase in intracellular cytokine IFN-β and decrease in TGF-β revealed by flow cytometric analysis. Corroborating to this, there is a significant increase of CD95 and IFN-β and a decrease in TGF-β observed with TANs infiltrated into β-lap treated TME compared to vehicle treated tumors. Furthermore,TLR4/MyD88 signaling deficiency or HMGB1 blockade significantly decreased the β-lap induced tumor infiltration of neutrophils. Blockade of HMGB1, depletion of neutrophils or TLR4/MyD88 deficiency also significantly decreases the antigen-specific T cell response. Altogether this present study clearly specifies that b-lap selectively kills $NQO1^+$ murine tumor cells through intensive ROS production and extensive DNA damage *in vitro* as well as neutrophils play an important role in β-lap mediated-antitumor effects *in vivo*.

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

POSTER #136

CLINICAL PHARMACOLOGY ANALYTICAL CORE

POSTER #137

IN VIVO THERAPEUTICS (IVT)

POSTER #138

BIOSTATISTICS & DATA MANAGEMENT CORE