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POSTER #1

EXPLORING THE IMPACT OF COMBINED INHIBITION OF STAT3 AND YAP1 ON THE GROWTH OF TRIPLE NEGATIVE BREAST CANCER CELLS

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Breast cancer is the most diagnosed cancer among women, resulting in over 40,000 deaths in the United States per year. Around 80 % of breast cancers are classified as invasive ductal carcinoma (IDC) and are further separated by immunohistochemical analysis into three molecular subtypes that have high clinical significance. Hormone receptor-positive (HR+) breast cancers show high levels of expression of the estrogen receptor (ER) and progesterone receptor (PR) but lack expression of the HER2 receptor. Triple-negative breast cancers (TNBC) do not express HER2, ER, or PR. No targeted therapies have been FDA-approved for TNBCs, and they have the poorest prognosis. A common driver of TNBC is circulating inflammatory cytokines such as interleukin-6 (IL6), which is expressed in over 80% of this subtype. IL6 initiates inflammatory-associated signaling upon binding to the glycoprotein 130 receptor complex on target cells. The IL6 bound receptors activate Janus kinases (JAKs) that, in turn, phosphorylate signal transducer and activator of transcription 3 (STAT3). Phosphorylated STAT3s often homodimerize and translocate into the nucleus to activate transcriptional programs that drive cell growth and invasion. Disruption of HIPPO signaling is also often observed in breast cancers where the transcriptional co-activator YAP1 may also promote pro-growth and invasion transcriptional programs. Data from our lab has recently shown that IL6/STAT3's effects on cell growth and proliferation require YAP1 activation. This has been shown to occur through IL6/STAT3 activating the transcription of the HIPPO pathway adaptor protein called Amot. Amot expression has been associated with tumor metastases and poor outcomes in breast cancer. Bioinformatics analyses by our group have further shown that Amot expression is strongly associated with the expression of IL-6 signaling. Amot expression levels may, therefore, be a biomarker of an IL6/STAT3/YAP1-dependent subtype of TNBC. My work will thus look at the effects of a combined treatment consisting of C188-9, a small molecule inhibitor of STAT3, and novel inhibitors of YAP1 developed by my laboratory may have synergistic effects on inhibiting the growth of the TNBC cell line MDA-MB-468. My lab has previously shown this cell line to be dependent on YAP1 and STAT3 for growth. Such work could potentially point to a clinical treatment for Amot high TNBCs.

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Anticancer drug target identification and/or validation ***Faculty***

POSTER #2

CMYC-MEDIATED METABOLIC REPROGRAMMING DRIVEN BY PIM2 INHIBITOR JP11646 PROMOTES CYTOTOXICITY IN TRIPLE-NEGATIVE BREAST CANCER

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cMYC-mediated Metabolic Reprogramming Driven by PIM2 inhibitor JP11646 Promotes Cytotoxicity in Triple-Negative Breast Cancer.

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Background: Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer with poor prognosis and lacks targeted therapies. Developing new targeted therapies for TNBC is critically important. Pim2, the pro-survival kinase, is overexpressed in breast cancer and other solid tumors compared to normal tissues. We have previously demonstrated that the PIM2 inhibitor, JP11646, targets the PIM2 protein, resulting in PIM2 degradation and showed enhanced cytotoxicity in TNBC cell lines and xenograft mouse models compared to the pan-PIM kinase inhibitor, AZD1208, supporting a kinase-independent function of JP11646.

Results: Our analysis of the breast cancer TCGA dataset showed that PIM2 is overexpressed in basal subtype as compared to other breast cancer subtypes suggesting that PIM2 may play an important role and could be a therapeutic target in that population. We demonstrated different effects on TNBC cell lines by pharmacological inhibition of the PIM2 kinase function using a pan-PIM kinase inhibitor AZD1208 and targeting the whole protein using JP11646. By overexpressing PIM2 wild-type (WT) and kinase-dead (KD) mutant proteins, we demonstrated that PIM2 has a pro-survival effect independent of its kinase activity. The KD mutant protein overexpressed cells were more resistant to JP11646 treatment to a similar degree as WT-protein overexpressed cells. JP11646 demonstrated a reduction in phosphorylations of known PIM2 substrates such as cMYC, 4EBP1, and BAD in similar fashion to AZD1208. Unexpectedly, JP11646 increased total cMYC protein level in a time-dependent manner but did not alter total 4EBP1 and BAD levels unlike AZD1208-treated controls. To explore if cMYC stability is influenced by JP11646, the cells were concurrently treated with JP11646 and cycloheximide. JP11646 reduced half-life of PIM2 but half-life of cMYC was unchanged. JP11646 treatment increased cMYC mRNA level suggesting cMYC increase is mediated through increase in transcription. There was no observed synergy between JP and cMYC inhibitor supporting that PIM2 and cMYC may work in the same pathway. Our global RNA seq of JP11646-and AZD1208-treated MDA-MB-231 cells, and analysis by KEGG pathway analysis, revealed that several cMYC regulated pathways were upregulated upon JP11646-treatment which were not altered in the AZD1208-treated cells. Among the top differences were oxidative phosphorylation (OXPHOS), ribosome function, cardiac muscle contraction, and downregulation of biosynthesis of nucleotide sugars, and aminoacyl tRNA.

Conclusion: JP11646 treatment results in upregulation of cMYC protein through increased mRNA in a time-dependent manner. We demonstrate that KD PIM2 decreases effectiveness of JP11646 treatment suggesting a kinase-independent function of PIM2. We hypothesize that JP11646 treatment reduces PIM2 levels resulting in cMYC pathway deregulation beyond previously described PIM2-mediated phosphorylation of cMYC. This results in the metabolic reprogramming of glycolytic cancer cells towards OXPHOS, reduces biosynthesis of nucleic acids, and aminoacyl t-RNA promoting cytotoxicity in TNBC cells.

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Anticancer drug target identification and/or validation ***Faculty***

POSTER #3

DIFFERENTIAL GENE EXPRESSION REGULATION BY THE RXR AGONIST V-125 IN MMTV-NEU MAMMARY TUMORS

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Retinoid X receptors (RXRs) are nuclear receptors that play a pivotal role in regulating gene expression by forming homodimer and heterodimers with other nuclear receptors. When activated, RXRs influence various cellular processes, including cell differentiation, proliferation, and apoptosis. This regulatory activity makes RXR agonists promising candidates in cancer research. However, the specific gene expression changes induced by RXR agonists can vary depending on the compound, reflecting their unique interactions within cellular environments and signaling networks. In this study, RNA sequencing was performed to compare the molecular pathways activated by the novel compound V-125 and FDA-approved drug bexarotene in mammary tumors of a mouse model of HER2+ breast cancer (MMTV-Neu mice). By analyzing the transcriptomic profiles, we identified that each treatment differentially regulated cancer-relevant gene categories, including focal adhesion, regulation of actin cytoskeleton, and extracellular matrix-receptor interaction. The most significantly altered genes by V-125, including Lrrc26, Mgarp, Aldh1a3, and Colca2 are positively correlated with improved survival in breast cancer patients. While V-125 and bexarotene act on many common pathways, they induce distinct gene expression profiles. V-125 primarily targets focal adhesion and extracellular matrix-receptor interaction pathways, whereas bexarotene affects regulation of actin cytoskeleton and NF-kappa B signaling pathways. We confirmed the significant differential expression ($p<0.05$) of key genes (Nrg1, Nfasc, Chi3l1, Slit2) at both the mRNA and protein levels using quantitative PCR (qPCR) and immunohistochemistry (IHC). Beyond cancer cells, RXR modulation influences the tumor microenvironment, including immune cells. Our transcriptomic analysis revealed that RXR agonist treatment affects pathways associated with macrophage activation and phagocytosis. This suggests the role of RXR in polarization of macrophage, an important immune population related to breast cancer. To investigate this further, we utilized bone marrow-derived macrophages (BMDMs) to evaluate the impact of V-125 and bexarotene treatment on macrophage polarization toward a tumor-suppressive phenotype. The polarization status of macrophages upon treatment was assessed through gene expression analysis by qPCR. We observed that BMDMs treated with 300nM of either RXR agonist significantly ($p <0.05$) increased mRNA expression of IRF7 and TLR9, associated with a pro-inflammatory, anti-tumor phenotype. This comprehensive approach allowed us to determine the relative effectiveness of V-125 and bexarotene in modulating gene expression and polarizing macrophages toward a tumor-suppressive phenotype, providing valuable insights into their therapeutic potential and the underlying molecular mechanisms.

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Anticancer drug target identification and/or validation ***Graduate Student***

POSTER #5

CROSSTALK BETWEEN ZNF217 TRANSCRIPTIONAL ACTIVITY AND IFN-Γ SIGNALING PATHWAY

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Interferon gamma (IFN-γ) has a profound impact on solid tumors by inhibiting tumor growth, development, and metastasis. Dysregulation of the interferon signaling pathway is linked to endocrine resistance and a pro-tumorigenic phenotype. ZNF217 is a transcription factor and oncogene that is predictive for resistance to breast cancer chemotherapy and endocrine therapies. Preliminary data from our lab shows that ZNF217 can modulate IFN-γ signaling in a NRG1-dependent manner and contributes to the regulation of cytokine secretion from ER+ breast cancer cells. Therefore, ZNF217 overexpression can induce a niche environment within the breast cancer epithelial cells to become nonresponsive to IFN-γ secreted from the cells in the tumor microenvironment. IFN-γ has both pro- and anti-tumorigenic roles, both increasing the expression of PD-L1 to prevent T cells from killing PD-L1 expressing cells and acting as a cytotoxic cytokine to induce apoptosis of cancer cells. Therefore, ZNF217 may contribute to induction of IFN-γ tumorigenic activity by modulating its signaling pathway.

To investigate whether the oncogene ZNF217 is responsive or nonresponsive to IFN-γ, we generated MDA-MB-231 cells overexpressing ZNF217 (NFL) or empty vector (NEV). We treated these cells with different concentrations of human recombinant IFN-γ for 6 hours and quantified gene expression of IFN-γ-regulated genes, including IRF1, PD-L1, and STAT1. Although gene expression of IRF1 was significantly increased after cell treatment with IFN-γ, ZNF217 overexpressing cells showed reduced IRF1 gene expression after treatment with IFN-γ. PD-L1 and STAT1 gene expression were significantly increased in NFL cells treated with IFN-γ as compared to IFN-γ-treated NEV cells, indicating that ZNF217 overexpressing cells respond to IFN-γ through upregulation of PD-L1 and STAT1 gene expression. These data suggest either crosstalk with stromal immune cells in vivo or alternative resistance mechanisms to overcome IFN-γ anti-tumorigenic roles. In addition, using gene expression data from ER+ breast tumors, we found that ZNF217's transcriptional target ERBB3 inversely correlates with expression of PD-1, PD-L1, and STAT1. ZNF217 suppresses IFN-γ signaling. Our data suggest that ZNF217 responds to IFN-γ by regulating its downstream gene targets such as IRF1, PD-L1, and STAT1. From our data, we can conclude that ZNF217 counteracts IFN-γ anti-tumorigenic effects by further upregulating PD-L1 and STAT1 gene expression and downregulating IRF1.

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Cell and molecular biology of breast cancer ***Graduate Student***

POSTER #6

MECHANISTIC INSIGHTS AND THERAPEUTIC TARGETS FOR TONSL-AMPLIFIED BREAST CANCERS WITH HOMOLOGOUS RECOMBINATION DEFICIENCY

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Breast cancer is a genetically heterogeneous disease in which genomic alterations can drive tumor progression and influence therapeutic outcomes. One critical factor that influences tumor progression and therapy outcome is homologous recombination deficiency (HRD). HRD is a clinically relevant biomarker associated with impaired DNA Repair. It is commonly used to identify tumors that may be responsive to HRD-targeted therapies, such as PARP inhibitors. However, not all tumors with HRD respond to PARP inhibitors suggesting control of HRD by additional unrecognized regulators.

TONSL (Tonsoku-like, DNA Repair Protein), located on chromosome 8q24.3, plays a key role in DNA repair by stabilizing replication forks and promoting double-strand break repair via Homologous Recombination (HR). TONSL is amplified in approximately 20% of breast cancers and is an identified oncogene. Despite this amplification, analysis of METABRIC datasets revealed that even in tumors with high TONSL expression, there remains significant HRD in certain breast cancer subtypes, particularly basal-like and luminal B breast cancer. $TONSL^{High}/HRD^{high}$ tumor characteristics are associated with poor outcome compared to $TONSL^{low}/HRD^{low}$, which is largely driven by HRD. This paradox suggests the presence of additional factors that modulate TONSL's role in HR. To delineate this complexity, we mapped the TONSL interactome using immunoprecipitation-mass spectrometry and identified ETV6, a transcriptional regulator, as a TONSL-binding partner.

In breast cancer cells with high TONSL expression but modified to express lower levels of ETV6, we observed increased DNA damage and reduced efficiency of homologous recombination repair. These findings suggest that TONSL's HR function is dependent on ETV6, and that loss of ETV6 disrupts TONSL-mediated repair processes. This relationship highlights an unrecognized vulnerability in TONSL-amplified tumors that may be exploited for therapeutic intervention.

Towards this goal, we performed an initial screen of 1500 compounds from NCI Diversity IV library and identified a potential hit compound that kills TONSL amplified cells. Computational modeling suggests that the drug binds and potentially disrupts TONSL:Histone H4:Histone H3.3:MCM2 complex, which is essential for HR. Our future studies will focus on further elucidation of therapeutic vulnerability of TONSL amplified breast cancers using the newly identified drug and other drugs with similar potential. Our future studies will focus on further elucidation of therapeutic vulnerability of TONSL amplified breast cancers using the newly identified drug and other drugs with similar potential. This study has the potential to redefine our understanding of HR regulation in breast cancer and contribute to the development of more effective targeted therapies for patients with HR-deficient and/or TONSL-amplified breast cancer.

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Cell and molecular biology of breast cancer ***Graduate Student***

POSTER #7

UNRAVELING THE MECHANISM OF AQP7 STRUCTURE WITHIN BREAST CANCER PROGRESSION

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Unraveling how the structure of Aquaproin-7 regulates its function in promoting breast cancer

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We discovered the water and glycerol channel protein Aquaporin-7 (AQP7/Aqp7) to be a marker of survival and metastasis in breast cancer patients. We found that AQP7 deficiency decreases proliferation and significantly decreases primary and lung metastasis tumor burden in vivo. In addition, Aqp7 deficiency resulted in highly dysregulated lipid metabolism. We currently are investigating AQP7 structure n by structure-function analysis to clarify the molecular mechanism by which AQP7 contributes to breast cancer. The NPA motifs of AQP7 channels are highly conserved. Mutation in NPA motifs decreases the water and glycerol permeability. In addition, we have generated additional point mutations that alter glycerol transport and oligomer formation. These mutants are being used to determine the mechanisms by which AQP7 contributes to breast cancer and both cellular and protein properties of AQP7 function, including protein folding properties, oligomer formation, and breast cancer cell proliferation. This research will examine how AQP7 structural perturbations affect AQP7 function, including to promote proliferation of breast cancer cells and breast cancer lipid metabolism. These experiments will determine how changes to AQP7 structure and expression contribute to breast cancer.

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Cell and molecular biology of breast cancer ***Graduate Student***

POSTER #8

ZNF217 PROMOTES BREAST TUMOR PROGRESSION BY FACILITATING ANGIOGENESIS

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The transcription factor ZNF217 is amplified in 20-30% of breast cancers and is identified as a putative oncogene. Its overexpression accelerates tumor progression, metastasis, and chemoresistance *in vivo* and correlates strongly with poor prognosis in patients. To identify growth factors that are released from mammary epithelial cells that overexpress ZNF217, we analyzed the conditioned media from MCF7 cells with or without knockdown of ZNF217 using a 71-Plex Discovery assay. Conditioned media from MCF7 cells deficient for ZNF217 showed a significant downregulation in the secretion of VEGF-A, PDGF-AA, and PDGF-AB/BB and was validated by ELISA in conditioned media from both MCF7 cells and MDA-MB-231 cells that overexpress ZNF217. VEGF-A, PDGF-AA, and PDGF-AB/BB are key components in promoting angiogenesis, a key hallmark of cancer. The transcriptional expression of VEGF-A and PDGF-AA/AB/BB after ZNF217 knockdown were not different by RNA sequencing data and qRT-PCR. To evaluate if the secretion of the identified angiogenic factors promotes endothelial cell sprouting, we co-cultured endothelial progenitor cells with the conditioned media from MCF7 cells ± siZNF217. Endothelial cells induced a sprouting phenotype when grown in conditioned media from MCF7 ZNF217 expressing cells compared to ZNF217 knockdown cells. Together, these data suggest that ZNF217 is a promotor of VEGF-A secretion and promotion of angiogenic sprouting in endothelial cells. We also evaluated the role of ZNF217 in VEGFR-2 signalling. Knockdown of ZNF217 decreased the expression of KDR (VEGFR-2 receptor for VEGF-A) in MCF7 cells in a NGR1-dependent manner. Knocking down KDR in MCF7 cells also decreased the live cell count. These data suggest a potential role for VEGF-A in autocrine signalling in the cancer cells. Single cell RNA sequencing data of mammary tumors derived from orthotopic injection of PyMT±Zfp217 revealed an increased population of endothelial cells in FVB mice injected with Zfp217 overexpressing cells compared to vector expressing control cells. These findings highlight a novel role for ZNF217 as a promoter of angiogenesis during breast cancer progression. Thus, unravelling the mechanisms of ZNF217-mediated angiogenesis will be an instrumental step in determining if anti-angiogenic therapies are an effective and novel strategy that can be used to overcome metastatic growth in breast cancer patients with tumors that overexpress ZNF217.

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Cell and molecular biology of breast cancer ***Graduate Student***

POSTER #9

THE ROLE OF ZEB1 IN LINEAGE SPECIFICITY AND CELL FATE CONVERSION OF AFRICAN-ANCESTRY ENRICHED BREAST MULTIPOTENT STROMAL CELLS

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Disparity in breast cancer outcomes in women of African Ancestry (AA) as compared to European Ancestry (EA) has been thought to derive from socioeconomic factors. However, emerging evidence demonstrating genetic ancestry-dependent differences in normal and cancer genome, cancer-associated mutation patterns and immunosuppressed tumor microenvironment suggests the involvement of biological factors. To investigate breast cancer outcome-associated biologic factors, we previously analyzed breast tissues of healthy donors of distinct genetic ancestry. We found that $PROCR^+$/ $ZEB1^+$/ $PDGFR\alpha^+$ stromal cells (hence, called PZP) are more abundant in normal breast tissues of women of AA as compared to EA.PZP cells display the properties of multi-lineage fibroadipogenic mesenchymal stromal cells that can differentiate into adipogenic and osteogenic lineages. Mechanistic studies of PZP cells in the context of breast cancer initiation and progression are yet to be reported. This study is focused on the PZP cell enriched Zinc finger E-box-binding homeobox 1 (ZEB1), which is a master transcription regulator of stemness and epithelial-to-mesenchymal transition (EMT). We generated ZEB1 gene knockout clones of PZP cells using CRISPR-Cas9 and performed RNA-sequencing of ZEB1 knockout clones and parental cells. The expression of epithelial markers KRT 7, 8, 18 and adipogenic markers PPARG and PPARGC1B were upregulated upon ZEB1 knockout. Additionally, ZEB1-deficiency increased the levels of inflammatory fibroblast markers (iCAFs), while downregulating the expression of myofibroblast marker genes encoding extracellular matrix proteins Tenascin-C (TNC), matrix metallopeptidase 1 (MMP1) and a surface protein,Leucine-rich repeat-containing protein 15 (LRRC15). These results suggest the role of ZEB1 in lineage specificity and cell fate conversion of PZP cells and shape the normal and breast tumor microenvironment. Consistent with this possibility, ZEB1 controlled the expression of prostaglandin-endoperoxide synthase 1 (PTGS1), which synthesizes immunosuppressive prostaglandin E2 (PGE2), in PZP cells. In support of the immunosuppressive role of PZP cells, PZP cell secreted factors reduced the fraction of Interferon γ and Granzyme B expressing CD8+ T cells in an *in vitro* T cell activation assay. Ongoing studies are investigating the potential contributions of ZEB1, PGE2, and TNC in the PZP cell-mediated decrease of CD8+ T cell effector functions. Thus, ZEB1 is a multifunctional transcription factor that controls various biochemical networks in the breast tumor microenvironment through PZP cell-intrinsic and extrinsic activities and is a candidate for therapeutic intervention.

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Cell and molecular biology of breast cancer ***Graduate Student***

POSTER #10

UNRAVELING MIRNA-PHOSPHOPROTEIN CROSSTALK IN BREAST CANCER EVS FOR INSIGHTS INTO METASTASIS

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Introduction

Breast cancer is a heterogeneous disease with distinct subtypes that require personalized treatments. These subtypes are driven by molecular alterations, including changes in gene expression and signaling pathways. MicroRNAs (miRNAs), small non-coding RNAs, play a key role in regulating these pathways by controlling gene expression at the post-transcriptional level. Phosphorylation, a common post-translational modification, disrupts normal signaling pathways in breast cancer, affecting cell growth, survival, and migration. While miRNAs in extracellular vesicles (EVs) are well-known for their role in cancer progression, their interaction with phosphorylated proteins in EVs is still being explored. Combining mass spectrometry-based proteomics with next-generation RNA sequencing of EVs can provide a detailed molecular profile of breast cancer subtypes, improving diagnosis and treatment strategies. Although EV miRNAs are crucial in cancer progression and metastasis, the exact mechanisms controlling their packaging into EVs remain unclear. Recent studies suggest that miRNAs may influence their sorting by regulating phosphorylation pathways, which affect RNA-binding proteins (RBPs) and miRNA processing machinery. This study aims to investigate how miRNAs interact with kinases and phosphatases to control their own EV packaging, shedding light on new regulatory mechanisms in breast cancer.

Methods

EVs were isolated from MCF-7, MDA-MB-231 breast cancer cells, MCF-10A mammary epithelial cells, and patient blood plasma samples using the EVtrap method. After EV lysis, proteins were extracted and peptides were digested with trypsin, using phase-transfer surfactants to improve digestion efficiency. Phosphopeptides in the EV samples were enriched using PolyMAC beads. Samples were analyzed using high-resolution liquid chromatography-tandem mass spectrometry with label-free quantification to identify differential phosphorylation patterns across breast cancer subtypes. Additionally, next-generation sequencing (NGS) was performed on EV-associated RNA from patient plasma samples to profile miRNA populations. Functional and pathway enrichment analyses were performed to identify key regulatory pathways involving EV miRNAs.

Preliminary Data

EVs were characterized and quantified using Western blotting and NTA. The release of EVs was higher in triple-negative breast cancer patients compared to other subtypes. NGS of EV miRNAs from blood plasma showed alterations in several intracellular pathways responsible for cellular processes. Phosphoproteomic analysis of EVs from MCF-10A, MCF-7, and MDA-MB-231 cells identified 11,576 unique phosphopeptides, with ~500 cancer-associated phosphoproteins differentially expressed in MCF-7 and MDA-MB-231 EVs compared to MCF-10A. Bioinformatics analysis revealed that specific miRNAs regulate their sorting into EVs by modulating phosphorylation pathways. Notably, miR-200 family members target AKT kinase, altering the phosphorylation of the RNA-binding protein hnRNPA2B1. Additionally, miRNA-mediated regulation of PTEN affects the phosphorylation of miRNA processing enzymes DROSHA and DICER, further influencing EV sorting.

Conclusions

Our findings reveal a novel mechanism where miRNAs regulate their sorting into EVs through phosphorylation-dependent pathways in breast cancer. This regulatory circuit involves kinases, phosphatases, and changes in RBPs and miRNA processing enzymes. Targeting this network could offer new therapeutic strategies to modify EV miRNA content, potentially inhibiting breast cancer progression and metastasis.

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Cell and molecular biology of breast cancer ***Graduate Student***

POSTER #11

LOSS OF DSRNA SENSOR PACT DRIVES PKR DEPENDENT GROWTH-INHIBITION IN TNBC.

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Triple Negative Breast Cancer (TNBC) has worse outcomes on average when compared to Estrogen/Progesterone Receptor Positive (ER/PR+) or Epidermal Growth Factor 2 Positive (HER2+) breast cancers. This discrepancy is partially due to TNBC lacking these receptors, which are the target of many cancer therapeutics. This leaves patients dependent on standard-of-care chemotherapy to treat TNBC.

A potential target for the treatment of TNBC is Adenosine Deaminase Acting on RNA 1 (ADAR1). ADAR1 is a double-stranded RNA (dsRNA) binding protein (dsRBP) responsible for deaminating Adenosine into Inosine (A-to-I editing) on most dsRNAs. The primary role of ADAR1 is to prevent the activation of dsRNA sensors MDA5 and PKR. These dsRNA sensors evolved to activate inflammatory responses upon sensing dsRNAs, which become abundant in the context of viral infection. However, since dsRNA sensors recognize the structure of dsRNA, they can be activated by viral and endogenous dsRNAs. Depletion of ADAR1 in ADAR1-dependent (cells that require ADAR1 for viability) cell lines is sufficient to activate MDA5 and PKR, resulting in immune signaling without viral infection, termed “viral mimicry.” However, therapeutically targeting ADAR1 comes with drawbacks. Not all cells are ADAR1-dependent, reducing the potential efficacy of targeting ADAR1. Additionally, ADAR1 depletion may be toxic, as A-to-I editing may play necessary roles outside of preventing “viral mimicry.” This need led us to search for other dsRBPs that suppress dsRNA sensing.

Our lab used publicly available DepMap data to identify genes with dependency scores that correlate with ADAR1-dependency. This revealed a correlation between ADAR1-dependency and PACT-dependency. PACT is a dsRNA binding protein with no known catalytic activity. In breast cancer, with one exception, all PACT-dependent cell lines are TNBC. Depletion of in PACT-dependent cell lines caused reduced viability and activation of the dsRNA sensor PKR. While depletion of PACT and often ADAR1 have little impact on PACT-independent cell lines, these cell lines were sensitive to depletion of both PACT and ADAR1, suggesting a partially redundant role. Through mechanistic studies in which we depleted both PACT and PKR, we found that reduced viability of PACT depleted cells is dependent on PKR. Using PACT mutant overexpression cell lines, we have also shown that dsRNA-binding incompetent PACT mutants are unable to prevent PKR activation. These data suggest that, like ADAR1, PACT binds dsRNA to compete with PKR for dsRNA binding sites to prevent PKR activation.

Currently, our lab is working to evaluate the effects of individual and combined depletion of PACT and ADAR1 in a mouse syngeneic TNBC model. Additionally, we are assessing if elevated PKR expression is sufficient to sensitize cells to depletion of PACT. If these experiments prove fruitful, our lab hopes to be able to develop small molecule PACT degraders, providing a new therapeutic for TNBC treatment.

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Cell and molecular biology of breast cancer ***Graduate Student***

POSTER #12

THE ROLE OF SGK1 IN GLUCOSE METABOLISM TO EVADE CELL DEATH BY ECM-DETACHED CELLS

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The ability of cancer cells to detach from the extracellular matrix (ECM) and inhabit distant regions of the body accounts for 90% of cancer mortalities. While detachment from the ECM induces caspase-dependent cell death, metastatic cancer cells undergo metabolic changes to circumvent death pathways such as anoikis. Cancer cells can acquire resistance to anoikis by activating pro-survival signaling pathways such as the PI(3)K signaling cascade. Many studies on effectors of PI(3)K signaling in cell proliferation heavily focus on AKT. Previous work in our lab discovered that serum and glucocorticoid kinase 1 (SGK1), a kinase with significant homology to AKT, is a driver of metabolic changes that promote survival during ECM-detachment. While AKT has been well-studied during tumorigenesis, it's becoming clear that SGK1 may also have a significant role in tumor progression and metastasis. This work seeks to understand how changes in the environmental composition of metabolites in the environment using physiologic media and glucose starved conditions will alter the capacity of SGK1 to mediate glucose metabolism. Using multiple cancer cell lines engineered to express constitutively active SGK1, our results demonstrate that SGK1 is more effective than AKT in promoting GLUT1 trafficking and activity in ECM-detached cells. In summary, these findings establish SGK1 as a key regulator of glucose uptake, offering new insights into how cancer cells adapt and survive beyond their native microenvironment.

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Cell and molecular biology of breast cancer ***Graduate Student***

POSTER #13

EVALUATION OF CXCR2 ON THE IMMUNE CELL LANDSCAPE OF THE METASTATIC NICHE IN BREAST CANCER BONE METASTASIS

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Bone is the most common site of metastasis for breast cancer patients, contributing to significant skeletal pain, risk of injury, and poor prognosis and survival. Since breast cancer that has metastasized to bone is currently incurable, an understanding of the factors enabling breast cancer bone colonization is critically needed to develop strategies to improve outcomes for cancer patients. Our previous study utilizing a novel *ex vivo* co-culture system of breast cancer cells in mouse bones identified chemokine Cxcl5 as a candidate regulator of metastatic colonization of cancer cells in bone. Addition of recombinant Cxcl5 was sufficient to promote proliferation of breast cancer cells in bone, while blockade of its receptor Cxcr2 with an antagonist reduced proliferation. These results indicate that inhibition of the Cxcl5/Cxcr2 signaling axis is a candidate therapeutic option for the treatment of breast cancer bone metastasis.

In order to evaluate Cxcr2 activation in the metastatic niche as a therapeutic vulnerability in ER+ breast cancer metastasis to bone, we aim to characterize the immune cell landscape in both Cxcr2 WT and KO mouse models. Cxcr2 KO has been shown to be sufficient in preventing the establishment of a metastatic niche. These niches are characterized by reduced neutrophil recruitment, increased macrophages, and increased CD3+ T-cells to create an immunosuppressive tumor microenvironment compared to WT mice. The recruitment of myeloid cells in association with Cxcr2 inhibition has not been evaluated in breast cancer bone metastasis.

My research will determine if Cxcr2 is sufficient to set up an immunosuppressive bone microenvironment, leading to increased breast cancer metastasis to bone and Cxcl5-induced cancer cell proliferation. Through the characterization of the immune cell landscape through multi-color flow cytometry panels, I will determine the impact of Cxcr2 and Cxcl5 on the immune cell populations from cells derived from both bone marrow and spleen of Cxcr2/Cxcl5 WT and KO mice ± IC injections of Cxcr2/Cxcl5 WT and KO cells. These findings could significantly affect the efficacy of immunotherapy in breast tumors with elevated expression of Cxcr2. Importantly, as breast cancer patients with metastasis to bone currently have few treatment options, this work has significant translational potential to improve the quality of life and provide new therapies to overcome bone metastasis dependent on the Cxcl5/Cxcr2 axis.

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Cell and molecular biology of breast cancer ***Post-Doctoral/Medical Fellow***

POSTER #14

TOWARDS UNDERSTANDING THE ANGIOMOTIN MEMBRANE FUSION ACTIVITIES

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In some epithelial cell derived carcinomas, overexpression of Angiomotins (Amots) has been found to promote cancer. Amots are a family of adapter proteins that play an important role in the localization and regulation of proteins involved in cellular polarity, differentiation, and proliferation. These functions have been attributed to Amot's coiled-coil homology (ACCH) domain's ability to selectively bind, deform, fuse, and reorganize membranes containing phosphatidylinositol lipids. However, it is unclear how the ACCH domain leads to tumorigenesis and metastasis. Based on our previous work, we hypothesized that disruption of the ACCH domain's ability to fuse membranes leads to a loss of normal cellular polarization and adhesion, thereby increasing the rates of cellular proliferation and migration (metastasis). To test this, we followed up on our previous mutation screens identifying mutations in The Cancer Genome Atlas—gastric adenocarcinoma R153H. We investigate the relationship between ACCH domain activity and downstream cellular effects using an in vitro approach. Utilizing fluorescence microscopy and cellular fractionation, we demonstrate how these mutations affect cellular trafficking by measuring Amot80 localization and local concentration of PI lipids within the plasma membrane, Golgi apparatus, ER, and endosomal vesicles. We use Student T-tests, Wilcoxon's Tests, and one-way ANOVAs to determine our data's significance and changes that would drastically impact cellular signaling. The results provide insight into how this individual ACCH domain residue might maintain normal phenotypes and how R153H may initiate tumorigenesis. Future work includes measuring the impact of these changes on cellular proliferation and migration, possibly indicating more aggressive cancer phenotypes.

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Cell and molecular biology of breast cancer ***Research Technician***

POSTER #15

GERMLINE LOSS-OF-FUNCTION REGULATORY VARIANT OF ACKR1 GENE AND ITS INFLUENCE ON NORMAL BREAST AND BREAST CANCER BIOLOGY

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The *Atypical Chemokine Receptor 1*(*ACKR1/DARC*) gene plays a major role in regulating immune/inflammatory pathways by functioning as a decoy receptor for several cytokines/chemokines including the breast cancer metastasis-associated CXCL12. *ACKR1* gene harbors several single nucleotide variants in the regulatory and coding regions. The regulatory region variant *rs2814778* is responsible for the Duffy-Null (*CC*) /heterozygous (*TC*) phenotype, which results in significant reduction in ACKR1 expression in both epithelial and non-epithelial cells. The *rs2814778* variant is enriched in African and Arab ancestry. ACKR1 germline variants are embedded in human population to protect against malarial infection but altered chemokine/cytokine signaling in individuals with these variants influence cancer progression pathways. It has recently been suggested that clinical trial design need to take Duffy phenotype/genotype into consideration to account for normal biological differences. We investigated the potential influence of *ACKR1* variant in breast cancer using three approaches. The first approach was analysis of the E5103 breast cancer clinical trial dataset, which showed enrichment of the *ACKR1 rs2814778* variant correlating with lower Disease-free survival (DFS) rate (HR = 1.5, p=0.0014 for TC/CC vs TT). The second approach was analysis of the UALCAN database, which showed lower expression of ACKR1 in breast cancer correlating with progression to brain metastases. The third approach was the establishment of a model system to determine the influence of rs*2814778* variants on breast progression with a long-term goal of identifying therapeutic vulnerabilities. We utilized breast tissues from the institutional resource of Komen Normal Tissue bank and generated immortalized breast epithelial cell lines with functional *TT* (wild type that express ACKR1 -African and European ancestry), heterozygous (*T/C*) and homozygous (*CC*). Cell lines with *TT* expressed higher levels of ACKR1 mRNA compared to those with *T/C* or *CC* in the regulatory region. We assessed the basal activity of receptor tyrosine kinases, which may contribute to cell-autonomous mechanisms in inflammation-driven cancer, and secreted chemokines/cytokines in these cell lines. We observed African ancestry and/or ACKR1 expression-dependent variations in phosphorylation of Hepatocyte Growth Factor Receptor (HGFR/c-Met), Insulin -like Growth Factor Receptor-1 (IGFR-1) and Fibroblast Growth Factor Receptor 3 (FGFR3) in these cell lines. *In vivo* studies are underway to determine whether ACKR1 expression levels in transformed cells correlate with distinct tumor characteristics including cancer stem cell properties, metastasis propensity and response to targeted and conventional chemotherapies. Comprehensive analyses of *ACKR1* germline variants may provide additional insights into whether these variants need to be taken into consideration for clinical decision-making including treatment options.

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Genetics of breast cancer ***Graduate Student***

POSTER #16

SINGLE NUCLEUS CHROMATIN ACCESSIBILITY AND TRANSCRIPTOME ANALYSES REVEAL ABERRANT INTER-CELLULAR COMMUNICATION IN INFLAMMATORY BREAST CANCER

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International efforts focused on discovery of biological targets in inflammatory breast cancer (IBC) have suggested that aberrant inter-cellular relationship instead of genomic aberrations is the key driver of IBC. To identify such IBC-specific aberrations, we generated single nucleus chromatin accessibility and transcriptome atlas of IBCs (9,628 nuclei from three donors) and compared IBC atlas with a similar atlas of the healthy breast (81,735 nuclei from 92 donors). We recently reported single nucleus atlas of breast tissues of healthy women of diverse genetic ancestry (Nature Medicine in press). In that report, we described markers that identify three major epithelial cell subtypes [Basal-myoepithelial (BM), luminal adaptive secretory precursor (LASP), luminal hormone sensing (LHS)], two endothelial cell subtypes, two adipocyte subtypes, fibroblasts, macrophages, and T cells of the healthy breast. We also showed that LHS cells are the likely cells-of-origin of Luminal A, Luminal B and HER2+ breast cancers. Unlike these breast cancer subtypes, IBC does not appear to have a cell-of-origin, as epithelial cells in IBCs shared gene expression pattern across BM, LASP and LHS cells. Similar results were obtained when comparison was restricted to ancestry-specific healthy breast atlas. However, individual gene level expression differences affecting specific signaling pathways were observed in epithelial cells of IBCs. LASP, and LHS cells of IBCs overexpressed GPR137C, a positive regulator of mTORC1 signaling, and HS6ST3, an enzyme required for heparan sulfate synthesis. LASP and LHS cells of IBCs displayed activation of mitotic and matrix metalloproteinase signaling, respectively. Endothelial cells of IBCs, which showed significant gene expression differences compared to endothelial cells of the healthy breast, displayed enhanced integrin cell surface interaction but loss of cell junction organization. Most critically, BM, LASP, LHS, and endothelial cells of IBCs compared to their counterparts in the healthy breast showed downregulation of TEX14, an inter-cellular bridge forming factor in germ cells and a regulator of mitosis. Downregulation of TEX14 expression in these cells was accompanied with changes in the chromatin accessibility patterns of this gene. These results reinforce the notion that defective inter-cellular communication is a hallmark of IBC and TEX14 expression levels may serve as a biomarker of this defect. We suggest that greater attention to vascular biology and vascular-epithelial cell communication has to be given for better understanding of IBC biology and therapeutic targeting.

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Genetics of breast cancer ***Laboratory Research specialist***

POSTER #17

PASSAGE NUMBER AFFECTS DIFFERENTIATION OF SENSORY NEURONS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Induced pluripotent stem cells (iPSCs) can be utilized in neurological disease-modeling and drug discovery due to their capability to differentiate into neurons which reflect the genetics of the patient from which they are derived. These cells demonstrate significant variability in culture, however, due to heterogeneity in culture conditions. In this study, we investigated the effect of passage number on the differentiation of iPSCs into peripheral sensory neurons (iPSC-dSNs) in order to optimize differentiation. Three iPSC lines were reprogrammed from the peripheral blood of three donors and differentiated into iPSC-dSNs at passage numbers within each of three ranges: low (5-10 passages), intermediate (20-26 passages), and high (30-38 passages). Prior to differentiation, the morphology and pluripotency of the parent iPSC lines were assessed. Differentiated iPSC-dSNs were evaluated based on electrophysiological properties and the expression of key neuronal marker genes.

Across passage number groups, all iPSC lines displayed a similar morphology and level of pluripotency. However, iPSC-dSNs differentiated from low-passage iPSCs exhibited expression levels of neuronal markers and sodium channel function most similar to the desired sensory neuron phenotype compared to those differentiated from intermediate or high passage numbers. These results indicate that lower passage numbers (5-10) may be more optimal for the differentiation of iPSCs into peripheral sensory neurons.

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Novel modalities or technologies for breast cancer diagnosis and treatment ***Faculty***

POSTER #18

THE IMPACT OF OXYGEN EXPOSURE ON CLINICAL BIOMARKERS – AN UNDERRECOGNIZED SOURCE OF PRE-ANALYTIC VARIABILITY

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When preclinical research fails to replicate human biology, scientific progress stalls, clinical trials falter, and patients continue to suffer. While many factors contribute to these failures, lack of attention to pre-analytic variability is a seminal issue. We recently reported that even short-term exposure to ambient air is sufficient to trigger signaling changes in tumor and non-malignant biospecimens. Those changes in turn alter their biology and responsiveness to targeted therapies. Thus, characterization of tumors collected and processed under physioxia (3% O_2) instead of current practice of collection and processing under ambient air (21% O_2) will help to identify clinically relevant biomarkers that are affected by O_2 tensions. This approach may help to reduce clinical trial failure rates and increase clinical translation of preclinical studies. Towards this goal, we collected human specimens (biopsies, ascites and pleural effusions) under physioxia, then divided the same specimen into two groups; one group maintained under physioxia for 45-60 minutes before fixing/processing and the other group exposed to ambient air for 45-60 minutes before fixing/processing. Samples were subjected to various biomarker analysis using immunohistochemistry, Western blotting, and qRT-PCR. Tumor cells cultured for few days under physioxia and ambient air and sorted for $EpCAM^+$ epithelial (potentially enriched for tumor cells) and non-epithelial cells were subjected to nanopore sequencing to determine O2 tension-dependent changes in DNA methylation. We found the levels of p-ERK and p-AKT, two clinically used biomarkers of targeted therapy response, are significantly higher in esophageal cancerbiopsies and lobular carcinoma of breast cancer biopsies under physioxia compared to those biopsies exposed to ambient air. Similar effects of O_2 tension on p-ERK and p-AKT levels were observed in cells isolated from ascites or pleural effusion. The effects of O_2 tension on biomarkers showed specificity as we did not observe significant differences in pEGFR under two conditions. The observed differences in signaling pathways extended to cultured cells from ascites fluids and pleural effusions. For example, phosphorylated p53 was increased in cultured $EpCAM^+$ epithelial cells under ambient air compared to cells under physioxia suggesting that exposure of tumor specimens to ambient air activates p53 pathways. Indirectly, these results suggest that p53 mutation status determines the effects of O_2 tension on levels of specific biomarkers in tumor specimens. O_2 tension-dependent differences in DNA methylation of specific CpG islands on chromosome 6, 10, 13, and 22 in $EpCAM^+$ cells and chromosome 17 in $EpCAM^-$ cells were also observed. Collectively, our current study lays out a new physiologically relevant biomarker validation/discovery platform, which may accelerate evaluation of physiologically relevant signaling networks, new drug discovery, and enhance clinical translation of preclinical observations.

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Novel modalities or technologies for breast cancer diagnosis and treatment ***Faculty***

POSTER #19

A BREAKTHROUGH IN BREAST CANCER TREATMENT: TARGETED DELIVERY OF DUALLY LOADED LIPID NANOPARTICLES ACHIEVES SYNERGISTIC EFFECT ACROSS ALL LUMINAL SUBTYPES

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Breast cancer is the most common type of cancer for women in the United States, with an estimated 287,850 new cases in 2022. Despite advances in medicine, approximately 15% of all cancer-related deaths were due to breast cancer, warranting the need for new treatments. Subpopulations of aggressive cancer cells have been shown to express stem cell marker receptors (SCMR) on the cell surface, making this receptor a good candidate for the targeted delivery of chemotherapies. Overactivation of phosphoinositide 3-kinase (PI3K) and the subsequent PI3K/AKT/mTOR pathway has been observed in many forms of cancer and resulted in the synthesis of various PI3K inhibitors. Nevertheless, poor drug tolerance and limited maximum tolerated dosages has hampered the availability of PI3K inhibitors to patients. Additionally, maytansinoids are a class of highly cytotoxic microtubulin inhibiting compounds, however, they are limited by a narrow therapeutic window and systemic toxicity. Our research has focused on engineering SCMR-targeted liposomal nanoparticles dually loaded with both PI3K-inhibitor prodrug and maytansinoid prodrug for selectively delivery to breast cancer cells. An SCMR-targeting peptide sequence was identified and synthesized for lipid nanoparticle (LNP) incorporation. *In vitro* studies were performed to ensure that cellular uptake of nanoparticles was enhanced by the presence of the SCMR-targeting peptide. Initial cytotoxicity experiments were conducted comparing the synergy between free PI3K inhibitor, free maytansinoid, and various combinations between the two drugs. A 10:1 ratio was selected for further evaluation with nanoparticles. Prodrug versions of the PI3K inhibitor and maytansinoid were synthesized for liposomal incorporation. Further cytotoxicity experiments compared SCMR-targeted and non-targeted nanoparticles loaded with one drug and nanoparticles loaded with both drugs. Breast cancer cells from all luminal subtypes were incubated with nanoparticles for 72 hours, cell viability was measured, and the Chou-Talalay method was used to evaluate the synergistic effects between the drugs. If the combination index (CI) value calculated is <1, there is a synergistic effect between the two drugs. If equal to 1 there is an additive effect, and if >1 an antagonistic effect. The CI values of non-targeted and SCMR-targeted singly loaded nanoparticles were 0.81-1.13 and 1.07-1.33, respectively. The CI values of non-targeted and SCMR-targeted dually loaded nanoparticles were 0.13-0.72 and 0.09-0.29. These findings have led us to believe that this combination of drugs in conjuncture with SCMR-targeted delivery to be pivotal in the treatment of breast cancer and in understanding multidrug resistance. Additionally, we are interested in evaluating our findings *in vivo* and investigating the efficacy of our nanoparticles in other forms of cancer.

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Novel modalities or technologies for breast cancer diagnosis and treatment ***Graduate Student***

POSTER #20

TOWARDS ADVANCING TARGETED DETECTION OF AGGRESSIVE TRIPLE-NEGATIVE BREAST CANCER AND ITS METASTASIS

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Each year in the US, over 100,000 new cases of brain metastasis are diagnosed.[1] Approximately 10-30% of women with aggressive subtypes of Triple Negative Breast Cancer (TNBC) experience metastasis to the brain[2,3]. TNBC has fewer targeted treatment options than any other type of invasive breast cancer, where treatment generally includes surgery and generalized, non-targeted chemotherapy that has an 80% incomplete response. Therefore, breast cancer brain metastasis (BCBM) has a high mortality rate, particularly due to chemotherapy agents having a limited permeability of the blood brain barrier (BBB) [5,4]. Previous research in the Kimble-Hill lab has shown specific Phosphatidylinositol (PI) to be potential biomarkers for breast cancer aggressiveness[5]. Therefore, we hypothesized that fluorescently labeled antibodies targeting these PI biomarkers can differentiate between co-cultured Neuro 2A cells, a mouse neural crest-derived cell line, and TNBC cell lines. We also hypothesized that intracellular signaling in the culture would result in higher Neuro 2A proliferation and higher TNBC migration rates towards these neuronal cells. Methodology includes fluorescent imaging of the cells constitutively expressing fluorescent tagged proteins and the MTT like CellTitre assay for cell counting within t-tests for statistically significant differences in proliferation and migration rates. We also use Pearson's correlation coefficients to determine the ability to discern the prevalence of PI lipids in each cell population using fluorescent imaging. Our study has therapeutic implications in understanding mechanisms underlying BCBM as well surgical applications for targeted removal of cancerous cells towards an overall goal of improving survival rates.

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Novel modalities or technologies for breast cancer diagnosis and treatment ***Graduate Student***

POSTER #21

EFFICIENT GENE DELIVERY FOR BREAST CANCER USING SMALL EXTRACELLULAR VESICLES: CHIRAL-ASSISTED SIRNA LOADING AND PH-RESPONSIVE PEPTIDE FUNCTIONALIZATION

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Small extracellular vesicles (sEVs) carry biomolecules sorted from their cell-of-origin and transport this diverse cargo for cell-to-cell communication. This intercellular interaction, along with their structural and physiological stability, highlights sEVs as promising nanocarriers for drug delivery. Although advances have recently been made in sEV-based drug delivery platforms, challenges from insufficient targeting and low therapeutic efficacy continue to hinder their practical clinical progress. Recent studies reported that sEV uptake depends on their cell origins and shows preferential uptake by parental cells, known as the homing effect, which presents an opportunity to utilize this feature to enhance its targeting ability. However, this enhanced uptake occurs through specific ligand-receptor interactions that facilitate endocytosis but only about 24% of endocytosed sEVs escape lysosomes and avoid undesirable degradation. This highlights the need for strategies to improve lysosomal escape to fully harness their homing effect for targeted drug delivery. Moreover, despite extensive exploration of drug loading methods, challenges such as lipid deterioration, protein denaturation, and low loading efficiency (<20%) contribute to the suboptimal efficacy of sEVs. In this study, we utilized chiral graphene quantum dots (GQDs) for siRNA loading into sEV via nanoscale chiral interactions, achieving > 60% loading efficiency while preserving sEV structural integrity. Additionally, we synthesized a cholesterol-conjugated pH-responsive peptide to functionalize sEV membranes through hydrophobic interactions, facilitating lysosomal escape in response to pH changes within the lysosome. We finely optimized the integration of these two non-aggressive and passive approaches, which, in turn, preserved the inherent bioactivity of breast cancer cell-derived sEVs to leverage the homing effect for targeted therapy. The GALA peptide decorated on sEVs facilitated lysosomal escape and enabled cytoplasmic cargo delivery through charge conversion, promoting fusion with the lysosomal membrane. This resulted in a 1.74-fold increase in cytoplasmic cargo delivery and achieved ~73% gene knockdown along with high-efficiency siRNA loading. To address chemotherapeutic resistance in breast cancer associated with a stiff extracellular matrix (ECM), we successfully delivered siTGF-β RNA intracellularly, resulting in reduced ECM stiffness and enhanced drug sensitivity. This was further demonstrated by a combination treatment resulting in low cell viability after two days in the cytotoxicity test, along with a notable inhibition of colony formation in the one-week clonogenic assay. Overall, our approaches have significant potential to enhance sEV-based gene delivery for breast cancer and accelerate clinical translation.

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Novel modalities or technologies for breast cancer diagnosis and treatment ***Graduate Student***

POSTER #22

PROFILING PROTEIN POST-TRANSLATIONAL MODIFICATIONS IN PLASMA-DERIVED EXTRACELLULAR VESICLES AS FINGERPRINTS FOR BREAST CANCER SUBTYPES

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Addressing tumor heterogeneity in breast cancer research is crucial, given the distinct subtypes like triple-negative, luminal A/B, and HER2, requiring precise differentiation for effective treatment. This study introduces a non-invasive method by analyzing post-translationally modified proteins in plasma extracellular vesicles (EVs), which play a role in immune regulation and intercellular communication. Examining modifications like phosphorylation, acetylation, and glycosylation in EVs provides insights into breast cancer dynamics. One hundred one plasma samples from luminal A/B, triple-negative breast cancer, and healthy individuals underwent discovery and validation experiments. Using data-dependent acquisition, the study identified over 28,000 unique non-modified peptides, 5,000 phosphopeptides, 680 acetyl peptides, and 1,300 glycopeptides that were successfully characterized. Bioinformatics analyses revealed significant overexpression of 815 non-modified proteins, 3,958 phosphopeptides, 352 acetyl peptides, and 895 glycopeptides in luminal A/B or triple-negative breast cancer subtypes. Phosphorylated and glycosylated PD-L1 peptides emerged as potential markers for breast cancer, regardless of subtype. Aligning findings with literature and PAM50 gene signatures highlighted markers correlated with lower survival rates. The study also conducted 123 scheduled parallel reaction monitoring (PRM) analyses, leveraging machine learning to pinpoint a panel of specific modification sites with high accuracy in subtype differentiation. This research reveals diagnostic markers and enhances understanding of the molecular landscape, contributing to more effective and personalized breast cancer diagnostics and treatments.

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Novel modalities or technologies for breast cancer diagnosis and treatment ***Post-Doctoral/Medical Fellow***

POSTER #23

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.

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Novel modalities or technologies for breast cancer diagnosis and treatment ***Research Technician***

POSTER #24

THE KOMEN TISSUE BANK: A HEALTHY TISSUE RESOURCE

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Breast cancer continues to be a significant source of morbidity and mortality in the United States and worldwide.As technology expands to better interrogate the genome, create predictive algorithms of risk beyond what the human eye can see, and develop methods to synthesize these data, it remainscrucial to have access to well-annotated biospecimens. Carefully collected cohorts that are representative of diverse ancestral backgrounds, ages, and exposures provide the platform from which innovation and discovery can arise. Early breast cancer research struggled to obtain breast tissue from healthy women, leaving researchers without control samples and limiting the ability to understand the biology of healthy breast tissue-- an important aspect of understanding early breast carcinogenesis.

In response to these problems, the Komen Tissue Bank was established in 2007 as the first—and still only—source of normal tissue from healthy individuals. Since that time, 5,173 breast tissue donors have allowed 126 unique primary investigators and their research teams to access data and/or biospecimens which have resulted in 108 publications to date. On average, 2,320 samples are sent to researchers annually, resulting in over 37,127 samples distributed since the inception of the KTB. Biospecimens are collected biannually at donation events in Indiana and across the United States, where tissue and whole blood are obtained from 100-120 donors without a history of breast cancer. During this process, each donor goes through informed consent, height and weight measurement, completion of a self-administered questionnaire, a blood draw, and tissue donation. A single donation yields whole blood, 5 aliquots of plasma, 5 aliquots of serum, 6 aliquots of DNA, 2 FFPE blocks, 4 fresh-frozen tissue cores, and a cryopreserved tissue core for cell isolation. Donors are asked to participate in annual follow-ups, which focus on changes in cancer status and medical history. This information enables researchers to examine factors in healthy tissue that may potentiate future breast cancer risk. In line with its mission of expanding access to the scientific community, the KTB operates an open-access virtual tissue bank, providing researchers with access to well-annotated data, including genomic,histological, and imaging data.

Here we highlight the KTB and how samples have enabled researchers to create unique cell lines and analyze diverse populations, leading to breakthroughs across the cancer control spectrum. The overall goal of the KTB is to facilitate the sharing of well-annotated biospecimens and data to assist investigators who are performing basic, translational, and clinical research, from primary prevention and early detection through metastatic disease.

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Novel modalities or technologies for breast cancer diagnosis and treatment ***Research Technician***

POSTER #25

SKIN TONE MODEL TO REMOVE BIAS FROM BREAST CANCER IMAGING USING DIFFUSE OPTICAL SPECTROSCOPY

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Breast cancer is the second most common cancer in women in the United States, accounting for about 30% of all new female cancers each year. Light-based imaging technologies, such as diffuse optical spectroscopy (DOS), are increasingly researched for noninvasive tumor detection, tissue characterization, and chemotherapy response monitoring. By measuring the absorption and scattering of near-infrared light, DOS quantifies the concentrations of oxygenated and deoxygenated hemoglobin, water, and lipids in tissue, enabling differentiation between benign and malignant tumors as well as assessment of neoadjuvant chemotherapy (NAC) response. However, melanin absorption in the skin can interfere with optical imaging signals, leading to reduced penetration depth, altered contrast, and potential inaccuracies in tumor detection and treatment monitoring, particularly in patients with darker skin tones. Seeking to improve imaging accuracy across diverse populations, researchers have used skin phantoms and tissue-mimicking models with tunable optical properties, to test and calibrate light-based imaging devices. Skin phantoms allow for controlled, repeatable experiments, making it possible to isolate the effects of melanin on optical measurements and develop compensation strategies such as wavelength adjustments and signal correction algorithms. However, traditional phantom fabrication methods are costly, labor-intensive, and require specialized equipment, limiting their widespread use in research and clinical settings.

Building on previously proposed methods of printing discrete patterns onto transparent polyurethane films to create low-cost, reproducible skin phantoms, our group has successfully fabricated a set of Tegaderm-based phantoms using a standard color laser printer. This approach offers a practical and accessible way to mimic melanin absorption without relying on complex chemical processes or specialized equipment. By printing precise toner patterns onto the polyurethane film, we control optical absorption properties without relying on complex chemical processes or specialized 3D printing techniques. Our group is now testing these phantoms with our DOS system to examine how varying pigmentation levels affect the estimated optical properties. This serves as a crucial first step toward developing strategies to compensate for melanin-induced bias and improve the accuracy of optical property recovery, ultimately ensuring more reliable imaging results across diverse skin tones. A scalable and accessible method enables systematic testing of breast cancer imaging devices across a wide range of melanin concentrations, ensuring that imaging systems remain effective regardless of skin tone. In the future, we will compare the performance of melanin-calibrated imaging systems against current clinical devices, assess their effectiveness in detecting tumors and predicting chemotherapy response, and evaluate their ability to ensure diagnostic accuracy across diverse patient populations.

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Novel modalities or technologies for breast cancer diagnosis and treatment ***Undergraduate***

POSTER #26

END-OF-LIFE FINANCIAL DECISION-MAKING AMONG RURAL BLACK AFRICAN AMERICAN WOMEN WITH ADVANCED OR LATE STAGED BREAST AND GYNECOLOGICAL CANCERS IN INDIANA

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ABSTRACT

Background

Gynecological and breast cancers are major health concerns for Black/African American (BAA) women, especially in rural areas. BAA women are twice as likely to develop these cancers compared to White women, with 20% higher incidence and mortality rates in Indiana. Alongside health disparities, BAA women and their families face severe financial challenges near the end of life, leading to debt, bankruptcy, treatment nonadherence, and poorer health outcomes. About one-third experience low incomes, limited assets, and racial discrimination, often forcing them to sell property, take high-interest loans, or leave jobs for end-of-life care. However, little is known about how financial decisions at the end of life affect financial well-being and health outcomes.

Aim

This study therefore aims to explore financial stressors, influencing factors and end of life financial decisions among BAA women with gynecological and breast cancers to develop strategies improving their financial well-being.

Method

This study will use a qualitative narrative inquiry approach with purposive and snowball sampling to recruit 20 participants: 10 BAA women (18+) with advanced-stage breast or gynecological cancers receiving palliative or end-of-life care, 5 family caregivers (spouses, children, or close relatives), and 5 healthcare providers involved in their care. After obtaining IRB approval, data will be collected through semi-structured interviews conducted in-person or online. Guided by the Fan and Henegar financial well-being framework, interviews will last 45-60 minutes, be audio-recorded, transcribed verbatim, and analyzed using structural and performative narrative analysis.

Relevance

Financial decision-making is increasingly recognized for its global impact on various life aspects, including health by shaping healthcare quality and outcomes and influencing crucial health-related decisions. This study addresses an urgent need to understand and reduce financial disparities in end-of-life care among BAA women, laying the foundation for future interventions and advancing health equity in cancer care.

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Nursing / Psychosocial Support / Genetic Risk Counseling ***Graduate Student***

POSTER #27

BREAKING THE SILENCE: UNVEILING BARRIERS TO MENTAL HEALTH SERVICE USE IN BREAST CANCER SURVIVORS WITH CLINICALLY SIGNIFICANT DISTRESS - A MIXED METHODS STUDY

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Background: Breast cancer survivors (BCS) have an increased risk of psychological distress, including symptoms of depression, anxiety, and post-traumatic stress, compared to healthy controls. Fear of cancer recurrence (FCR) is an especially prevalent form of distress, with approximately 50% of BCS reporting clinically significant FCR. This mixed methods study explored relationships between psychological distress, mental health service (MHS) use, and barriers to MHS use among BCS.
Methods: A mixed methods sequential explanatory design was utilized. Initially, baseline data from 384 early-stage, post-treatment BCS with clinically significant FCR at screening enrolled in a randomized controlled trial comparing 3 FCR interventions were analyzed. The prevalence of clinically significant FCR, anxiety, depression, and post-traumatic stress symptoms was measured. Associations between distress measures and MHS use were assessed. The quantitative findings prompted qualitative follow-up consisting of interviews with 24 distressed BCS to elucidate barriers hindering their use of MHS.
Results: Clinically significant levels of at least one form of distress besides FCR were reported in 226 (58.85%) BCS. Of 298 (77.60%) BCS with at least one significant distress score including FCR, only 61 (20.47%) reported using any MHS within the 3 months before baseline. Clinically significant anxiety ($p = 0.0027$), depression ($p = 0.0015$), and post-traumatic stress symptoms ($p = 0.0227$) were significantly associated with MHS use. Conversely, FCR was significantly associated with fewer visits to certain MHS. Qualitative interviews revealed personal and systemic barriers contributing to underutilization of MHS in BCS, including avoidant coping, financial and logistical constraints, inaccessibility to providers with certain patient-preferred skillsets or backgrounds, such as speaking the survivor's first language, and limited timely care options. In addition to pinpointing such barriers, valuable suggestions for improvement were elicited that may allow current and future providers to better meet the needs of BCS.
Conclusions and Implications: Only a minority of clinically distressed BCS use MHS. Anxiety, depression, and post-traumatic stress symptoms may be better predictors of MHS use than FCR given the tendency for fearful survivors to cope with avoidance. Interventions emphasizing alternatives to avoidant coping may benefit BCS with FCR. Further research is needed to identify solutions to the multifaceted barriers impeding MHS use among BCS.
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Nursing / Psychosocial Support / Genetic Risk Counseling ***Medical Student***

POSTER #28

TOWARDS UNDERSTANDING TRENDS PREVENTING PATIENT SUCCESS OUTCOMES IN ONCOLOGY CARE

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Individuals who are impacted by social determinants of health (SDH) account for 50% of cancer cases mortality. Research indicates that this and other cancer health disparities persist partly because physicians reinforce cultural biases and are not given adequate training to address SDH in oncology. This results in lower-quality care and worse health outcomes for people of color and those from economically disadvantaged areas, creating a critical need for physicians to gain the knowledge and skills necessary to overcome barriers in underserved communities, improve communication, and build trust with the patient population. The current study aims to understand the factors that contribute to those barriers during the cancer journey by examining the differences in help-seeking perceptions between patients, patient support, and clinicians.Study participants were recruited from social media and public space marketing campaigns and asked to complete a population-specific survey on Qualtrics. All three surveys consisted of standard demographic questions, followed by questions taken from the UK Cancer Awareness Measure (CAM) to examine help-seeking barriers from the perspective of each group. The patient and patient support surveys also contained questions from the Brief Coping Orientation to Problems Experienced (COPE) Inventory to examine coping patterns following the cancer diagnosis. The last section of the survey measured familiarity and the impact of education, communication approaches, and social determinants of health for diverse patients. The study found essentialthemes for treating and supporting these patients from the perspective of each population. The results identified 13 themes related to help-seeking, eight themes related to cultural competence, seven themes related to coping, and one theme related to social determinants of health. The current study illuminates shortcomings in medical care that reflect the literature on inconsistent training that results in healthcare inequities while highlighting new areas for intervention. Future research includes studying population-specific barriers and developing resources to improve the quality of oncology care, patient experience, and health disparities.

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Nursing / Psychosocial Support / Genetic Risk Counseling ***Postbaccalaureate Fellow***

POSTER #29

HEALTH AND BREAST CANCER AWARENESS AMONG ADULTS AT THE DOMINICAN-HAITIAN BORDER: A PILOT STUDY

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While breast cancer mortality rates have decreased in developed countries, low- and middle-income countries are experiencing risingbreast cancer deaths. In the clinic in Rosa La Piedra—a mountain village located in the Dominican Republic near the border of Haiti— women with breast cancer often present with very advanced disease, a trend attributed to significant socioeconomic and educational challenges. Delays in seeking medical care have been linked to limited resources, cultural beliefs, geographical constraints, low levels of health literacy, and stigma.

A pilot study was initiated to assess existing knowledge, health perceptions, and obstacles related to breast cancer awareness among adults in Rosa La Piedra. The study employs a cross-sectional survey administered verbally to overcome literacy barriers. Survey items focus on awareness of breast cancer risk factors, recognition of early signs, understanding of disease etiology, and perceived obstacles to seeking timely care. Volunteer medical teams administered the survey during two brief trips to Rosa La Piedra in May and November of 2024. A total of 24 surveys were completed.The findings of these surveys support the initial assessment that there are critical knowledge gaps and misconceptions about breast cancer. For instance, of the 18 participants who were asked, “what do you think causes breast cancer?” Ten participants did not know, seven participants believed that an injury to the breast caused the cancer, and one believed that the cancer occurred from not taking their medication.

The results of this study have provided valuable information on the local beliefs and challenges of Rosa La Piedra that can delay medical care. These insights and additional results collected as the survey continues to be administered will be used to develop targeted educational interventions and support strategies, such as establishing a medical fund to facilitate access to diagnostic and treatment services. Increasing breast cancer awareness in underserved communities worldwide, such as Rosa La Piedra, is essential to promoting early detection and preventing the rising rates of both incidence and mortality of breast cancer.

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Nursing / Psychosocial Support / Genetic Risk Counseling ***Pre-Medicine Undergraduate***

POSTER #30

EFFECTS OF GLOBAL AND PERIPHERALLY-RESTRICTED INHIBITORS OF ENDOCANNABINOID DEACTIVATION ON THE DEVELOPMENT OF CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY AND TUMOR GROWTH IN A MOUSE BREAST CANCER MODEL

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Chemotherapy produces anti-cancer effects in breast cancer but also produces dose-limiting side effects such as neuropathic pain. Inhibition of the primary enzymes that degrade endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide (AEA) suppress neuropathic pain behavior in models of chemotherapy-induced peripheral neuropathy (CIPN) while also displaying anti-cancer properties. 2-AG is hydrolyzed by the enzyme monoacylglycerol lipase (MGL), while AEA is degraded by fatty-acid amide hydrolase (FAAH), respectively. Our groups previously discovered a novel CNS impermeable MGL inhibitor, LEI-515, that was able to reverse neuropathic nociception in paclitaxel-treated mice in the absence of cancer. In the current studies, we assessed peripheral MGL inhibitor LEI-515, global MGL inhibitor JZL184, and peripheral FAAH inhibitor URB937 for their respective abilities to block development of CIPN and reduce tumor size in a mouse model of breast cancer. First, LEI-515, JZL184, URB937 or vehicle was administered prophylactically with the chemotherapeutic agent paclitaxel in a cohort of non-tumor bearing mice. Mechanical (assessed using the von Frey assay) and cold (assessed using the acetone test) hypersensitivities were then assessed. A separate cohort of BALBc mice received 4T1 tumor cell inoculation into the mammary fat pad. Pharmacological treatments began once tumors were palpable. Treatments were administered once daily for LEI-515, JZL184, URB937, or their vehicle, before paclitaxel treatment. Paclitaxel treatment was every two days. Mechanical and cold hypersensitivities were assessed every 4 days throughout the study, and tumor volumes were measured daily. At the terminal end point, tumor weight and colonic content in small and large intestine were assessed. Both peripheral MAGL inhibitor LEI-515 and peripheral FAAH inhibitor URB937 prevented the development of paclitaxel-induced mechanical and cold hypersensitivity in non-tumor mice. JZL184 did not prevent the development of mechanical or cold hypersensitivity induced by paclitaxel. LEI-515 did not interfere with the ability of paclitaxel to kill tumor cells in the MTT assay. In 4T1 tumor-bearing mouse studies, LEI-515 prevented the development of chemotherapy-induced mechanical and cold hypersensitivity, and reduced tumor size when combined with paclitaxel compared to chemotherapy alone. Global MGL inhibitor JZL184 reduced tumor size when combined with paclitaxel compared to chemotherapy alone, but did not prevent chemotherapy-induced mechanical or cold hypersensitivity. Peripheral FAAH inhibitor URB937 attenuated chemotherapy-induced mechanical and cold hypersensitivity but did not enhance paclitaxel's ability to reduce tumor volumes when compared to paclitaxel alone. Paclitaxel mice that received LEI-515 showed reduced tumor weights. Colonic weights of all paclitaxel-treated mice increased. Our findings indicate that peripheral MGL inhibition holds therapeutic potential for cancer patients to treat both their cancer and CIPN. Our findings also indicate that peripheral FAAH inhibition holds therapeutic potential for the treatment of CIPN.

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Synthesis and/or pharmacology of novel potential antitumor agents ***Graduate Student***

POSTER #31

PROTACS FOR IMMUNE CHECKPOINT PROTEIN PD-L1 TO COMBAT TRIPLE-NEGATIVE BREAST CANCER

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Programmed death protein 1 and its ligand (PD-1/PD-L1) make up one of the most important immune checkpoints that attenuate inflammatory responses. PD-1/PD-L1 blockade therapies have demonstrated superior efficacy in the clearance of multiple cancers by unleashing the endogenous immune response against tumors. Currently, all 9 of the FDA-approved anti-PD-1/PD-L1 therapies are monoclonal antibodies, which suffer from large molecular size, low tissue penetration, short circulating half-life, and relatively low bioavailability. Given the significance of PD-1/PD-L1 signaling in immuno-oncology, novel approaches to modulate this pathway that overcome the abovementioned disadvantages are in demand.

Proteolysis targeting chimeras (PROTACs) are heterobifunctional molecules designed to hijack the cellular ubiquitin-proteasome system (UPS) to degrade intracellular proteins. The ideal protein target for PROTACs should be cytoplasmic and readily accessible by proteasomal and lysosomal degradation systems. Hence, relatively few studies have demonstrated therapeutic degradation of membrane proteins, even though many membrane proteins such as PD-L1 are strongly implicated in many diseases.

In this study, we designed a series of membrane-targeted degradation (MemTAD) molecules for PD-L1. Our molecules were tested on the BT-549 cell line, derived from a triple-negative breast cancer (TNBC) patient, and the degradation efficiency of PD-L1 was measured using both flow cytometry (for membrane PD-L1 levels) and Western blot (for total PD-L1 levels). Our candidates have significantly reduced PD-L1 levels in BT-549 cells with DC50 values < 1 μM and maximal degradation of total PD-L1 proteins up to 72%. This work provides critical insights into designing more effective degraders targeting PD-L1, especially for aggressive cancers like triple-negative breast cancer. The PD-L1 degraders described here are a valuable tool for research and promising candidates for clinical development.

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Synthesis and/or pharmacology of novel potential antitumor agents ***Graduate Student***