



# Amelia Project

## 2023 Abstract Book

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# POSTER #1

## THE HISTONE DEACETYLASE INHIBITOR ENTINOSTAT AS AN INHIBITOR FOR HSF1 AND MYC

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Breast cancers are often classified into subtypes by the expression of hormone receptors without consideration of clonal amplifications of other genes. Our lab has found that 14% of breast cancer patients have copy-number amplifications of the two oncogenes Heat shock factor 1 (HSF1) and Cellular myelocytomatosis (c-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 indirectly 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 cells with amplifications of the HSF1 and c-MYC genes using ovarian cancer cell lines as a model. In the drug screen we found that the cell lines that carry the HSF1-MYC coamplification are more sensitive to Histone Deacetylase (HDAC) inhibitors than cancer cell lines without this coamplification. Our preliminary results show that the HDAC inhibitor Entinostat, decreases HSF1 and c-MYC protein levels as well as decreases the mRNA expression of their direct downstream targets. Our lab will further investigate the mechanism of how the Histone Deacetylase inhibitor Entinostat decreases HSF1 and c-MYC in the context of cancers that contain HSF1 and c-MYC amplifications and the efficacy of using Entinostat in these cancers.

*Anticancer drug target identification and/or validation*

*Graduate Student*

# POSTER #2

## DEVELOPMENT OF MCAK INHIBITORS THAT INDUCE ANEUPLOIDY IN TRIPLE NEGATIVE BREAST CANCER MODELS

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The standard of care for triple negative breast cancer, the most lethal breast cancer subtype, is chemotherapy using a combination of microtubule poisons and DNA damaging agents. Microtubule poisons, like paclitaxel, have been 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 to be upregulated across all breast cancer subtypes, with the highest levels in triple negative breast cancer. 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<sub>50</sub> for paclitaxel, but there was no change in normal diploid lines, indicating that 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 that another mechanism is likely 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 screened two drug libraries using a FRET-based assay that detects MCAK conformation and an image-based microtubule depolymerization assay and identified three candidate inhibitors; 2030-1 B4 (B4), 2021-4 C4 (C4), and 2042 H9 (H9). While all three drugs inhibited MCAK's microtubule depolymerization activity *in-vitro*, C4 was the most potent, as it inhibited microtubule depolymerization activity at lower concentrations. These drugs induced similar levels of aneuploidy in both taxane-sensitive and taxane resistant breast cancer cells. C4 also caused an approximate 2-fold reduction in the IC<sub>50</sub> of paclitaxel, similar to the effects seen in knockdown experiments. All three drugs also caused a potent reduction in colony formation assays in both taxane-sensitive and resistant cells, suggesting that MCAK inhibition causes a loss of viability over successive cell divisions through successive rounds of aneuploidy induction. Collectively our work will expand the field of precision medicine to include aneuploidy-inducing drugs, while giving treatment options to breast cancer patients with relapsed or drug-resistant disease.

*Anticancer drug target identification and/or validation*

*Graduate Student*

# POSTER #4

## OXYGEN TENSION - DEPENDENT DIFFERENCES IN CANCER CELL KINOME

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Current approaches to preclinical cancer research often fail to consider the impact of maintaining cancer cells under ambient oxygen (O<sub>2</sub>) tension (~21%). This is also true for hypoxia studies that typically involves cancer cells previously grown in ambient O<sub>2</sub> before subsequent transfer to hypoxic conditions. However, the tumor microenvironment is characterized by significantly lower O<sub>2</sub> levels. We have previously demonstrated the impact of ambient O<sub>2</sub> tension on stem cell populations, signaling pathways and resistance to therapy. We developed an experimental approach that allows us to collect and process tumor tissues from transgenic mammary tumor mouse models under physioxia (3% O<sub>2</sub>) such that they are never exposed to ambient O<sub>2</sub>. In the present study, our goal was to explore kinase signaling pathway alterations that occur due to physioxic and ambient O<sub>2</sub> tensions and to determine how these pathways are influenced by treatment with targeted drugs in the context of these O<sub>2</sub> levels.

Our studies revealed increased basal phosphorylation levels of EGFR (Y1068) in cells processed and propagated in ambient air (AA), relative to physioxia. However, downstream signaling effectors AKT and ERK showed higher phosphorylation levels under physioxia, compared to AA, suggesting that their activation is independent of EGFR signaling. These findings correlate with the decreased sensitivity of the tumor cells under physioxia to target drugs lapatinib and alpelisib. We then sought to examine basal and target 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 ambient air showed very minimal changes, pairwise comparison between lapatinib treated physioxia cells and vehicle treated AA cells revealed an increase in the activity of PDGFRB in lapatinib treated physioxia cells. Similarly, a receptor tyrosine kinase (RTK) array and western blotting showed increased basal and lapatinib induced phosphorylation of PDGFRB (Y751) under physioxia. Next, we determined the potential role of PDGFRB in downstream signaling pathway activation of AKT and ERK and resistance to lapatinib. We found that sunitinib, a multitarget RTK inhibitor with high affinity for PDGFR effectively decreased PDGFRB 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. Furthermore, 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. Therefore, it may be necessary to carry out preclinical cancer studies in the context of physiologically relevant oxygen tensions to aid translatability of these studies.

*Cell and molecular biology of breast cancer*

*Graduate Student*

# POSTER #5

## REGULATION OF CELLULAR METABOLIC STRESS BY AQUAPORIN-7 IN BREAST CANCER

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The complex yet interrelated connections between cancer metabolism, gene expression, and oncogenic driver genes have the potential to identify novel biomarkers and drug targets with prognostic and therapeutic value. We effectively integrated metabolomics and gene expression data from breast cancer mouse models through a novel unbiased correlation-based network analysis. This approach identified 35 metabolite and 34 gene hubs with the most network correlations. These hubs have prognostic value and are likely integral to tumor metabolism and breast cancer. We focused on the gene hub Aquaporin-7 (human AQP7/mouse Aqp7), a water and glycerol channel, as a novel regulator of breast cancer. We identified AQP7 as a negative prognostic marker of overall survival and metastasis in breast cancer patients. Both in vitro and in vivo experiments showed that AQP7 is required for proliferation, primary tumor progression, and metastasis. Metabolomics on Aqp7 knockdown cells and tumors revealed significantly altered lipid levels, redox, and urea/arginine metabolism. Given the correlation of AQP7 with breast cancer and its involvement in glycerol transport and lipid homeostasis, we investigated AQP7 as a metabolic target for cancer therapy.

We find that AQP7 is a critical regulator of metabolic and signaling responses to environmental cellular stresses and of endocrine therapy efficacy in breast cancer metastasis to lung. Our data support AQP7 expression being higher in tumors from breast cancer patients that do not respond to endocrine therapy compared to those that do respond. We investigated whether AQP7 inhibition can increase the therapeutic efficacy of endocrine therapy in breast cancer. Interestingly, we see the aquaporin inhibitor Auphen cooperates with endocrine therapy tamoxifen to reduce the viability of breast cancer cells, which suggests that Auphen treatment makes the cells more susceptible to tamoxifen. Aqp7 is a novel therapeutic target that provides a metabolic vulnerability in breast cancer and can be targeted to inhibit breast cancer metastasis in combination with endocrine therapy. Together, this study highlights AQP7 as a potential cancer-specific therapeutic vulnerability, and AQP7 inhibition can be exploited for therapeutic benefit in overcoming endocrine therapy resistance.

*Cell and molecular biology of breast cancer*

*Graduate Student*

# POSTER #6

## INVESTIGATING THE BIOLOGICAL BASIS OF RACIAL DISPARITIES IN BREAST CANCER PROGRESSION.

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Breast cancer continues to be one of the highest incident cancers in the United States. A critical statistic of note is that African American (AA) women have a higher incidence of aggressive cancers and 41 percent higher death rate from breast cancer. Although multiple factors including socio-economic backgrounds contribute to this disparity, the molecular and cellular basis underlying this striking disparity is not well understood. Breast tumor cell invasion is critical in breast cancer progression and requires a complex signaling and communication network. Communication between the tumor stroma and ductal epithelium is of great interest to understanding the advancement of disease. Extracellular vesicles (EVs) have drawn attention as modes of communication through their ability to shuttle molecular cargoes between various cell types in the tumor microenvironment. Using human immortalized luminal breast epithelial cells and a stromal cell population particularly increased in breast AA tissues, we are examining the role and mechanism of paracrine signaling that modulates the behavior and tumorigenic potential of normal luminal epithelial cells. Using these cell lines, derived from tissues obtained from the Komen Tissue bank, we found that in a three-dimensional spheroid model, the epithelial cell line alone is noninvasive, but co-culture with stromal cells enhanced invasion by the ductal cells. Additionally, treatment of the ductal cells with EVs isolated from the stromal cells, increased invasive activity of the epithelial cells as well as their mammosphere-forming efficiency. This demonstrates important crosstalk between the ductal epithelium and tumor stroma, and may explain the striking disparity observed in the acquisition of aggressive tumor phenotypes in AA women. Further work is aimed at delineating the mechanisms underlying EV-facilitated crosstalk between the tumor and stroma.

*Cell and molecular biology of breast cancer*

*Graduate Student*

# POSTER #7

## HUNK PHOSPHORYLATES RUBICON TO SUPPORT AUTOPHAGY, PROMOTING TUMORIGENESIS IN HER2+ BREAST CANCER

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Human epidermal growth factor receptor 2-positive (HER2+) breast cancer is defined by having HER2 gene amplification that coincides with HER2 protein overexpression. HER2 is amplified in 15-20% of breast cancers and overexpression of this gene is a predictor of survival in breast cancer patients. HER2-targeted therapies have been successful in treating HER2+ breast cancer; however, HER2+ breast cancer can develop resistance to these therapies establishing an urgent need for novel targets within HER2+ breast cancer to inhibit tumorigenesis. Previous work in our lab establishes that Hormonally Upregulated Neu-associated kinase (HUNK) is a Serine/Threonine (S/T) protein kinase that is overexpressed in HER2+ breast cancer and is responsible for promoting autophagy, thereby leading to therapeutic resistance and tumorigenesis in HER2+ breast cancer. Our previous work shows that HUNK phosphorylates the autophagy protein, Rubicon, at S92 on its' N-terminal domain promoting autophagy in 293T cells. However, we have yet to establish a role for this phosphorylation site within HER2+ breast cancer cells. Therefore, the objective of this study is to identify if this phosphorylation event plays a role in promoting tumorigenesis in HER2+ breast cancer. To further elucidate phosphorylation of Rubicon at S92 in the underlying mechanisms of tumorigenesis in HER2+ breast cancer, we generated multiple scientific tools: a phospho-specific antibody to detect Rubicon phosphorylation at S92, a phospho-deficient (S92A) Rubicon mutant, and a phospho-mimetic (S92D) Rubicon mutant. We have established an autophagy phenotype within a set of cell lines derived from the MMTV-neu mouse model. These cells are derived from MMTV-neu HUNK wild type (WT) or MMTV-neu HUNK knockout (KO) tumors. These cell lines will be utilized alongside our phospho-deficient and phospho-mimetic Rubicon mutants to determine the role that Rubicon S92 phosphorylation plays in promoting tumorigenesis in HER2+ breast cancer.

*Cell and molecular biology of breast cancer*

*Graduate Student*

# POSTER #8

## CXCR2 AS A THERAPEUTIC TARGET 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. An innovative *ex vivo* culture system was developed in which breast cancer cells are grown within mouse bones, which enables exploration of conditions that promote cancer cell colonization of bone without confounding variables of the primary tumor and earlier stages of metastasis. By profiling the soluble factors present in the media from cancer-primed bones, the chemokine Cxcl5 was identified as a candidate regulator of metastatic colonization of cancer cells in bone. Addition of recombinant Cxcl5 is sufficient to both maintain proliferation in active cancer cells and overcome dormancy to activate proliferation of quiescent cancer cells, suggesting that Cxcl5 is involved in the dormancy switch that enables colonization. Since Cxcr2 is the only functional receptor of Cxcl5, treatment with a Cxcr2 antagonist was evaluated and significantly decreased cancer cell 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.

Current studies utilize genetically modified cell lines and transgenic mouse models to understand the specific contributions of cancer- and stromal-cell derived Cxcr2 in bone colonization. Additionally, Cxcr2 antagonist treatment is now being evaluated as a therapeutic option for breast cancer bone metastasis. These studies utilize *in vivo* imaging in mouse models of prostate and breast cancer bone colonization to evaluate the efficacy of Cxcr2 inhibition in reducing metastatic tumor burden. Furthermore, microCT trabecular analysis is used to characterize bone responses to MMTV-PyMT tumors, an ER+ murine mammary carcinoma model. After 4 weeks, bones from mice that received intratibial injection with MMTV-PyMT cells show both osteolytic and osteoblastic tumor morphology, indicating the relevance of this model for further studies of ER+ cancer in bone. 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.

*Cell and molecular biology of breast cancer*

*Graduate Student*



# POSTER #9

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

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Endoreplication is an alternative cell cycle wherein cells undergo alternating G and S phases without dividing. While endoreplication can occur naturally to generate polyploid cells in growth and development, it can also be co-opted by cancer cells, leading to genome instability, tumor evolution, and cancer relapse; however, the mechanisms leading to these outcomes are unknown. We developed a cell culture method to generate induced endoreplicating cells (iECs) from diploid RPE-1 cells by treating cells with cell cycle inhibitors to study how changes in cell cycle dynamics lead to genomic heterogeneity and how cells with increased ploidy return to division. We found that Aurora B kinase inhibition resulted in a subtype of endoreplication called endomitosis, wherein cells enter mitosis but do not divide. Cells treated with a high concentration of Aurora B inhibitor failed at the metaphase/anaphase transition, resulting in daughter cells with multi-lobed nuclei. In contrast, cells treated with a lower concentration of Aurora B inhibitor failed at cytokinesis and resulted in binucleate cells. We found cells with multi-lobed nuclei preferentially undergo DNA synthesis, suggesting nuclear phenotype and/or the level of Aurora B inhibition may play a critical role in determining whether a cell can bypass the tetraploid checkpoint to become polyploid. To address this question, tetraploid binucleate G1 cells were treated with high or low concentrations of Aurora B kinase inhibitor and their ability to undergo DNA synthesis was assessed. We found similar abilities to bypass the G1 tetraploid checkpoint with both high and low Aurora B kinase inhibitor treatment, suggesting Aurora B kinase inhibition during mitosis is likely the reason for differential ability of cells to bypass the tetraploid checkpoint. To understand the molecular pathways contributing to endoreplication, we performed RNA-seq on iECs and found transcriptomic changes consistent with senescence. Using fluorescence microscopy, we found that some, but not all, cells were positive for senescence markers, suggesting the response to Aurora B inhibition is heterogeneous. Additionally, we found induction of endoreplication generates distinct high- and low-p21 populations. To ask whether polyploid iECs can resume proliferation, iECs were flow-sorted based on DNA content and plated in the absence of inhibitor. After an initial lag phase, a subset of cells began to rapidly proliferate and form colonies of mitotically dividing cells. We are currently assessing why only some cells return to division, and if senescence is a determining factor. We are also re-expressing genes downregulated in iECs to ask if we can establish a gene re-expression network that stimulates return to division. Our studies provide a potential model for tumor heterogeneity that will help define molecular mechanisms that lead to endoreplication and promote return to division and may provide new insights into how cancer cells evade normal cell cycle control.

*Cell and molecular biology of breast cancer*

*Graduate Student*

# POSTER #10

## 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.

*Cell and molecular biology of breast cancer*

*Graduate Student*

# POSTER #11

## TONSL IS AN IMMORTALIZING ONCOGENE OF THE CHROMOSOME 8Q24.3 AMPLICON AND NEW THERAPEUTIC TARGET IN BREAST CANCER

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Study of genomic aberrations leading to immortalization of epithelial cells has been technically challenging due to lack of appropriate isogenic model system. To address this technical challenge, we utilized primary breast luminal epithelial cells propagated from healthy donors of different genetic ancestry and their hTERT-immortalized counterparts to identify functional gene expression changes associated with immortalization. We identified elevated expression of TONSL (Tonsoku Like, DNA Repair Protein) as one of the earliest events during immortalization. TONSL alone immortalized primary breast epithelial cells and increased telomerase activity. While TONSL overexpression alone was insufficient for neoplastic transformation, TONSL-immortalized primary cells modified to overexpress defined oncogenes generated estrogen receptor-positive adenocarcinomas in NSG mice. Analysis of breast tumor microarray with ~500 tumors revealed poor overall and progression free survival of patients with TONSL-overexpressing tumors. TONSL is located on chromosome 8q24.3 and amplified in ~20% of breast cancers with significantly higher amplification in metastatic tumors. TONSL forms a complex with FACT and MMS22L1 to modulate multiple cellular pathways including DNA replication, repair through homologous recombination (HR) and functions as a post replicative histone/chromatin reader. TONSL increased chromatin accessibility to pro-oncogenic transcription factors including NF- $\kappa$ B and limited access to the tumor suppressor p53. Most importantly, TONSL overexpression resulted in significant changes in the expression of genes associated with DNA repair hubs, including upregulation of several genes in HR and Fanconi Anemia pathways. Consistent with the effects of TONSL on HR-associated genes, TONSL overexpressing primary cells exhibited upregulated DNA repair via HR. Moreover, TONSL is an essential gene for growth of TONSL-amplified breast cancer cell lines in vivo. Breast cancer cell lines with TONSL/chr8q24.3 amplification were sensitive to TONSL-FACT complex inhibitor CBL0137, both in vitro and in vivo. To our knowledge, TONSL is the only gene other than telomerase with immortalizing function and represents a new therapeutic target for breast cancer with chr8q24.3 amplification.

*Cell and molecular biology of breast cancer*

*Graduate Student*

# POSTER #12

## HUNK REGULATION OF IL-4 PROMOTES POLARIZATION OF TUMOR ASSOCIATED MACROPHAGES IN TRIPLE NEGATIVE BREAST CANCER

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Triple-negative breast cancer (TNBC) is a type of breast cancer that does not express hormone receptors (estrogen receptor (ER) or progesterone receptor (PR)) or human epidermal growth factor receptor 2 (HER2). Therefore, contrary to other types of breast cancer, TNBC is not sensitive to endocrine therapy or HER2-targeted inhibitors. Some new FDA approved treatments for TNBC include immunotherapy targets such as immune checkpoint inhibitors (ICI). Unfortunately, inadequate anti-tumor T-cell effector function and high abundances of tumor-associated macrophages (TAMs) have limited the efficacy of ICI therapy. TAMs constituted one of the most abundant immune cell populations in mammary tumors. Within the tumor, TAMs can be polarized to classically activated M1-like and alternatively active M2-like phenotypes. M2-like macrophages contribute to cancer progression by inducing tumor angiogenic responses, promoting tumor growth and metastasis. In cancer, the polarization of macrophages toward an M2 phenotype is directed by cancer-cell-derived factors such as pleiotropic cytokines like interleukin-4 (IL-4). New knowledge suggests that IL-4 expression in cancer cells is regulated by the signal transducer and activator of the transcription 3 (STAT3) transcription factor. However, it is still undescribed what signaling pathways are responsible for mediating IL-4 in breast cancer cells. Intriguingly, we observed that Hormonally Up-Regulated Neu-Associated Kinase (HUNK), is responsible for IL-4 production in the 4T1 mammary tumor cell line, which is considered a metastatic TNBC model. We engineered 4T1 cells expressing HUNK and HUNK knockdown by shRNA. Our current data shows that 4T1 cells expressing HUNK, have elevated levels of STAT3 phosphorylation, IL-4 production, and secretion compared to 4T1 cells where HUNK has been downregulated by shRNA. Furthermore, the loss of IL-4 secretion in 4T1 HUNK knockdown cells corresponds to a reduced ability of conditioned medium from HUNK knockdown cells to induce alternative activation of macrophages. We also observed that HUNK has a significant effect on M2-like TAM's presence in the tumor microenvironment, where tumors derived from HUNK knockdown 4T1 cells have reduced M2-like TAMs compared to control tumors. Therefore, our results proposed the identification of a HUNK signaling pathway that is responsible for pro-metastatic TAM function in TNBC. Our study will evaluate HUNK as a therapeutic target for TNBC metastasis by modulating the TAM population within the tumor microenvironment.

*Cell and molecular biology of breast cancer*

*Graduate Student*

# POSTER #13

## THERMAL PROTEOME PROFILING OF IB-DNQ AND RUCAPARIB TREATED TRIPLE NEGATIVE BREAST CANCER CELLS REVEALS THERMAL STABILITY CHANGES IN CELL CYCLE KINASES AND SWI/SNF SUBCOMPLEXES

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Triple negative breast cancer (TNBC) accounts for 15-20% of all breast cancer cases, and patients have a higher risk of relapse and decreased survival. TNBC lacks the estrogen, progesterone, and epidermal growth factor 2 receptors; consequently, commonly used hormonal therapies are ineffective. One potential TNBC-specific target is NAD(P)H:quinone oxidoreductase 1 (NQO1), which is often highly expressed in TNBC tissue. The NQO1 bio-activatable drug isobutyl-deoxyxyboquinone (IB-DNQ) acts as a futile cycling substrate for NQO1 that leads to an accumulation of reactive oxygen species and subsequent DNA damage. Upon this damage, PARP1 is hyperactivated to initiate DNA repair mechanisms. PARP1 inhibition (via Rucaparib) combined with IB-DNQ should increase DNA damage to synergistically induce cell death specifically in cells highly expressing NQO1.

To further understand the mechanism of Rucaparib and IB-DNQ-induced cell death, MDA-MB-231 TNBC cells expressing endogenous NQO1 or the rapidly degraded NQO1\*2 variant were treated with lethal and sublethal doses of IB-DNQ, Rucaparib, or a combination treatment. Samples were analyzed using global proteomics, phosphoproteomics, and thermal proteome profiling (TPP). Kinase substrate enrichment analysis of phosphoproteomics data identified changes in kinase activity. Following TPP, the R-based analysis workflow Inflect-SSP generated melt curves using the protein abundance values from each temperature point and calculated z-scores to assign p-values to shifts in melt temperature.

We hypothesized that in the presence of NQO1, IB-DNQ and Rucaparib treatment would result in changes in phosphorylation and protein thermal stability, illuminating how the drugs alter the TNBC proteome. IB-DNQ and Rucaparib combination treatment of TNBC cells triggered large changes in the phosphoproteome while low dose IB-DNQ treatment alone did not induce extensive changes in phosphorylation networks. Histone H2AX was among the several proteins identified to have increased phosphorylation when cells were treated with the combination of IB-DNQ and Rucaparib. This finding validates that the drugs induced persistent DNA damage as phosphorylated H2AX at Serine 139 is a marker of DNA damage. We also observed a decreased melt temperature for H2AX following combination treatment suggesting that increased phosphorylation of H2AX may destabilize the protein. Thermal proteome profiling identified significant protein stabilization within the cell cycle kinase network when cells were treated with the sublethal dose of IB-DNQ. Together, these results indicate that IB-DNQ and Rucaparib impact cell cycle progression. Thermal proximity co-aggregation analysis of TPP data revealed a change in stability of SWI/SNF subcomplexes following combination treatment suggesting changes in chromatin remodeling. Our findings illustrate that paired TPP-phosphoproteomics datasets reveal complementary insights into cell signaling following drug treatment.

# POSTER #14

## 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.

*Cell and molecular biology of breast cancer*

*Graduate Student*

# POSTER #15

## 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 prolonged tumor relapse and metastatic disease progression. Our understanding of the factors that contribute to tumor dormancy and metastatic outgrowth remains 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 of developing metastasis at 10 years follow-up compared to normal-weight women. Therefore, we sought to investigate 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 an 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 become obese over a 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 were 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 fibroblast growth factor 2 (FGF2). Using dormancy-inducing 3D culture and *in vivo* approaches, we demonstrate that serum from obese 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 us 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.

*Cell and molecular biology of breast cancer*

*Post-Doctoral/Medical Fellow*

# POSTER #16

## THE ADAPTOR PROTEIN AMOT MEDIATES IL-6 SIGNALING IN BREAST CANCER

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The adaptor protein Amot coordinates the regulation of proteins that control cell growth and polarity. This underlies observations that overexpression of Amot in breast cancers correlates with an increased likelihood of metastasis and reduced survival. Our lab previously found that overexpression of Amot promotes the proliferation of both hormone receptor-positive and triple-negative breast cancer cells. These effects were linked to the dysregulation of cell polarity and HIPPO signaling via increased activity of the transcriptional co-activator YAP1. To date, no studies have described how Amot expression is induced in breast cancers. Therefore, it is critical to elucidate the mechanisms that control the expression

of Amot in breast cancer. In this study, we build upon preliminary data showing that the pro-inflammatory cytokine IL-6 induces Amot expression in breast cancer cells. Because Amot activates YAP1, which in turn, stimulates IL-6 expression, we hypothesize that Amot induction can result in a positively reinforcing feedback loop for IL-6 expression and secretion. We validated Amot overexpression and silencing in human MCF7 and MDA-MB-468 breast cancer cells after transduction with lentivirus containing recombinant Amot or shRNA targeting Amot. Using the ELISA technique, we found that Amot overexpression in these breast cancer cells resulted in significantly increased IL-6 secretion. We are currently in the process of isolating protein from these cells to measure changes in protein levels using Immunoblot methods. Additionally, we will isolate mRNA to measure changes in IL-6 transcript levels using RT-PCR. If successful, this project will demonstrate that Amot expression is sufficient and required for the endogenous stimulation of IL-6 expression and secretion in breast cancer cells. These results will support the overall hypothesis that Amot is an essential element whereby breast cancer cells gain autonomous IL-6 secretion after exposure to IL-6 from tumor-infiltrating inflammatory cells. This could potentially explain a mechanism through which hormone receptor-positive breast tumors transition to highly malignant hormone receptor-negative tumors that are often associated with tumor recurrence following anti-estrogen treatment.

*Cell and molecular biology of breast cancer*

*Post-baccalaureate Fellow*



# POSTER #17

## 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.

# POSTER #18

## PROTEOLYTIC CLEAVAGE OF ZNF217 GENERATES TRUNCATED PROTEINS THAT PROMOTE BREAST CANCER

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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. Due to the high expression and heterogeneous localization of ZNF217 in some human breast tumors, both ZNF217 expression levels and localization may be critical determinants of ZNF217's function. However, little is known about how ZNF217 is regulated as a protein to promote breast cancer. We discovered that breast tumors and cell lines express both full length and smaller ZNF217 proteins generated by a protease-dependent mechanism. Although the smaller ZNF217 proteins are even more prominent than full length ZNF217 in both human and mouse breast tumors and cell lines, the importance of these smaller forms remain unknown. To investigate the function of smaller ZNF217 proteins, we generated a panel of truncation mutants of ZNF217 and overexpressed them in human breast cancer cells. Interestingly, removal of the N-terminus increased the cytoplasmic ZNF217 and significantly increased primary and metastatic tumor burdens *in vivo*. We used recombinant proteins to determine if Calpain 1 or Calpain 2 is sufficient to cleave ZNF217 protein. Our results show, that Calpain 2 but not Calpain 1 cleaves ZNF217 into its smaller fragment of 75kDa. These findings suggest that calpain 2-induced cleavage is a potential mechanism that contributes to how ZNF217 promotes oncogenic functions. Identification of the smaller ZNF217 oncoproteins as tumor promoting factors may be clinically valuable as a biomarker to influence personalized treatment strategies for patients with advanced metastatic breast cancer and high ZNF217 expression.

*Genetics of breast cancer*

*Graduate Student*

# POSTER #19

## SINGLE NUCLEI CHROMATIN ACCESSIBILITY AND TRANSCRIPTOMIC MAP OF BREAST TISSUES OF WOMEN OF DIVERSE GENETIC ANCESTRY

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Single nuclei analysis is allowing robust classification of cell types in an organ that helps to establish relationships between cell-type specific gene expression and chromatin accessibility status of gene regulatory regions. Using the institutional resource of breast tissues of healthy donors of various genetic ancestry, we have developed a comprehensive chromatin accessibility (snATAC-seq) and gene expression (snRNA-seq) atlas of human breast tissues. Our analyses included 51,367 nuclei with average sequence coverage of 1195 genes per nuclei. These nuclei were derived from 22 donors of Ashkenazi descent, 20 of European non-Ashkenazi ancestry, 10 of Asian ancestry, 10 Hispanic/Latina, 10 Native American, 6 from BRCA1 mutation carriers, and 5 from BRCA2 mutation carriers. Although tissues from 20 women of African Ancestry were included in sequencing, poor quality sequencing reads prevented inclusion of data from those samples in the final analysis. Integrated analysis revealed 10 distinct cell types in the healthy breast, which included three major epithelial cell subtypes (mature luminal, luminal progenitor, basal), two endothelial subtypes, two adipocyte subtypes, fibroblasts, T-cells, and macrophages. Mature luminal cells could be further divided into two distinct hormone sensitive subtypes, HS $\alpha$  and HS $\beta$ , with HS $\alpha$  subtype expressing higher levels of Estrogen Receptor alpha (ESR1) and NEK10, a tyrosine kinase that controls p53 activity and limits cell proliferation. The luminal progenitors could be broadly classified into alveolar progenitors (AP) and basal-luminal hybrid progenitors (BL). AP cells expressed higher levels of ELF5, a known marker of alveolar cells, compared to BL cells. Basal cells could be classified into two subtypes with Basal-BA $\alpha$  cells being the most dominant and expressing higher levels of TP63 and NFIB compared to Basal-BA $\beta$  cells. ESR1 expression pattern was distinctly different in tissues from Native Americans compared to the rest, with a high level of ESR1 expression extending to AP cells. In fact, overall AP cell numbers were ~3-fold higher in Native Americans compared to others (18.9% versus 1.5-8.8%). Furthermore, Ingenuity pathway analysis of differentially expressed genes revealed elevated Estrogen Receptor signaling in ML and AP cell types of Native Americans compared to those of other genetic ancestry. Despite significant differences in expression and activity in Native Americans compared to others, the chromatin accessibility map of the ESR1 gene regulatory regions in ML and LP cells did not show any genetic ancestry dependent variability. In general, cell subtype-specific gene expression did not correlate with chromatin accessibility differences, suggesting that transcriptional regulation independent of chromatin accessibility governs cell type-specific gene expression in the breast. Collectively, these results reveal complexities in gene expression in different cell types of the breast.

*Genetics of breast cancer*

*Research Associate*

# POSTER #20

## ENHANCED REACTIVE OXYGEN SPECIES PRODUCTION AND CELL DEATH IN TRIPLE-NEGATIVE BREAST CANCER CELLS TREATED WITH ELECTRIC PULSES AND RESVERATROL

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**Aim:** Triple negative breast cancer (TNBC) is difficult to treat since it lacks all the three most commonly targeted hormone receptors. Patients afflicted with TNBC are treated with platinum core chemotherapeutics, such as cisplatin. Despite the initial effective anticancer effects of cisplatin, TNBC attenuates its effect and develops resistance eventually, which results in tumor reoccurrence. Hence, there is a critical demand for effective, alternative, and natural ways to treat TNBC. Towards this, a promising technique for inhibiting TNBC cell proliferation involves promoting the production of reactive oxygen species (ROS), which triggers pro-apoptotic caspases 9 and 3. Resveratrol (RESV), an active bio compound found in naturally available fruits, such as grapes, is utilized in this research for that. In addition, electrochemotherapy (ECT), which involves the application of electrical pulses (EP), was utilized to enhance the uptake of RESV.

**Methods:** MDA-MB-231, human TNBC cells were treated with/out RESV, and eight 600–1,000 V/cm, 100  $\mu$ s pulses at 1 Hz. The cells were characterized by using various assays, including viability assay, and ROS assay.

**Results:** A TNBC cell viability of as low as 20% was obtained at 24 h (it was 13% at 60 h), demonstrating the potential of this novel treatment. ROS production was the highest in the combination of EP at 1,000 V/cm along with RESV at 100  $\mu$ mol/L.

**Conclusions:** Results indicate that RESV has the potential as an anti-TNBC agent and that EP + RESV can significantly enhance the cell death to reduce MDA-MB-231 cell viability by increasing ROS production and triggering apoptosis.

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

*Graduate Student*

# POSTER #21

## COMPUTATIONAL MECHANOBIOLOGY MODEL TO ASSIST WITH PREDICTION OF HEALING AND COSMETIC OUTCOMES FOLLOWING BREAST-CONSERVING SURGERY

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### Introduction:

The creation of surgical computational models is a relatively new field aimed in predicting the patient-specific response to a procedure. For breast-conserving surgery (BCS; otherwise known as lumpectomy), patient-to-patient variation in breast and tumor characteristics makes it difficult for surgeons to predict postoperative healing, oncologic, and cosmetic outcomes. This creates a need for a tool that assists surgeons and patients in creating a comprehensive individualized treatment plan. The majority of previously developed computational breast healing models emphasize only biochemical rather than integrated biochemical and biomechanical processes. Therefore, with the goal of improving model accuracy and utility, we are developing a predictive computational mechanobiology model that simulates breast healing following BCS using coupled biochemical-biomechanical parameters focused on multi-scale mechanics, including cell contractility and collagen remodeling.

### Methods:

The computational model was adapted from previous work evaluating three-dimensional (3D) cutaneous wound healing. Model parameters were modified to be breast tissue specific while implementing a unique generalized breast geometry derived from clinical data averages. Time-dependent changes in fibroblast and collagen densities were informed through an experimental porcine lumpectomy study by applying image analysis techniques to histological cross-sections obtained 1, 4, and 16 weeks after surgery. To optimize model biochemical parameters, Gaussian Process (GP) was fitted to experimental fibroblast and collagen data. The GP is a machine learning method that incorporates past collected data, allowing for the prediction of non-tested parameter sets. The same methodology was also performed to inform mechanobiological parameters using human clinical post-surgical cavity contraction data.

### Results:

The fitting using the biochemical GP allowed the fibroblast and collagen density profiles to be within standard deviations of experimental data, which provided confidence in the robust selection of biochemical parameters. Using the mechanobiology GP, optimal mechanobiological parameters were then identified by fitting human clinical contraction data. Along with this fit, mechanistic parameter relationships were inferred, indicating that cavity contraction was largely dependent on fibroblast and myofibroblast contractile forces, with an interesting coupling identified between myofibroblast force and collagen force saturation parameters. The fully informed human mechanobiology model yielded a contraction profile that paralleled human clinical outcomes, with substantial cavity contraction (36% of original volume) over the first 4 weeks before reaching a steady-state and a visible divot formed on the breast surface by week 16.

## **Conclusion:**

Through fitting preclinical porcine data and human clinical data, the computational model proved to effectively simulate the breast healing response following BCS, including fibroblast infiltration, collagen remodeling, and breast permanent deformation. Compared to previous BCS wound healing models, we implemented a more detailed mechanobiological model coupled with the nonlinear mechanics of breast tissue. In the future, this model will be applied to patient-specific geometries and design of therapeutic approaches (e.g., regenerative breast fillers).

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

*Graduate Student*

# POSTER #22

## LABEL-FREE TRACKING OF MDA-MB-231 CELL PROLIFERATION USING IMAGE SEGMENTATION-BASED MACHINE LEARNING TECHNIQUE

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Machine learning, a subset of artificial intelligence, is a versatile tool to analyze images. In this work, we deployed an image segmentation-based machine learning (ML) technique to identify the growth stage of MDA-MB-231, human Triple Negative Breast Cancer (TNBC) cells. This method is a label-free method of tracking cell proliferation without chemicals or biological assay. A specific chemical assay for cell growth and viability takes years of development and is very expensive too. Moreover, available assays will chemically interfere with the intercellular mechanism of the cells. In contrast, we use a non-invasive technique by acquiring cell images at a particular interval under a conducive growth environment. The image acquisition does not require any chemicals to interact with cells. The raw images are pre-processed using the most updated convolutional neural network (CNN) based image-segmentation model before feeding into the ML. CNN was chosen because it automatically detects significant cell features and classifies the growth stage without any manual supervision. We chose the model to learn by using around 80% of the data and testing on the rest of the randomly selected 20%. The generalizability of the learning model enhances due to data augmentation and by the random 80-20% split between the training and test set. The images are acquired every hour, and the classification has 16 classes from  $t=0h$  to  $t=15h$ . The model automatically detects the growth stage of TNBC cells. We obtained an accuracy of 92%, which is considered to be very good. We found that using an 8-class dataset has higher accuracy than the 16-class dataset. The learning models are scalable and can be used for other types of cells after completing appropriate pre-training. Further, it opens a more comprehensive array of research opportunities, which can help understand morphological changes.

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

*Graduate Student*

# POSTER #23

## BREAST CANCER IMAGING USING NONINVASIVE DIFFUSE OPTICAL SPECTROSCOPY

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Almost 300,000 new cases of invasive breast cancer are expected to be diagnosed in the United States this year. Neoadjuvant chemotherapy (NAC) is an increasingly used pre-surgical systemic treatment that serves to decrease the size of the tumor before lumpectomy or mastectomy. The tumor's response during NAC can also be monitored by oncologists, for example to detect tumor sensitivity to specific chemotherapy drugs and inform future drug use, or to identify those among the 10-35% of patients that are insensitive to NAC. Thus, by monitoring the tumor response during NAC, the treatment can be modified accordingly to increase its efficacy. Seeking to assess the response to NAC noninvasively and more accurately than with palpation, various established and emerging imaging modalities have been investigated, including ultrasound, PET, MRI, and diffuse optical spectroscopy (DOS). Of these modalities, DOS is advantageous because it is a safe, compact, relatively low-cost functional technique that can be performed frequently at the bedside or point of care and does not require contrast injection or exposure to ionizing radiation. It also offers excellent contrast in dense breast tissue (BIRADS C/D) and in young women (unlike mammography). Based upon the absorption and scattering of near-infrared light in tissue, DOS provides a macroscopic quantitative measure of the tissue's molecular components, such as oxy- and deoxyhemoglobin, water, and lipid, which are directly related to breast tissue metabolism and vascular characteristics. It is thus applicable to risk assessment, screening, differential diagnosis (offering >90% specificity and sensitivity to differentiate benign and malignant tumors) and predicting chemotherapy response.

Previously, we showed our group's contributions to the first multi-center, prospective clinical study of DOS technology sponsored by an independent oncology research group (ECOG-ACRIN 6691). The study demonstrated the promising usefulness of DOS to predict NAC response. In this presentation, we discuss the development and technical validation of a novel handheld DOS device for tissue imaging that will be the first clinically compatible device of its kind. As the scan is performed with its portable, handheld probe, the data and images quantifying breast composition and metabolism are generated in real-time and immediately displayed on a mobile device, such as a smartphone or a tablet. The handheld DOS imager has the highest 2D spatial resolution for this modality reported to date. In the near future, prospective, observational clinical studies will compare its performance against the gold-standard DOS system, assess its performance to predict the pathological complete response of breast cancer tumors during NAC, as well as evaluate its ability to distinguish benign and malignant breast lesions.

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



# POSTER #24

## BENEFICIAL EFFECTS OF LOW-INTENSITY VIBRATIONS ON THE MSC-DERIVED PROTEOMES AND THE DEVELOPMENT OF ANTI-TUMOR PEPTIDES

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Advanced breast cancer metastasizes to many organs including the lung, liver, brain, and bone, and it is difficult to treat. As a protein-based novel therapeutic option, we previously developed induced tumor-suppressing cells (iTSCs) and evaluated the possibility of using their proteomes as anti-tumor agents. iTSCs were generated by activating oncogenic signaling in various tumor and non-tumor cells. As mechanical signals have beneficial effects in various cell types, we examined the effects of low-intensity vibration (LIV) on the tumor-suppressive capabilities of mesenchymal stem cell (MSC)-derived iTSCs. We also evaluated the anti-tumor efficacy of three peptides that were enriched in the trypsin-digested secretomes of iTSCs. LIV was applied using a custom-made vibration table, and a group of anti-tumor peptides was predicted by global mass spectrometry-based proteomics. The anti-tumor capabilities were evaluated using breast cancer cell lines as well as other cancer cell lines via *in vitro* assays. The results revealed that delivering 2 twenty-minute bouts of low-magnitude vibration (0.3 gravity, 90 Hz) separated by 3 hours elevated the anti-tumor effects of the MSC-derived conditioned medium. Furthermore, the three peptides (P02, P04, and P05) presented significant anti-tumor actions and suppressed proliferation and migration of breast cancer cells. P04 and P05 are derived from aldolase A (ALDOA), one of the glycolytic enzymes linked to the Warburg effect. Collectively, the present study demonstrated that LIV is effectively enhances the tumor-suppressive capabilities of MSC iTSCs and ALDOA-derived peptides presented the anti-tumor actions. These results are the first steps towards developing a peptide-based therapy for breast cancer.

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

*Graduate Student*

# POSTER #25

## **A Human Skeletal Muscle Stem-Myotube Model Reveals Multiple Signaling Targets Of Cancer Secretome In Skeletal Muscle**

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Skeletal muscle dysfunction due to the effects of cancer secretome 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 mouse C2C12 mouse myoblast cell line for in vitro studies. Because of 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 in hMuSCs 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.

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