55th Annual Midwest Student Biomedical Research Forum

Saturday, March 2, 2024

ROOM 3047

1:45 p.m. **O-01** NADPH OXIDASE 4 (NOX4) KNOCKOUT REDUCES MIGRATION OF PROSTATE CANCER CELLS
Presenter: Oluwaseun Adebisi, UNMC

2:00 p.m. **O-08** TARGETING MAP3K1 IN PANCREATIC CANCER
Presenter: Lidia Boghean, UNMC

2:15 p.m. **O-10** TUMOR INITIATION IN LUNG CANCER
Presenter: Deepan Chatterjee, Creighton University

2:30 p.m. **O-23** ESTABLISHING THE INTERACTION MECHANISM OF MUC1 AND ALIX IN PANCREATIC CANCER
Presenter: Kristine Hoagstrom, UNMC

2:45 p.m. **O-31** MUCIN 5AC MODULATES CANCER-ASSOCIATED FIBROBLAST HETEROGENEITY THROUGH EPIGENETIC REGULATION
Presenter: Rachel Kehrberg, UNMC

3:00 p.m. **O-32** STEMNESS MACHINERY IN PANCREATIC CANCER: A NOVEL ROLE OF GLYPICAN 6
Presenter: Nivedeta Krishna Kumar, UNMC

3:15 p.m. **O-34** RADIATION TO ADIPOCYTES ALTERS SECRETOME INCREASING PROSTATE CANCER MIGRATION
Presenter: Kia Liermann-Wooldrik, UNMC

3:30 p.m. **O-38** TARGETING THE ONCO-GOLGI IN PROSTATE CANCER PROGRESSION VIA AUTOPHAGY AND ER STRESS INHIBITION
Presenter: Amanda Macke, UNMC

3:45 p.m. **O-51** ELUCIDATING THE ROLE OF *CBFB-MYH11* IN MAINTENANCE OF Inv(16) AML
Presenter: Sipra Panda, UNMC

4:00 p.m. Break

NADPH OXIDASE 4 (NOX4) KNOCKOUT REDUCES MIGRATION OF PROSTATE CANCER CELLS

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Background

Prostate cancer has an incidence rate of approximately 12.5% and a recurrence rate of 30% after five years of initial therapy. Metastatic prostate cancer is highly lethal and there is currently no cure. The standard treatment for prostate cancer patients includes radiation therapy. However, radiation therapy can have an adverse impact on the surrounding tissues, including fibroblasts in the extracellular matrix (ECM), which could potentially promote tumor recurrence and metastasis. Production of reactive oxygen species (ROS) is increased during radiation therapy, both in the tumor and its adjacent tissues. This upregulation may be due to electron leakage from the mitochondria or the activity of NADPH oxidases. NOX4 is a member of the NADPH oxidase family that produces hydrogen peroxide (H_2O_2). Notably, the expression of NOX4 is also found to be upregulated in prostate tumor samples as well as its adjacent tissues. This highly oxidative environment is believed to play a crucial role in prostate cancer progression and metastasis.

Significance

Radiotherapy increases the amount of ROS generated in the tumor microenvironment which could promote prostate cancer metastasis. Developing therapies that reduce reactive oxygen species in the tumor ECM could prevent tumor spread.

Hypothesis

We hypothesized that knocking out NOX4 (NOX4 KO) reduces metastasis in radiated prostate tumor-bearing mice by decreasing ROS and its associated signaling.

Experimental design

Our study involved culturing mouse primary fibroblasts (MPF) from WT or NOX4 KO mice with RM1 cells *in vitro.* We first seeded the MPFs on a six-well plate, inserted a transwell, and then seeded the RM1 cells on the transwell. After 24 hours, we radiated the cells with 3 Gy and estimated the number of migratory cells after 48 hours. We also performed a scratch assay by mixing RM1 cancer cells with WT and NOX-4KO MPFs (1:10). The cells were radiated, and a scratch was made after 24 hours. Images of the cell migration were taken at different time points after the scratch (0 hours, 6 hours, and 24 hours). For our *in vivo* study, we implanted RM1 cells into C57BL/6 mice, exposed the tumor to radiation (8 Gy for 3 days) and waited for 3 weeks before harvesting the tumors. Tumor weight, tumor size, and metastasis were used as endpoints for the *in vivo* study. We also performed immunofluorescence staining on tumor samples harvested from irradiated RM1 implanted mice and assessed vimentin and E-cadherin expression in the tumor microenvironment.

Results

We found that NOX4 KO reduces the number of migratory radiated RM1 cells when co-cultured with MPFs ($p < 0.0001$). We also observed that NOX4 KO MPFs had a lower percentage of wound closure when compared to wild type ($p = 0.0128$). We observed a significant metastasis decrease in irradiated RM1 implanted mice when compared to radiated control ($p<0.001$). Immunofluorescence staining of prostatic tissue harvested from irradiated RM1 implanted mice showed a decreased vimentin expression compared to wild type ($p=0.019$).

Conclusion

Our studies demonstrate a reduction in prostate cancer metastasis in a NOX4 KO tumor microenvironment. We have previously shown that NOX4 KO reduces the expression of p-SMAD2/3 downstream of the TGF-beta signaling pathway. Our future studies will be focused on understanding the interplay of NOX4 KO and EMT via the TGF beta signaling pathway.

TARGETING MAP3K1 IN PANCREATIC CANCER

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MAP3K1, a mitogen-activated protein kinase, is involved in various cancer signaling networks including the NFκB, JNK, ERK, and p38 pathways. Functioning as a signaling kinase in these oncogenic pathways, MAP3K1 contributes to tumor growth and metastasis, and high MAP3K1 expression at the transcript level in pancreatic cancer patient tumors is associated with poorer (50% vs. 15%) 5-year survival. NF-κB kinase subunit-β (IKKβ), an important phosphorylation target of MAP3K1, was shown to be important in pancreatic cancer disease onset and progression. Direct pharmacological inhibition of IKKβ through ATP-competitive small molecule inhibitors has previously proven to be unsuccessful. Targeting an upstream kinase is a viable method of limiting IKKβ activity, thus making MAP3K1 an attractive therapeutic target. We previously reported the discovery of IKKβ Activity Modulator 1 (IKAM-1), an ATP-competitive MAP3K1 inhibitor. Structure-guided design using AlphaFold MAP3K1 and Schrödinger GLIDE led to the development of 51-106, an ATP-competitive selective MAP3K1 small molecule inhibitor. 51-106 blocked TNFα-induced MAP3K1-IKKβ-mediated NFκB activity, inhibited pancreatic cancer cell growth and migration, and induced a dose-dependent S-phase arrest. Quantitative mass spectrometry analysis using tandem mass tag (TMT) labeling of phosphopeptides from PANC-1 cells treated with 51-106 identified Nucleophosmin 1 (NPM1) as a novel member of the MAP3K1 signaling pathway as 51-106 treatment decreased NPM1 T199 phosphorylation in a dose-dependent manner. In combination studies, 51-106 synergistically inhibited growth with gemcitabine in LSL-KrasG12D/+, LSL-Trp53R172H/+, Pdx1-Cre (KPC) cell lines *in vitro* and in a KPC syngeneic subcutaneous implantation mouse model of pancreatic cancer *in vivo*. Using 51-106, a MAP3K1 ATP-competitive small molecule inhibitor, our study is the first to show a putative relationship between MAP3K1 and NPM1 and our *in vitro* and *in vivo* results warrant further investigation into the inhibition of MAP3K1 as a therapeutic option in cancer.

TUMOR INITIATION IN LUNG CANCER

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Background: Lung cancer is the leading cause of cancer-related deaths worldwide. Non-small cell lung cancer (NSCLC) is the most common form, representing about 85% of all lung cancer cases, with the remaining 15% of cases being small-cell lung cancer (SCLC) (Vyse *et al.*, *Signal Transduct Target Ther* 2020). Almost all SCLC tumours are genetically defined by inactivating mutations in tumor suppressors TP53 and Rb1 (Gazdar *et al.* 2017; George *et al.* 2015). SCLC arises from pulmonary neuroendocrine cells (PNECs) or lung epithelia that convert to the neuroendocrine phenotype (Yang *et al.* 2018; Sutherland *et al*. 2011). SCLC can be initiated from tumor-propagating cells (TPCs), which can self-renew and differentiate into the bulk tumor population and are highly tumorigenic in transplantation assays. SCLC tumours can be divided into subtypes, including SCLC-A and SCLC-N, based on whether they express the transcription factor ASCL1 or NeuroD1, respectively. Previous studies have shown, as tumours initially form, they express the ASCL1 transcription factor, but as they become more aggressive and metastatic, they undergo a transcriptomic shift to express the NeuroD1 transcription factor. (Ireland *et al.*, *Cancer Cell* 2020). In contrast to NSCLC, SCLC tumours have wild type-RAS. Downstream of RAS, KSR1 regulates efficient signaling through the RAF/MEK/ERK kinase cascade. The RAF/MEK/ERK pathway is a critical regulator of lung cancer stem cell (CSC) proliferation (McCubrey *et al.*, *Biochim Biophys Acta* 2009). CSCs are the major contributor to therapy resistance in tumours (Hanh Phi *et al.*, *Stem Cells Int.* 2018). The selective targeting of CSCs and their contribution to intrinsic and acquired resistance necessitate novel therapeutic approaches in SCLCs.

Significance of problem: Small-cell lung cancer (SCLC) contributes to 15% of all lung cancers almost all of whom are smokers. The prevalence of therapeutic resistance to SCLC led to its classification as a recalcitrant cancer in 2012 (Gazdar *et al.*, *Nat Rev Cancer*2017). The RAF/MEK/ERK pathway is a critical regulator of lung CSC proliferation (McCubrey *et al.*, *Biochim Biophys Acta* 2009). Consequently, effective broad targeting of the RAS pathway has the potential to block tumor regrowth by limiting CSC survival (Fu *et al.*, *Clin Cancer Res* 2019). The possibility that targeting KSR1 might yield a high therapeutic index led us to test the **hypothesis** that KSR1 is necessary for tumor initiation and chemotherapeutic resistance in the more aggressive, metastatic subtype of SCLC, SCLC-N. Improved mechanistic understanding of KSR1's role in self-renewal and clonogenicity may yield a therapeutic approach for targeting CSCs, with potential to improve survival in these cancers.

Experimental Design: Using CRISPR/Cas9, we targeted KSR1 (KSR1 KO) in three non-KRAS- mutated Neuro-D1 specific SCLC cell lines (H82, H524 and HCC33). To confirm the effect of KSR1 on CSC frequency *in vitro* single-cell colony forming assays and *in vitro* Extreme Limiting Dilution Analysis (ELDA) were performed for control and KSR1 knockout cells. To identify the subset of cells responsible for tumor initiation and uncover genes that characterize CSCs, we performed single-cell RNA sequencing in the SCLC cell line, H82 ± KSR1. To understand how these stem cells contribute to therapy resistance, we grew these cells in the presence of cisplatin, a chemotherapy drug routinely used for the treatment of SCLC. For dose-response curves, SCLC-N cell lines ± KSR1 KO were treated with different doses of cisplatin, followed by multi-well drug resistance assays. For the latter, 2000-3000 cells were plated per well in a 96 well plate and the appropriate concentration of the drug is added to each well. Each well was scored weekly over a period of 10 weeks and the data rendered as a Kaplan-Meyer plot. Wells were scored as resistant if they were >50% confluent.

Results: The colony forming assays revealed that KSR1 KO decreases clonogenicity, a characteristic of CSCs, of single cells cultures of H82, H524 and HCC33 cells. *In vitro* Extreme Limiting Dilution Analysis (ELDA) showed that *KSR1* KO decreased the frequency of CSCs 3-4-fold, suggesting that KSR1 is required for stem-like properties even in human SCLC cells. UMAP and RNA velocity analysis of single-cell RNA sequencing data revealed that KSR1 disruption dramatically altered H82 heterogeneity, suggested the identity of CSC-containing subclusters and identified cell surface makers enriched within specific subclusters which should make possible the enrichment, isolation and characterization of SCLC CSCs. When the SCLC-N cell lines ± KSR1 were treated with different doses of cisplatin, KSR1 KO modestly decreased the ED50 of cisplatin treated H82, HCC33 and HCC33 cells. However, KSR1 disruption dramatically enhances the ability of cisplatin to kill CSCs. *In-vitro* ELDA showed that, compared to control HCC33 cells, KSR1 KO increased the ability of four different doses of cisplatin to kill CSCs 3-fold. Furthermore, multi-well drug resistance assays revealed that KSR1 knockout almost completely prevents the onset of cisplatin resistance in both H82 and HCC33 cells for at least six weeks.

Conclusions and future directions: These data show that KSR1 is required for viability of the clonogenic and self-renewing tumor-initiating population essential for SCLC formation and that disruption of KSR1 may enhance the efficacy of first line chemotherapeutics used to treat SCLC with specific lethality directed towards the tumor-initiating subpopulation within SCLC. KSR1 knockout sensitizes SCLC-N and decreases TIC frequency in response to cisplatin treatment. These data reveal KSR1 as an important regulator of tumor initiation and therapy resistance in SCLC-N CSCs. Further studies will focus on isolating CSC from the NeuroD1 subtype of SCLCs along with identifying the KSR1 dependent mechanisms that underly their formation and confer therapy resistance.

ESTABLISHING THE INTERACTION MECHANISM OF MUC1 AND ALIX IN PANCREATIC CANCER

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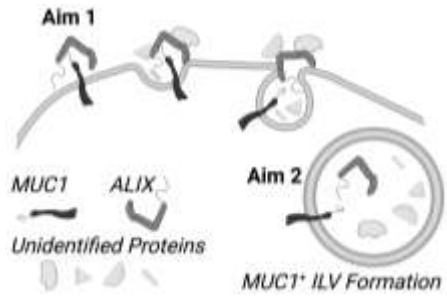
Background: Insufficient understanding of pancreatic ductal adenocarcinoma (PDAC) progression contributes to the lack of early detection and continued rise in PDAC-related deaths. Small extracellular vesicles (sEVs) play a key role in this progression process from early stage to metastasis. PDAC-derived sEVs show a selective enrichment of the oncogenic transmembrane protein Mucin1 (MUC1), and our preliminary MUC1 knockout studies demonstrate that it impacts sEV cargo, suggesting that MUC1 potentially regulates sEV formation. The cellular location where sEVs form and the proteins involved in their formation influence cargo packaging, resulting in tumor-derived EVs (TEVs) comprised of different cargo than non-cancerous sEVs. A cargo sorting mechanism alternate to the canonical ESCRT-dependent pathway involves the cytoplasmic adaptor protein ALG-2-Interacting Protein X (ALIX), which plays a prominent role in regulating TEV formation. Our preliminary ALIX knockdown studies revealed that loss of ALIX resulted in decreased MUC1 presence in sEVs. *We propose that MUC1 is sorted into sEVs using this alternative ALIX-dependent mechanism.*

Significance: A distinct sEV biogenesis pathway used by cancer cells has not been defined. These studies will provide evidence of a novel interaction between the MUC1 cytoplasmic tail (MUC1.CT) and ALIX and its potential role in sEV formation in PDAC. Defining this critical step in sEV cargo sorting will advance our understanding of PDAC progression, provide insight into the potential sEV sorting mechanism of MUC1 and other cancer biomarkers, and drive the development of a targetable early-detection pathway. We **hypothesize** that the interaction between MUC1.CT and ALIX alters ALIX intracellular trafficking through a conserved binding motif that targets ALIX to the endosomal membrane and contributes to the formation of MUC1$^+$ sEVs.

Experimental Design: To define the MUC1.CT-ALIX interaction mechanism, we will identify residues involved in the interaction and visualize the interaction's structural components using canonical, structural-biochemical techniques. To determine the location where MUC1 and ALIX interact, we will conduct fluorescence resonance energy transfer (FRET) with subcellular localization. To decipher alterations in sEV cargo and morphology dependent on this interaction, we will inhibit binding between MUC1 and ALIX and compare the isolated sEVs.



Schematic 1. Illustrative representation of the specific aims and central hypothesis. The role of the MUC1.CT-ALIX interaction in MUC1$^+$ sEV formation. **Aim 1**: Define the mechanism of interaction between MUC1.CT and ALIX to highlight conserved binding interactions between ALIX and sEV-sorted proteins. **Aim 2**: Decipher the impact of the MUC1.CT-ALIX interaction on sEV cargo protein sorting.

Results: Proteomics analysis recognized ALIX as an interacting partner of MUC1.CT. Further analysis identified a novel MUC1.CT-ALIX interaction. Immunofluorescence microscopy (IFM) of ALIX colocalized with several organelle markers showed that MUC1 altered ALIX cellular distribution and revealed a potential role of MUC1 in recruiting ALIX to selected membranes to instigate sEV biogenesis. *In silico* modeling revealed the possibility of a conserved binding mechanism between MUC1.CT and ALIX.

Conclusions: Our current and proposed studies present a potentially novel mechanism for sorting protein content into sEVs following a conserved targeting mechanism by ALIX. Characterizing the mechanism by which ALIX-dependent sorting of MUC1$^+$ sEVs occurs will lay a foundation for identifying new cancer-specific markers for PDAC progression, instigating the development of targetable early detection in the novel pathway.

MUCIN 5AC MODULATES CANCER-ASSOCIATED FIBROBLAST HETEROGENEITY THROUGH EPIGENETIC REGULATION

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Background
Pancreatic cancer (PC) is a deadly malignancy characterized by the expression of mucins and a highly desmoplastic stroma comprising up to 90% of the tumor. Cancer-associated fibroblasts (CAFs), a significant component of this stroma, contribute to cancer pathobiology through the secretion of extracellular matrix (ECM), growth factors, metabolites, and cytokines. It is now well known that CAFs are a heterogeneous population with both tumor-promoting and tumor-restraining roles; however, the source of this heterogeneity remains poorly understood. In PC, CAFs are derived from local pancreatic stellate cells (PSCs) and distant adipose mesenchymal stem cells (AD-MSCs). Emerging evidence suggests CAF maturation is mediated through epigenetic mechanisms, including DNA methylation and histone acetylation. Our previous study using murine models demonstrated the critical role of mucin 5AC (Muc5ac) in developing a heterogeneous stroma. However, the molecular mechanisms involved in AD-MSC and PSC maturation into different subsets of CAFs remain elusive.

Hypothesis
Muc5ac is responsible for epigenetic modifications during CAF maturation into heterogenous, precursor-based subtypes.

Experimental Design
Primary AD-MSCs and PSCs were isolated from C57BL/6 mice and exposed to conditioned media (CM) from KPC-3266 or KPC-3266 Muc5ac KO cells or co-cultured with these cells. Cells were pretreated with a pharmacological inhibitor of CXCR2, 5ng/ml SCH527123. RNA was isolated, and qRT-PCR was performed for transcriptome analysis. Protein lysate was collected, and western blots were used to examine protein expression. IHC staining of the active transcription mark, histone 3 K27 acetylation (H3K27ac) was performed on *Kras^{G12D}, Pdx1-Cre* (KC) and *Kras^{G12D}; Pdx-1cre; Muc5ac$^{-/-}$* (KCM) tissue.

Results
Co-culture with Muc5ac expressing, but not Muc5ac KO, cancer cells or treatment with CM from these cells increased the expression of CAF subtype markers, including inflammatory CAF (iCAF) markers (*Il6*, *Cxcl1,* and *Cxcl2*) and antigen-presenting (apCAF) markers (*Cd74* and *H2-ab1)* in primary AD-MSCs. Muc5ac carries tumor cytokines trapped in its glycan tree, contributing to the microenrichment of C-X-C motif chemokine receptor 2 (CXCR2)-ligands at the cell surface. Pharmacological inhibition of CXCR2 decreased levels of CAF markers, suggesting that Muc5ac-trapped ligands induce AD-MSC CAF maturation via the CXCR2 signaling axis. Treatment of AD-MSCs and PSCs with CM derived from Muc5ac proficient, but not deficient, cancer cells induced Stat-3 phosphorylation. Mechanistic study indicated that AD-MSCs epigenetically mature into CAFs by Muc5ac mediated initial upregulation of DNA methyltransferase 3b (DNMT3b) and ten-eleven translocation 1 (TET1) dioxygenase followed by downregulation of DNMT1. Treatment of PSCs with CM derived from Muc5ac proficient, but not deficient, PC cells increased histone H3K27 acetylation marks, a common histone mark of active transcription, which increases during CAF maturation. Similarly, higher immunoreactivity to H3K27ac was seen in KC pancreatic tissues compared to KCM pancreatic tissues, both in the ductal and stromal compartments.

Conclusions
This study suggests the importance of Muc5ac in regulating CAF heterogeneity by modulating the biology of CAF precursor cells through epigenetic regulation. Further, it indicates that AD-MSCs and PSCs have distinct mechanisms of CAF maturation and unique CAF marker expression, demonstrating CAF heterogeneity based on cellular origins.

STEMNESS MACHINERY IN PANCREATIC CANCER: A NOVEL ROLE OF GLYPICAN 6

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Background and Significance: Cancer stem cells (CSCs) are a small sub-population of cells that have the ability to self-renew and differentiate into various cell types within a tumor. In pancreatic cancer (PC), CSCs are accountable for recurrence, therapy resistance, disease aggressiveness, and metastasis. The renewal of CSCs is a complex and multifaceted process that involves several intricate mechanisms. Despite significant progress, molecular mechanism for the pancreatic **CSC renewal is poorly understood**. Recent evidence suggests that normal embryonic and adult progenitor stem cells share common characteristics with CSCs. In this study, we aim to identify and investigate molecular mechanisms of novel stemness regulators that govern the self-renewal of CSCs in PC. Understanding the precise regulatory mechanisms controlling self-renewal and plasticity is crucial for targeting CSCs effectively.

Hypothesis: *Reactivation of pancreatic pluripotent stemness signatures in tumors could reveal cellular origin and contribute to aggressiveness and therapy resistance in pancreatic cancer.*

Experimental Design/Methods: In this study, we performed Bulk RNA sequencing to compare and analyze the transcriptome of induced pluripotent stem cells (iPSCs), pancreatic CSCs (PCSCs), pancreatic cancer cells (PCCs), and normal pancreatic cells (NPs). We identified gene signatures specific to the self-renewing pancreatic stem cells. We then analyzed the expression of these putative stemness candidates in different CSC-subtypes in PC using single cell RNA-seq dataset (GSE155698) available publicly online . We then utilized mouse Kras;p53;PdxCre (KPC), and human pancreatic tumor tissue samples for confocal immunofluorescence microscopy and immunohistochemistry to compare the top-hit expression in the stem versus parent/ non-stem counterpart. Pancreatic CSCs were grown under spheroid culture with stem cell conditions. Pancreatic CSCs were then used for further analysis (western blotting and PCR) of stemness candidates' expression with the parent/non-stem cells as controls.

Results/Data: Our *in-silico* analysis revealed a novel candidate, Glypican 6 (GPC6), a member of heparan sulfate proteoglycans family, to be upregulated in the stem populations. GPC6 has been identified as a key factor involved in maintaining the pluripotency of embryonic stem cells. It can modulate signaling pathways like Wnt and FGF (Fibroblast Growth Factor), which are crucial in determining the fate of multipotent stem cells—whether they continue to self-renew or differentiate into various cell lineages. Histological analysis revealed an increased expression of GPC6 in mouse and human pancreatic tumor tissues compared to normal pancreas tissue, which was also mirrored through co-immunofluorescence imaging with stemness markers. Aligning with our RNA-sequencing results, pancreatic spheroids / pancreatic CSCs showed a significantly higher protein expression of GPC6 compared to the non-stem cell counterpart *in vitro*. Interestingly, the stem population showed a specific increased N-terminal expression of GPC6 compared to the parent in the western blot. Using confocal immunofluorescence microscopy, we found that the PCSCs also showed an increased GPC6 expression compared to the parent.

Conclusions and Future Perspectives: Our results demonstrate that the CSCs sub-type population shows a unique gene signature: Glypican-6. GPC6 showed specific expression in pancreatic stem cells *in vitro.* Further mechanistic and in vivo studies on GPC6 and analysis of changes in stemness status is required to establish its role as a potential stem cell regulator of self-renewal in PC.

RADIATION TO ADIPOCYTES ALTERS SECRETOME INCREASING PROSTATE CANCER MIGRATION

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Background
Prostate cancer is one the most diagnosed cancers in the male population. While the overall 5-year survival rate is 97%, 20-30% of prostate cancer patients will experience signs of recurrence in their lifetime. One of the main risk factors associated with prostate cancer is obesity, or excess adipose tissue. Radiotherapy is a vital component of the treatment of many types of cancer, especially those in the pelvic region, such as prostate cancer.

Significance
Common radiotherapy treatments do not spare adipose tissue found in the abdominal region and, thus, are exposed to radiation. Understanding how radiation can alter adipose tissue, specifically in the context of cancer, could explain how and why metastasis and recurrence occur.

Hypothesis
We hypothesize that irradiated adipose tissue creates a pro-tumorigenic landscape by altering the secretome of adipocytes, triggering prostate cancer progression.

Experimental Design
In vitro studies designed to evaluate the impact of irradiated adipocytes on prostate cancer cells have been conducted using a transwell system. In the bottom well, 3T3-L1 MBX cells were chemically differentiated into mature adipocytes and then subjected to 3 Gy of radiation for 3 consecutive days, or SHAM irradiated, before PC3, C42B, and LNCaP prostate cancer cells were seeded into a migration chamber above the adipocytes. The prostate cancer cells were allowed to migrate through the chamber towards the adipocytes for 48hrs and were collected, counted, and maintained in culture for further analysis of pro-tumorigenic qualities. The 3T3 adipocytes, following co-culturing, were assayed for lipase and fatty acid synthase activity. *In vivo*, C57BL/6 mice received 7.5 Gy of radiation to their inguinal fat pads using a Small Animal Radiation Research Platform (SARRP) for 5 consecutive days. A month later, RFP-expressing RM1 prostate cancer cells were injected orthotopically. Tumor burden was assessed using IVIS imaging, measuring primary, and enumerating metastatic tumors. Using immunofluorescence, oxidative damage in the fat pads of irradiated mice was assayed.

Results
In vitro, co-culturing of prostate cancer cells with irradiated adipocytes (3T3-MBX cells) promotes a significant 2- to 3- fold increase in migration compared to co-cultured with unirradiated 3T3 cells, when validated across three different prostate cancer cell lines. The prostate cancer cells co-cultured with the irradiated adipocytes have a significant increase in intracellular lipid content ($p=0.0216$, 0.0045 observed in two different cell lines) when compared to the parental cells and pronounced morphological changes associated with an epithelial-to-mesenchymal transition. Specifically, cancer cells co-cultured with the irradiated adipocytes have significantly less E-cadherin staining (-67.16± 3.129) compared to the parental cell line. We determined that the adipocytes have increased lipase activity ($p=0.0120$) in both a time and radiation dependent manner, allowing the prostate cancer cells to take up free fatty acids from the adipocytes. This was verified by the presence of Bodipy lipid staining in the prostate cancer cells following co-culturing with Bodipy stained adipocytes. The media from the irradiated versus the non-irradiated adipocytes revealed a 2-, 4-, and 5- fold up-regulation in ESM-1, IGF-1, and IGF-2, adipokines associated with tumor progression. *In vivo,* when fat pads are irradiated prior to orthotopic injection with prostate cancer cells, there is a 4-fold increase in tumor burden and number of metastases per mouse but no difference in the size of the primary tumor. Further analysis of the fat pads revealed irradiated adipose tissue has a significant 2-fold or 3-fold increase in lipid peroxidation and DNA oxidative damage, respectively, compared to unirradiated adipose tissue. Irradiated adipocytes also exhibit an increased immune cell infiltration and secretion of lipids, as well as a 4-fold decrease in fat cell area when compared to unirradiated adipocytes.

Conclusions
Given this data, we postulate that irradiated adipose tissue alters the secretome of adipocytes by inducing oxidative damage to the cells, increasing the amount of released free fatty acids and dysregulating adipokine secretion, causing prostate cancer progression.

TARGETING THE ONCO-GOLGI IN PROSTATE CANCER PROGRESSION VIA AUTOPHAGY AND ER STRESS INHIBITION

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Background Fragmentation of the Golgi apparatus, termed "onco-Golgi," is implicated in several cancer types, including aggressive castration-resistant prostate cancer (CRPC). This leads to abnormal post-translational modification of pro-metastatic proteins, including α_v integrins. We previously found that the onco-Golgi expedites the activating transcription factor 6 (ATF6) branch of the endoplasmic reticulum (ER) stress response. Moreover, we demonstrated structural and functional alterations in Golgi matrix proteins (golgins) within the fragmented Golgi.

Problem However, the underlying causes of these phenomena remain unclear. Additionally, autophagy inhibitors have been proposed to enhance existing CRPC therapies, but the mechanism is poorly understood. In our study, we observed that treatment with the autophagy inhibitor hydroxychloroquine (HCQ) restores a compact Golgi morphology and normal processing of proteins passing through the Golgi of CRPC cells.

Method Here, we investigated Golgi morphology, Golgiphagy, and protein glycosylation using a variety of microscopy and biochemical approaches including electron and high-resolution microscopy, lectin staining and lectin-affinity chromatography, and endoglycosidase sensitivity.

Results We identified the recruitment of fragmented Golgi membranes for phagophore formation. Inhibition of autophagy by HCQ, Bafilomycin A1, or depletion of Autophagy Related 5 (Atg5) restored Golgi morphology and reduced the association of golgins with autophagosome membranes. We propose that Golgiphagy drives CRPC-associated Golgi fragmentation. Additionally, to elucidate the role of onco-Golgi in tumor progression and metastasis, we investigated alterations in the glycosylation of α_v integrins. Golgi fragmentation causes mislocalization of the glycosyltransferase, Beta-1,4-Mannosyl-Glycoprotein 4-Beta-N-Acetylglucosaminyltransferase (MGAT3), resulting in integrin glycosylation by Alpha-1,6-Mannosylglycoprotein 6-Beta-N-Acetylglucosaminyltransferase (MGAT5), the N-glycosylation competitor of MGAT3. Importantly, MGAT5-modified integrins (but not MGAT3-modified integrins) are recognized by Galectin-3 at the plasma membrane (PM), promoting integrin clustering and retention. Our data revealed an increased prevalence of MGAT5-modified integrins on the PM of CRPC cells and in high-grade prostate tumors. Additionally, these integrins are reduced on the cell surface after HCQ treatment. Furthermore, ATF6 depletion significantly reduces Golgi fragmentation, leading us to test the simultaneous ATF6 KD and HCQ treatment in CRPC cells. This combined treatment prominently affected Golgi reorganization, decreased expression of MGAT5-modified integrins on the PM, diminished colocalization of Integrin α_v with Galectin-3, and reduced fibronectin adhesion in CRPC cells. In an orthotopic tumor model, ATF6 depletion and HCQ treatment also significantly reduced orthotopic tumor growth and metastasis formation individually and eradicated metastases when combined.

Conclusion Therefore, targeting autophagy with HCQ and the ATF6-mediated ER stress response may serve as a strategy to counter onco-Golgi-associated tumor progression.

Elucidating the role of *CBFB-MYH11* in maintenance of Inv(16) AML

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ABSTRACT

Background & Significance- Acute myeloid leukemia (AML) is a heterogenous disease arising from the immature blood cells in the bone marrow. One of the most common chromosomal aberrations, inversion of chromosome 16 [inv(16)], generates the fusion gene *Cbfb-MYH11 (CM)* which is known to initiate leukemogenesis. Because the fusion protein is invariably found in patients at relapse, it is assumed that *CM* is also required after leukemic transformation. This raises the possibility that *CM* may be dispensable after leukemic transformation. However, ME-1 cells are known to carry additional mutations that cause immortalization, and, unlike patient samples, can spontaneously lose the inverted chromosome 16, so may not accurately model the role of *CM* after leukemic transformation. We hypothesize that *Cbfb-MYH11* induces gene expression and are required for the maintenance of primary leukemia cells.

Experimental design & Results- To address the role of *CM* after initiation, we used a new knock-in mouse model (*Cbfb*$^{+/flMYH11}$) where the *CM* is deleted in presence of *Cre* after leukemic transformation. To test the effect of *CM* deletion *in vitro* and evaluate the leukemic stem cell (LSC) activity we performed colony assays. We observed a statistically significant decrease in colony forming ability in *Cbfb*$^{+/flMYH11}$*Cre*$^{+}$ cells as compared to control *Cbfb*$^{+/flMYH11}$ cells. To determine if the colonies that did grow from *Cre*-infected cells had successfully deleted *CM*, individual colonies were picked. We found that majority of the colonies retained the unexcised *CM* allele, but 5-20% of colonies had successfully deleted *CM*, indicating that a population of leukemia cells can survive without *CM in vitro*. To understand if the *CM* is essential for survival *in vivo,* we used a shRNA knockdown model. We transduced *CM* expressing leukemic cells with a doxycycline (Dox) inducible shRNA against *MYH11* (*shMYH11*). To test the feasibility of this model, we evaluated the effect of knockdown (KD) of *CM* by qRT-PCR and western blot. We found that expression of *CM* was significantly lower in cells treated with Dox as compared to untreated ones. To test the effect of *CM* KD *in vivo*, we transplanted mice with transduced leukemia cells, allowed the cells to engraft and then treated mice with control or Dox water. We found that decreased *CM* expression almost eliminated the leukemic burden in the peripheral blood and spleen. In the BM, there was a statistically significant decrease in leukemic burden starting on day 14 of Dox treatment, but the leukemia cells remained at 30-40% through day 21. RT-PCR of the leukemia cells in the BM revealed significant KD of *CM* at day 7 and day 21 of treatment. Using BrdU incorporation assays, leukemia cells in the BM of Dox treated mice demonstrated significantly lower proliferation as compared to control. To characterize the immunophenotype of the *CM* KD leukemic cells in the BM, we tested the cell surface expression of LSC markers (CKIT, Cd59a, IL1RL1, CD123). We found a small but statistically significant increase in CD123^{+} population of leukemia cells in Dox treated mice. To test if the *CM* KD cells in the BM could cause relapse, we maintained transplanted mice on Dox and monitored for signs of leukemia. We observed 5 of the 12 dox treated mice relapsed by Day 42 of treatment. Western blot and qRT-PCR confirmed KD of the fusion protein in the relapsed samples. These findings indicate that leukemic cells are able to survive in the BM with reduced *CM* and may give rise to relapse.

Conclusion- Taken together, our results imply that *CM* is important for the maintenance of leukemia and could be a potential drug target, but that inhibition of the fusion protein alone is unlikely to be curative. We are currently investigating the mechanisms that allow leukemia cells with reduced CM to survive in the bone marrow, with the goal of curing inv(16) AML.