56th ANNUAL MIDWEST STUDENT BIOMEDICAL RESEARCH FORUM

Saturday, March 8, 2025

ROOM 3047

8:00 a.m. O-32 LAMA5: A NOVEL PLAYER IN MICROENVIRONMENT-MEDIATED PANCREATIC CANCER PROGRESSION

Presenter: Annant Bir Kaur

8:15 a.m. O-48 KINASE SUPPRESSOR OF RAS 1 (KSR1) IS REQUIRED FOR KEAP1-DEPENDENT TUMOR-INITIATING CELL FORMATION IN LUNG ADENOCARCINOMA CELLS.

Presenter: Eric Mpingirika

8:30 a.m. O-69 IDENTIFYING METASTASIS ASSOCIATED KINASE NETWORKS IN PROSTATE CANCER

Presenter: Sriya Sridhar

8:45 a.m. O-10 NEGATIVE REGULATION OF PR55α BY THE P53 TUMOR SUPPRESSOR

Presenter: Alison Camero

9:00 a.m. O-18 UNVEILING THE ROLE OF B7H3 IN DRIVING THE PROGRESSION OF SMALL CELL LUNG CANCER

Presenter: Mahek Fatima

9:15 a.m. O-14 TARGETED DEGRADATION OF KSR1 INHIBITS COLORECTAL CANCER TRANSFORMATION

Presenter: Sarah Dharmaji

9:30 a.m. O-02 NADPH OXIDASE 4 (NOX4) KNOCKOUT REDUCES METASTASIS OF PROSTATE CANCER CELLS

Presenter: Oluwaseun Adebisi

9:45 a.m. BREAK

LAMA5: A NOVEL PLAYER IN MICROENVIRONMENT-MEDIATED PANCREATIC CANCER PROGRESSION

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Background and Significance: Pancreatic cancer is the fourth leading cause of cancer-related deaths in the US, with a dismal 5-year survival rate of 12.8%. The most common type of pancreatic cancer, Pancreatic Ductal Adenocarcinoma (PDAC), often presents with multi-organ metastasis at the time of diagnosis, with limited treatment options and a high mortality rate. A defining feature of PDAC is its dense desmoplastic stroma, which can make up 90% of the tumor mass in late stages. This tumor microenvironment (TME) comprises various cellular and acellular components, including cancer-associated fibroblasts (CAFs), immune cells, extracellular matrix (ECM), cytokines, growth factors, etc. CAFs (the predominant stromal cells in PDAC), secrete ECM components like laminin, collagen, etc that mediate tumor-stroma interactions. One such factor, Laminin subunit alpha 5 (LAMA5), an integral part of the Laminin heterotrimer, interacts with the cell-surface receptors to regulate ECM-driven cellular processes such as proliferation and metastasis in various cancers, but its role in PDAC remains unexplored. This study aims to investigate the LAMA5-mediated mechanism of initiation and progression of pancreatic ductal adenocarcinoma.

Hypothesis: LAMA5 PROMOTES INITIATION AND PROGRESSION OF PANCREATIC CANCER BY FACILITATING TUMOR MICROENVIRONMENT-CANCER CELL CROSSTALK

Experimental Design: A mass spectrometry-based proteomics analysis of mouse CAF-conditioned media identified top CAF-secreted proteins in early stages of PDAC. These proteins were further subjected to gene expression and relative survival analysis via publicly available PDAC RNA seq datasets from TCGA and GEO (number of samples: 804). An amalgamation of *in vitro* and *in vivo* experiments detailed the expression profile of the protein of interest, LAMA5. Protein expression was confirmed in mice autochthonous models of pancreatic cancer progression (KrasG12D/+; Pdx1Cre) and human tumor vs normal samples in human tissue microarray using immunofluorescence (IF) and immunohistochemistry (IHC). To observe the localization of the protein in early PDAC development, a cerulein treatment model was used to induce acute pancreatitis in mice. Since CAFs are the main producers of ECM proteins and are involved in exosome mediated cellular crosstalk, exosomes were isolated from conditioned media of normal and cancer-activated fibroblasts in both mice and human. Exosomal fractions from pancreatic cancer and normal cell lines were also subjected to immunoblot analysis to get a comprehensive view of the CAF-cancer cell crosstalk. Extracted exosomes were confirmed by the presence of exosomal marker proteins such as TSG101, CD63, Hsp70.

Results/Data: Proteomics analysis of mouse CAF-conditioned media identified elevated ECM components, with LAMA5 emerging as one of the significantly overexpressed proteins in the activated fibroblast secretome. Publicly available RNA seq data analysis revealed a significant increase in LAMA5 expression in cancer as compared to normal pancreas, with a high LAMA5 expression correlating with a low survival rate in PDAC patients. IF and IHC analysis of LAMA5 expression revealed a gradual increase in LAMA5 levels with higher PANIN stages and finally PDAC. Cerulein treatment-induced acute pancreatitis in WT and KC mice also drove an enhanced expression of LAMA5 in CAF and epithelial cancer cell compartments. Further, as confirmed by IF, human and mouse CAFs depicted a more prominent LAMA5 expression than normal human fibroblast and mouse stellate cells. An immunoblot analysis of the isolated exosome fractions from normal and cancer-activated fibroblasts revealed substantially elevated LAMA5 protein in the CAF samples of both mice and humans compared to normal fibroblasts. To further characterize this crosstalk, exosomal fractions from different pancreatic cancer cell lines were also analyzed for the presence of LAMA5. Indeed, a high expression of LAMA5 was observed in both primary tumor and metastatic cell lines. However, LAMA5 is constantly overexpressed only in the secreted exosomal fractions compared to the cellular lysates.

Conclusions: Our results show evidence of a potential role of LAMA5 in facilitating intercellular communication between CAFs and cancer cells, thus promoting the aggressiveness of the cancer. In conclusion, LAMA5 may function as a "**matrix modulating molecule**" to alter the extracellular environment at local and distant metastatic sites in pancreatic cancer.

KINASE SUPPRESSOR OF RAS 1 (KSR1) IS REQUIRED FOR KEAP1-DEPENDENT TUMOR-INITIATING CELL FORMATION IN LUNG ADENOCARCINOMA CELLS.

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Background, Significance, and Hypothesis. Lung adenocarcinoma (LUAD), a leading cause of cancer-related mortality, frequently harbors concurrent mutations in Kelch-like ECH-associated protein 1 (KEAP1) and serine/threonine kinase 11 (STK11), which contribute to aggressive tumor phenotypes and poor prognosis. KEAP1, a critical regulator of the nuclear factor erythroid 2-related factor 2 (NRF2) antioxidant pathway, facilitates NRF2 degradation under normal conditions to maintain redox homeostasis. Loss-of-function mutations in KEAP1 stabilize NRF2, driving the transcription of antioxidant, metabolic, and detoxification genes that enhance resistance to oxidative stress and tumor survival under therapy. STK11, encoding the serine/threonine kinase LKB1, functions as a tumor suppressor by regulating energy metabolism through AMP-activated protein kinase (AMPK) signaling. Loss-of-function mutations in STK11 disrupt this pathway, leading to metabolic dysregulation, immune evasion, and tumor progression. Co-mutations in KEAP1 and STK11, observed in approximately 10.2% of LUAD cases, synergistically activate pathways that enhance oxidative stress resistance and metabolic reprogramming, resulting in markedly reduced median survival (7.3 months). Emerging evidence highlights kinase suppressor of Ras 1 (KSR1), a scaffold protein that coordinates RAF/MEK/ERK signaling, as a key modulator of these processes. KSR1 amplifies ERK activity, sustaining tumor-initiating cells (TICs) that drive tumor maintenance, relapse, and therapy resistance. KSR1 knockout (KSR1 KO) disrupts TIC viability by impairing ERK-mediated oncogenic signaling. Elucidating the interplay between NRF2, STK11, and KSR1/ERK signaling is essential to understanding the vulnerabilities of STK11/KEAP1-mutant LUAD upon KSR1 abrogation.

TICs are drivers of tumor maintenance, therapeutic resistance and relapse, and are synonymous with drug-tolerant persister cells (DTPs). TICs are maintained at high levels in KEAP1 and STK11-mutant LUAD. KSR1 amplifies TIC proliferation and survival, supporting tumor progression. KSR1 KO reduces TIC and DTP formation. Understanding the interplay between KEAP1, STK11, and KSR1/ERK signaling could reveal novel therapeutic vulnerabilities to improve outcomes in this challenging LUAD subset. We **hypothesize** that KEAP1/NRF2 and STK11/AMPK pathways converge with KSR1/ERK signaling to sustain TIC populations and promote therapy resistance, and that KSR1 abrogation reverses the oncogenic phenotypes driven by KEAP1 and STK11 mutations. Understanding this interplay will reveal critical vulnerabilities to improve therapeutic outcomes of these aggressive LUAD subtypes.

Experimental design. To identify pathways that are significantly altered by KEAP1 and STK11 disruption, we compared RNA sequencing data from LUAD H358 KEAP1 KO or STK11 and KEAP1 double knockout (DKO) cells to non-targeting control (NTC) H358 cells using gene set enrichment analysis (GSEA). We performed CRISPR/Cas9 targeting of KSR1 in H358 control, KEAP1 KO, or DKO cells. To evaluate the impact of KSR1 disruption in combination with KEAP1 and STK11 disruption on TIC frequency we performed in vitro Extreme Limiting Dilution Analysis (ELDA) on KSR1 KO, KSR1/KEAP1 KO, KSR1/KEAP1/STK11 KO and control H358 cells. Serial dilutions of cells (ranging from 1,000 to 1 cell per well) were plated in 96-well round-bottom 3D culture plates and spheres with diameters greater than 100 µm were scored as TICs after 7–9 days of incubation. These counts were analyzed by regression analysis using ELDA software to determine TIC frequency for each condition.

Results. Pathway analysis using GSEA on H358 KEAP1 KO and DKO cells relative to their NTCs revealed significant enrichment of antioxidant and pentose phosphate metabolic pathways consistent with the ability of KEAP1 disruption to promote NRF2 expression and relieve oxidative stress. These pathways could play a role in TIC/DTP formation and/or function. While KEAP1 KO cells exhibited a global down regulation of ribosomal genes and pathways related to translation, DKO cells displayed significant upregulation of eukaryotic initiation factor (eIF) genes. This observation highlights distinct differences in translational reprogramming in the KEAP1 KO ^{and} DKO cells. ELDA demonstrated that KEAP1 KO and DKO dramatically increase TIC formation (11.5 and 8.5-fold respectively) compared to control H358 cells. KSR1 KO significantly reduced TIC frequency by 86.78% in control H358 cells and prevented the increase in TIC levels caused by KEAP1 KO, while having no significant effect on DKO TIC frequency.

Conclusion and future directions. These data suggest that KSR1 interacts with KEAP1/NRF2 pathways to maintain TICs potentially through the attenuation of antioxidant and translational reprogramming mechanisms identified in GSEA. These findings underscore the therapeutic potential of targeting KSR1 to suppress TIC maintenance and overcome therapy resistance in LUAD. Future studies will focus on elucidating the mechanisms by which KSR1 supports TIC maintenance. We will investigate why KSR1 KO depletes TICs in control and KEAP1KO cells but not in the DKO. Polysome profiling will be performed to assess contribution of KSR1 on translational reprogramming in KEAP1 KO, DKO, and control H358 cells. Single-cell RNA sequencing (scRNA-seq) will be used to identify subpopulations of KSR1- and KEAP1-dependent TICs within H358 and other LUAD cells and identify transcriptional changes associated with KEAP1 and KSR1 regulation.

IDENTIFYING METASTASIS ASSOCIATED KINASE NETWORKS IN PROSTATE CANCER

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Background: Prostate cancer (PCa) is the second leading cause of death in American men. Time of diagnosis and treatment play an important role in determining the severity and consequences of the disease. While most men with early diagnosis and treatment of the disease have an almost hundred percent chance of survival, those who are diagnosed after the disease progression and metastases have a lower than thirty percent rate of survival. The metastasized cells display genetic changes that increase their ability to survive, including resistance to androgen deprivation therapy (ADT), transition from epithelial to mesenchymal state, and dormancy. Previously published literature as well as data generated from our lab suggests that the receptor tyrosine kinase AXL is co deleted with tumor suppressors PTEN and TP53 in aggressive metastatic PCa. Additionally, this genetic signature gives rise to bone and visceral metastases in mouse models.

Significance of Problem: AXL has been shown to crosstalk within a network of RTKs such as EGFR, HER2, RON, VEGFR and PEAK1. However, in PCa, the role of RTKs that functionally contribute towards metastatic progression in the context of AXL loss is unknown. Likewise, identifying therapeutic agents targeting metastasis associated RTKs can be a beneficial strategy for treating aggressive PCa.

Hypothesis: We hypothesize that the activation of metastasis associated RTKs promote an aggressive disease phenotype in AXL null PCa. Thus, we aim to identify such kinases that can functionally take over in the absence of AXL and establish kinase inhibitor based therapeutic modalities to preferentially target PTEN/TP53/AXL null PCa.

Experimental Design: A transcriptomic approach to identify the upregulated kinases in PTEN/TP53/AXL null cell was performed using isogenic mouse and human cell lines. Differentially expressed genes from the transcriptome analysis was verified against promoter acetylation status (H3K27ac CHIP sequencing) and a list of kinases upregulated in Axl null cells were established. Next an orthogonal pharmacological approach was performed using bone derived metastatic PCa cell line - PC3 (PTEN/TP53 null) and an isogenic CRISPR mediated AXL knockout line. The PC3 and PC3 AXL knockout cells were then treated with a library of 2047 known kinase inhibitors (Selleck#L1200) at two concentrations of 500nM and 2.5uM. The cell viability after 72 hours of drug treatment was then measured via MTT assay. The drugs that preferentially killed AXL null PC3 cell were analyzed and validated.

Results/Data: The upregulated RTKs identified through the omics approach were validated by immunoblotting. Metastasis associated RTKs such as Pdgfra/b, Fgfr1, Vegfr1 (Flt1) and Ror2 were found to be upregulated. Additionally, genes within the mTORC1 signaling pathway, MYC and E2F targets appeared to be enriched in the context of AXL loss. Interestingly results from the complementary pharmacological screen highlighted inhibitors against PDGFR, VEGFR, mTOR and MEK to be effective against AXL null cells.

Conclusions: The goal of identifying the kinase dependencies of an AXL null cell is to determine therapeutic agents to target a pathway that compensates in the absence of AXL. From the kinase screen, we were able to identify kinases that take over when AXL is lost as well as drugs that can target them. Using data from both mouse transcriptomic studies as well as human pharmacological studies, we suggest that upregulation of PDGFR and VEGFR can potentially compensate for AXL loss. Furthermore, targeting mTOR pathway presents as an effective therapeutic strategy for targeting AXL null cells.

NEGATIVE REGULATION OF PR55α BY THE P53 TUMOR SUPPRESSOR

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Background: Pancreatic cancer is among the deadliest cancers, with an overall five-year survival rate of only 12% in 2024. Its high mortality rate stems from aggressive progression, late detection, limited treatment options, and a lack of targeted therapies. PR55α is a regulatory subunit of protein phosphatase 2A (PP2A), which supports multiple tumorigenic pathways including YAP, c-Myc, β-catenin, and ERK, and has been identified as a critical promoter of pancreatic cancer. PR55α contains seven WD40 domains essential for its structural stability, interaction with other proteins, and function. Recent studies from our lab discovered a novel pathway by which p53-controlled FBXL20, a substrate adaptor of an SCF ubiquitin E3 ligase complex, negatively regulates PR55α protein stability via proteasomal degradation. These findings have established an essential role of PR55α in promoting pancreatic cancer development and progression, therefore PR55α is a potential therapeutic target for pancreatic cancer.

Significance of Problem: Although the p53/FBXL20 pathway has been identified as the negative regulator of PR55α protein stability, the precise molecular mechanism underlying this regulation remains unknown. Given the great opportunity of developing PR55α-based targeting therapies for pancreatic cancer, there is a critical need to understand how FBXL20/SCF mediates PR55α degradation, which includes identifying the binding motifs between FBXL20 and PR55α, the FBXL20-targeted ubiquitination sites in PR55α, and the additional members of the SCF complex involved in the regulation. A clear understanding of this mechanism can potentially lead to the development of PR55α-targeted therapeutics for pancreatic cancer treatment.

Question: This project aims to elucidate the molecular mechanism by which the p53/FBXL20 cascade mediates PR55α proteasomal degradation, to develop PR55α-based targeted therapies for pancreatic cancer.

Experimental Design: PR55α consists of seven WD40 domains, which are versatile motifs that contribute significantly to its protein-protein interactions, structural stability, substrate recognition and targeting, and isoform specificity. To identify the recognized binding motifs responsible for PR55α degradation by the p53/FBXL20 cascade, we generated a series of myc-tagged PR55α truncates encompassing various WD40 domains. Myc-PR55α truncates were subcloned into the pCS2-MT expression vector. Co-immunoprecipitation (co-IP) was performed to assess the binding between FBXL20 and full-length or truncated PR55α.

Results/Data: All Myc-PR55α truncates were successfully expressed in HEK-293T, HPNE, and CD18 cells. Co-immunoprecipitation (IP) confirmed the interaction of PR55α full-length with FBXL20 in HEK-293T cells. Furthermore, co-IP identified that truncates containing WD40 domain #5 consistently bound FBXL20, while truncates lacking this domain did not. Additional domains #4, #6, and #7 appear to enhance binding stability. These results suggest that WD40 domain #5 is critical for FBXL20-mediated PR55α degradation.

Ongoing Studies: 1) We are performing a cell-free binding assay to determine whether the interaction between FBXL20 and PR55α is direct or indirect. In the case of the latter, we would need to further identify the binding intermediate(s) between PR55α and FBXL20 using proteomics. 2) We are doing functional tests are ongoing to validate the role of specific WD40 domains identified by co-IP within the binding motif in mediating PR55α ubiquitination and degradation by the FBXL20/SCF E3 ligase. 3) We will perform internal deletions to further validate the binding motif function. 4) We will elucidate the biological significance of the negative regulation of PR55α stability by the p53/FBXL20 pathway in oncogenesis.

Conclusions: The goal of this study is to identify the binding motif of PR55α that interacts with FBXL20. This binding interaction is required for the p53/FBXL20 cascade to negatively regulate PR55α levels by ubiquitination and degradation. Understanding the specifics of this pathway can help advance PR55α-targeted therapies, homing in on the specific ubiquitination sites of PR55a. This research bears significant relevance in the realm of pancreatic cancer, a field that is currently limited by few therapeutic options. This study lays the groundwork for a potential breakthrough intervention. We will continue to explore and elucidate the broader functions of the binding motif and its impact on cancer development and progression.

UNVEILING THE ROLE OF B7H3 IN DRIVING THE PROGRESSION OF SMALL CELL LUNG CANCER

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Background, Significance, Hypothesis: Lung cancer is the leading cause of cancer-related deaths globally, with small cell lung cancer (SCLC) representing a highly aggressive subtype. The prognosis for SCLC remains dire due to early metastasis, chemoresistance, high recurrence rates, and limited therapeutic options. Despite the approval of some immunotherapeutic drugs for SCLC, their efficacy is restricted to a small subset of patients. This limited success is attributed to low expression of PD-L1 and the immune-cold tumor microenvironment in SCLC, underscoring the urgent need for new therapeutic targets. Bioinformatic analysis of SCLC datasets, focusing on immune checkpoint regulators, identified B7H3 (CD276) as a promising candidate upregulated in SCLC. This finding was confirmed in SCLC cell lines and human tissue samples. B7H3 is a cell surface protein overexpressed in multiple tumor types, functioning as both an immune modulator and an oncogenic driver. However, its role in SCLC tumorigenesis remains unclear. Our preliminary data shows that B7H3 knockout (B7H3 KO) results in decreased functional activity of SCLC cells. Based on our findings and published reports our central hypothesis is B7H3 drives the growth and metastasis of SCLC.

Experimental design: To investigate the functional involvement of B7H3 in SCLC, we performed various *in vitro* and *in vivo* experiments. We employed CRISPR-Cas9 to knock out the *B7H3* gene in SCLC cell lines. To study the role of B7H3 in the growth and metastasis of SCLC, we performed cell viability assay, transwell migration assay, and colony formation assay in B7H3 proficient and deficient SCLC cell lines (SBC3, SBC5, H1688). To assess the role of B7H3 in the tumorigenesis of SCLC, we injected H1688 parental and B7H3 KO cells subcutaneously in NSG mice and measured the tumor growth.
To therapeutically target B7H3, we treated the spontaneous SCLC mouse model RPM (Adeno-CGRP-Cre; $Rb1^{flox/flox}$; $TP53^{flox/flox}$; $LSL\text{-}Myc^{T58A}$) with antibody-drug conjugate (ADC) m276-SL-PBD and assessed tumor growth, metastasis, and T cell infiltration. To further elucidate the role of B7H3 in SCLC progression, we generated a novel spontaneous mouse model, RPMC ($Rb1^{flox/flox}$; $TP53^{flox/flox}$; $LSL\text{-}Myc^{T58A}$; $Cd276^{fl/fl}$). We have further performed RNA-seq analysis on H82 parental and B7H3 KO -cells to investigate the underlying mechanisms of B7H3 mediated SCLC progression that we have observed in our *in vitro* and *in vivo* experiments.

Data and Results: The *in vitro* assays showed a decrease in cell proliferation, migration, and clonogenicity of B7H3 deficient SCLC cell lines compared to the parental cell lines. We also observed a significant decrease in tumor volume and tumor weight in the B7H3 deficient SCLC xenograft models. In our m276-SL-PBD ADC treatment studies, we observed a significant decrease in tumor burden in the RPM mice that were treated with the ADC compared to the PBD and vehicle treated groups. Immunohistochemical analysis revealed a decrease in tumor-associated angiogenesis, stemness, and an increase in apoptosis in the ADC treated group. Immunofluorescence staining also revealed a significant increase in T-cell infiltration in lung and brain tissues of mice treated with B7H3 targeting m276-SL-PBD ADC. Our novel spontaneous mouse model RPMC exhibited a marked decrease in tumor burden, tumor-associated angiogenesis, and metastasis, with a
significantly increased T-cell infiltration compared to controls. Transcriptomic analysis of B7H3-proficient/-deficient cells revealed NFIB among the top 50 downregulated genes following B7H3 deletion. NFIB, a key driver of SCLC metastasis, was significantly reduced in B7H3-deficient human and syngeneic (RPMC) cell lines. Metastatic tissues from RPMC mice also showed lower NFIB expression compared to the RPM model. In conclusion, this study highlights the critical role of B7H3 as well as modulation of NFIB expression in regulating SCLC progression.

Conclusion: Our findings establish B7H3 as a promising therapeutic target and demonstrate the potential role of CD276 ADCs in overcoming the limitations of current treatments of SCLC. By advancing our understanding of the role of B7H3 in promoting tumor growth, this study paves the way for designing more effective therapeutic strategies to combat SCLC.

TARGETED DEGRADATION OF KSR1 INHIBITS COLORECTAL CANCER TRANSFORMATION

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Background, Significance of problem, Hypothesis: Colorectal cancer (CRC) is the second most common cancer diagnosed in both men and women in the United States (American Cancer Society, 2024). KRAS is the most frequently mutated oncogene, with mutations occurring in approximately 45 to 50% of colorectal adenocarcinomas. KRAS-mutant cancers are highly dependent on receptor tyrosine kinase (RTK)/RAS signaling. Kinase Suppressor of Ras 1 (KSR1) functions downstream of Ras as a molecular scaffold for RAF, MEK, and ERK, regulating ERK signaling crucial for cell fate decisions. In CRC, MEK inhibitors promotes tumor-initiating cell (TIC) populations, contributing to acquired resistance. KSR1 disruption enhances the efficacy of MEK inhibitor trametinib to overcome both intrinsic and acquired resistance in CRC (Daley et al., PNAS 2023). KSR1 knockout (KO) also sensitizes small cell lung cancer cells to cisplatin (Chatterjee et al., in press). Despite the potential of KSR1 as a therapeutic target, no KSR1-targeting drugs are currently in clinical use. To evaluate KSR1 as a viable drug target, we used the (degradation TAG) dTAG system, a proteolysis- targeting chimeric molecule (PROTAC) -based approach that induces rapid, reversible degradation of target proteins. The dTAG system uses a generic heterobifunctional molecule that binds to and links FKBP12^{F36V}. FKBP12^{F36V} is expressed in-frame to the protein of interest. The dTAG compound links the chimeric protein to either cereblon or Von-Hippel Lindau (VHL) ubiquitin ligases, to promote ubiquitination and proteasomal degradation. We **hypothesize** that using this dTAG system for targeted degradation of KSR1 will suppress the transformed phenotype of CRC cells, suggesting the potential of KSR1-directed PROTACs or molecular glues to impair CRC tumor development.

Experimental design: We generated an HA-tagged FKBP12^{F36V}-KSR1 fusion construct that is rapidly degraded in response to ligands dTAG-13 and dTAG^{V}-1, which target fusion constructs to E3 ubiquitin ligases cereblon and VHL, respectively. KSR1 KO HCT116, SW620, T84, and SW480 CRC cells were generated using CRiSPR/Cas9. Lentivirus was used to infect control and KO cells and express the FKBP12^{F36V}-KSR1 construct. To determine the optimal dose for FKBP12^{F36V}-KSR1 degradation, cells expressing FKBP12^{F36V}-KSR1 were treated with dTAG-13 at concentrations ranging from 0.1nM to 100nM for 4 hours. For dTAG^{V}-1, FKBP12^{F36V}-KSR1-expressing KSR1 KO CRC cells were treated with doses ranging from 0.5nM to 500nM. A 7-day time course in HCT116 and T84 cells expressing FKBP12^{F36V}-KSR1 and treated with one dose of 500nM dTAG^{V}-1 and 100nM dTAG-13 evaluated the duration of dTAG-mediated degradation. p-ERK levels were assessed by western blot after 4-hour treatments with dTAG^{V}-1 or dTAG-13. To assess the impact of KSR1 degradation on colony formation, KSR1 KO + FKBP12^{F36V}-KSR1 SW480 cells were treated with dTAG^{V}-1 or DMSO control for 72 hours and plated in soft agar. A short-term *in vivo* study was performed by subcutaneously injecting KSR1 KO + FKBP12^{F36V}-KSR1 HCT116 cells ($1x10^{6}$) into NCG mice, followed by treatment with 40 mg/kg dTAG^{V}-1 in 5% DMSO/20% solutol/saline or vehicle control, delivered intraperitoneally (IP) every 24 hours, over 3 days. Tumor tissue was collected for analysis after 4 hours post-final injection.

Results: Exogenous expression of FKBP12^{F36V}-KSR1 in KSR1 KO HCT116 and SW480 CRC cells successfully rescued ERK activation, demonstrating that FKBP12^{F36V}-KSR1 can restore Raf/MEK/ERK pathway signaling disrupted by the loss of endogenous KSR1. Complete degradation of FKBP12^{F36V}-KSR1 in colorectal cancer cells was achieved using 100nM dTAG-13, which was subsequently used in all experiments. Time-course analyses revealed degradation within 2 hours of treatment with 100nM dTAG-13 across all cell lines. Similarly, treatment with 500nM dTAG^{V}-1 resulted in complete degradation after 24 hours. In HCT116 and T84 cell lines, treatment with both dTAG^{V}-1 and dTAG-13 resulted in sustained FKBP12^{F36V}-KSR1 degradation, which persisted for at least 7 days post-treatment. However, withdrawal of the dTAG ligand led to restoration of FKBP12^{F36V}-KSR1 within 2 hours. These data demonstrate that long-lasting protein degradation can be achieved with this system, and re-treatment is not required for at least 7 days in functional *in vitro* assays. Treatment with 100nM dTAG-13 and 500nM dTAG^{V}-1 for 4 hours reduced the ratio of p-ERK to total ERK, indicating that KSR1 degradation effectively diminishes signaling through the Raf/MEK/ERK cascade. Expression of FKBP12^{F36V}-KSR1 in SW480 KSR1 KO cells fully rescued soft agar colony formation, confirming the biological functionality of the chimeric protein. Treatment with dTAG^{V}-1, resulted in a ~83% reduction in colony formation at 14 days compared to the DMSO control, indicating that KSR1 degradation inhibits the soft agar colony-forming capacity of these cells. Immunohistochemical analysis of *in vivo* tumor xenografts revealed that dTAG^{V}-1 effectively degraded FKBP12^{F36V}-KSR1, as indicated by diminished HA staining in dTAG-treated tumors compared to control tumors. Control tumors exhibited more foci of strongly HA-positive cells, while dTAG-treated tumors displayed reduced staining intensity. Histochemical scoring (H-score) quantification confirmed ~50% decrease in the mean H-score for dTAG-treated tumors, demonstrating that dTAG^{V}-1 administered at 40 mg/kg via intraperitoneal injection every 24 hours for 3 consecutive days, effectively mediates FKBP12^{F36V}-KSR1 degradation *in vivo.*

Conclusion: Our findings establish proof-of-concept for KSR1 degradation using the dTAG system in both *in vitro and in vivo* models. The *in vivo* tumor xenograft study lays a foundation for evaluating the long-term effects of KSR1 degradation on tumor growth and therapy resistance. While further technical studies could enhance delivery of dTAG to optimize KSR1 degradation *in vivo*, our results strongly support continued exploration of the dTAG approach and the development of KSR1-targeting PROTACs for therapeutic applications.

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

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Background

Prostate cancer (PCa) is a type of cancer that is primarily observed in men aged 55 years or above. It has an incidence rate of approximately 12.5% and a recurrence rate of 30% after five years of initial therapy. The standard treatment for prostate cancer patients includes radiation therapy. However, radiation therapy can have an adverse impact on the surrounding tissues, including fibroblasts and endothelial cells in the tumor microenvironment (TME), which could potentially promote tumor recurrence and metastasis. The 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 and certain ROS producing enzymes increases the amount of ROS generated in the tumor microenvironment which could culminate in cancer metastasis. Developing therapies that could potentially reduce reactive oxygen species in the tumor ECM could help prevent tumor spread.

Hypothesis

We hypothesized that knocking out NOX4 in PCa TME could reduce prostate cancer metastasis by decreasing ROS and its associated signaling.

Experimental design

We cocultured wildtype (WT) or NOX-4 knockout (NKO) mouse primary fibroblasts (MPFs) with PCa cells *in vitro* and tested their migration. We also performed a scratch assay by mixing PCa cells with WT and NOX-4KO MPFs (1:10). We have also knocked down NOX4 via siRNA treatment in human umbilical vein endothelial cells (HUVECs) and performed binding assays using labelled PCa cells. For our in vivo study, we implanted PCa cells in WT or NKO C57BL/6 mice. We used tumor weight, tumor size, and metastasis as endpoints for the in vivo study. We also performed immunofluorescence staining on normal tissues surrounding the tumor harvested from PCa implanted mice and assessed vimentin, e-cadherin and 4-hydroxylnonenal expression.

Results

The implantation of NOX4 competent PCa cells (RM1 or TRAMPC2) in NOX4 knockout (NKO) C57BL/6 mice showed a significant decrease in tumor weight and size compared to wild type. We also observed a significant decrease in metastasis of radiated NKO tumor-bearing mice when compared to the control ($p<0.001$). Immunofluorescence staining of normal prostatic tissue harvested from irradiated tumor-bearing mice showed a decreased vimentin expression compared to wild type ($p=0.019$). We have also demonstrated that $NOX4^{-/-}$ mice have reduced lipid peroxidation in prostatic tissues when compared to the wild type. In vitro, we found that NOX4 knockout mouse primary fibroblasts (MPFs) reduced the number of migratory RM1 cells when co-cultured as compared to wildtype ($p<0.0001$). Furthermore, NKO MPFs had a lower percentage of wound closure when compared to wild type (57.8 vs 41.4; $p = 0.0128$). NOX4 knockdown in HUVECS correlated with lesser binding of PCa cells ($p = 0.02$).

Conclusion

Together, our studies have begun to correlate a reduction in prostate cancer metastasis via NOX4 KO in the tumor microenvironment.