55th Annual Midwest Student Biomedical Research Forum

Saturday, March 2, 2024

ROOM 3042

8:00 a.m. **O-07** MUCIN 16 PROMOTES PROTUMOR DESMOPLASIA TO INCREASE PANCREATIC CANCER AGGRESSIVENESS
Presenter: Namita Bhyravbhatla, UNMC

8:15 a.m. **O-12** EVALUATING KIT PROTO-ONCOGENE TRANSCRIPTIONAL MECHANISMS
Presenter: Venkatasai Rahul Dogiparthi, UNMC

8:30 a.m. **O-27** TARGETING TRANSLATIONAL CONTROL IN CANCER
Presenter: Kayla Jonas-Breckenridge, UNMC

8:45 a.m. **O-52** TARGETING HOMOLOGOUS RECOMBINATION DEFICIENT CANCERS WITH SMALL MOLECULE INHIBITORS OF RPA:RAD52 PROTEIN-PROTEIN INTERACTION
Presenter: Sneha Pandithar, UNMC

9:00 a.m. **O-55** THE RE-CLASSIFICATION OF A *PMS2* VARIANT OF UNDETERMINED SIGNIFICANCE USING OUR FUNCTIONAL GENOMICS MISMATCH REPAIR *IN VITRO* MODEL
Presenter: Jocelyn Plowman, Creighton University

9:15 a.m. **O-58** MUC4 ALTERS THE COURSE OF PROGRESSION OF PDAC PRECURSOR LESIONS AND FAVORS IPMN-MEDIATED PDAC DEVELOPMENT
Presenter: Pratima Raut, UNMC

9:30 a.m. **O-75** NUCLEAR PHOSPHOINOSITIDE SIGNALING REGULATES LSD1'S ASSOCIATION WITH EPIGENETIC COMPLEXES
Presenter: Colin Wooldrik, UNMC

9:45 a.m. **O-76** TARGETING CYCLIN K ATTENUATES TUMOR GROWTH AND CONFERS THERAPEUTIC VULNERABILITY IN PANCREATIC CANCER
Presenter: Yi Xiao, UNMC

10:00 a.m. BREAK

MUCIN 16 PROMOTES PROTUMOR DESMOPLASIA TO INCREASE PANCREATIC CANCER AGGRESSIVENESS

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Background

Pancreatic ductal adenocarcinoma (PDAC) is a lethal malignancy characterized by dense desmoplasia and aberrant expression of multiple mucins. Among various mucins, we previously reported that *de novo* MUC16 expression increases progressively with the PDAC stage and correlates with poor prognostic outcomes. Further analysis solidified the involvement of MUC16 in metastasis, specifically through the modulation of cancer-associated fibroblasts (CAFs). Recent studies have shown the heterogeneous nature of the desmoplastic stroma with both tumor-promoting and restraining roles, especially through metastatic dissemination. This is dictated by the cancer cell-derived factors and their crosstalk with different cells. These findings are crucial in identifying drivers of desmoplasia, which may reveal new therapeutic targets that modify the environment of the tumor.

Significance of Problem

MUC16 has emerged as a regulator of PDAC metastatic events and the desmoplastic stroma. Despite existing studies on MUC16, little is known about how MUC16 controls desmoplasia in a manner that facilitates metastatic events. Delineating this axis could improve our understanding of the desmoplastic reaction in PDAC and provide new opportunities to manage this disease.

Hypothesis

MUC16-CAF crosstalk develops an obstructive stroma which facilitates metastatic dissemination in PDAC.

Experimental Design

Transcriptomic analysis was done on patient-derived cancer-associated fibroblasts (CAFs), murine-derived CAFs, and murine-derived mesenchymal stem cells cultured with KPC and KPCM16-derived conditioned media to assess for CAF signatures. Tumor samples from autochthonous murine models of PDAC [KPC (K-ras^{G12D}; TP53^{R172H}; Pdx-1cre) and KPCM16 (K-ras^{G12D}; TP53^{R172H}; Muc16$^{-/-}$; Pdx-1cre)] were subject to second harmonic generation microscopy and analyzed to evaluate collagen architecture. Additional *in silico* analysis was performed using publicly available TCGA datasets, RNASeq data derived from KPC and KPCM16 KO murine models, and publicly available scRNAseq datasets.

Results

Using autochthonous murine models, we discovered that MUC16 promotes fibrosis and deposition of extracellular matrix proteins in PDAC. Furthermore, MUC16 knockout cancer cell secretome significantly decreases collagen production by cancer associated fibroblasts and their precursors. These findings mirror scRNAseq and RNASeq analysis, wherein MUC16/CA125 high patients have significantly upregulated collagens. Next, due to the extended structure of MUC16 and its ability to modify the expression of metalloproteases, we evaluated the biophysical characteristic of ECM and noted prometastatic alterations in collagen microstructure in the presence of MUC16.

Conclusion

Our preliminary findings suggests that MUC16 can increase collagen expression and alignment, in order to facilitate metastatic spread. Delineating this axis could improve our understanding of the pathobiology of PDAC and provide new opportunities to manage this disease.

EVALUATING KIT PROTO-ONCOGENE TRANSCRIPTIONAL MECHANISMS

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ABSTRACT:

Hematopoiesis is maintained by hematopoietic stem and progenitor cells (HSPCs), which balance the production and destruction of all blood cells throughout the body. HSPCs also respond to stress induced by anemia, infections, and cancer, showcasing their role in maintaining hematological balance. Hematopoietic growth factors are essential to promote blood-producing HSPC activities. Stem Cell Factor (SCF) – a growth factor mediating cell signaling through the proto-oncogene receptor Kit - promotes HSPC survival and proliferation in normal and stress contexts. Activating mutations in the Kit receptor or downstream signaling mediators which deregulate Kit signaling causes hematologic diseases including leukemia. While many cellular outcomes of Kit signaling are established, chromatin targets of this pathway are poorly understood. Associating chromatin sites with Kit-response signatures (Kit Response Elements, KREs) can elucidate signal activities and mechanisms of transcriptional control. ***I hypothesize that defining chromatin states at Kit response elements will reveal essential mechanisms controlling gene transcription and HSPC activities.*** Understanding how (and where) KREs regulate transcription will uncover fundamental principles and novel therapeutic approaches to target malignant and non-malignant hematological disorders.

To identify and prioritize KRE's (cis-elements) regulating critical hematopoietic processes, we stimulated human CD34+ HSPCs with 10 ng/mL SCF and collected RNA after 1 hour. The SCF-stimulated RNA-sequencing data was compared to hundreds of published datasets that analyze chromatin state in hematopoietic cell types. Using this approach, we generated individual signatures of putative KRE accessibility, activity, and occupancy of known hematopoietic transcription factors (e.g. GATA2, GATA1, TAL1, LDB1, LMO2 and others). Annotating chromatin signatures to SCF-regulated gene loci resulted in a list of putative KREs, representing a repertoire of Kit signaling activity. We previously used similar prioritization approaches to identify an enhancer within Sterile Alpha Motif (SAM) domain protein-14 (Samd14) protein which is required for red blood cell regeneration in acute anemia. Mechanistically, the Samd14 is an important component of SCF/Kit signaling. To prioritize cis-elements and identify features of cis-element enriched at SCF-regulated genes, I developed a predictive model trained by integrated -omics data. We have identified features predictive of SCF-response by Random Forest approach. The predicted KREs were ranked based on occupancy of GATA1, TAL1, LDB1 and ATRX transcription factors, histone marks associated with active enhancer regions, chromatin accessibility, and enhancer-promoter interactions predicted by promoter capture HiC analysis. SCF upregulated genes were compared with gene sets enriched downstream of other Receptor Tyrosine Signaling (RTK) pathways, revealing only a small subset (24 out of 90) that shared overlapping targets with Kit signaling. Notably, a substantial portion of these genes are associated with the inflammatory response, suggesting crosstalk between Kit signaling and inflammatory pathways. This comprehensive exploration helps uncover KRE activators controlling intricate molecular dynamics of Kit signaling activity. Among transcriptional targets, SCF regulates transcription factors such as EGR1 (Early Growth Response - 1), with known roles in promoting cell survival, proliferation, and differentiation of HSPCs. To identify whether KRE activity is dependent on SCF-mediated EGR1 transcription, I developed a model using CRISPR-interference to repress its expression, revealing cohorts of EGR1-sensitive KREs. As one example, I discovered that the ETS2 gene, another important hematopoiesis-promoting transcription factor, contains an EGR1-sensitive KRE. We are currently testing whether additional predicted KREs with unique molecular signatures have similar functional properties, or whether distinct regulatory mechanisms are operating within subsets of KREs to regulate HSPC activities. By preventing the SCF-mediated upregulation of EGR1, we can confirm a direct link between EGR1 and Kit regulation. However, since many predicted KREs are not EGR1-occupied, we expect to identify additional mechanisms that are operating at the transcriptional level.

Conclusions: The chromatin profiles associated with signal responsive cis-elements (downstream of Kit activation) were unknown. We identified 280 KREs annotated with their chromatin status, and prioritized the chromatin features that were predictive of SCF response - GATA1, TAL1, ATRX and LDB1 - using a Random Forest Model. Moreover, we functionally validated a SCF-dependent transcription factor, EGR1, and linked its activity to regulation of subsets of SCF-regulated genes. Of the identified KREs, 16 of them were occupied by at least one of the predicted transcription factors along with accessibility and enhancer activity. Our ongoing work will evaluate changes in chromatin accessibility and occupancy induced by SCF stimulation at KREs, and determine the extent of EGR1-dependency genome-wide. Our long-term goals include linking these transcription prediction models to identifying new regulatory mechanisms of hematopoiesis. KREs may represent new susceptibility loci for investigating onset of hematological diseases that involve Kit signaling, including Acute Myeloid Leukemia, myelodysplastic syndrome, and anemias.

TARGETING TRANSLATIONAL CONTROL IN CANCER

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Background: Cancer cells rely on hyperactive protein synthesis to reprogram the proteome to sustain the transformed phenotype. By enabling upregulation of oncogenes such as Cyclin D1, Snail, VEGF, etc., aberrant eIF4E-dependent translation (commonly referred to as cap-dependent translation) is a major contributor to various hallmarks of cancer, ultimately driving tumorigenesis, metastasis, and therapeutic resistance. Thus, aberrant protein translation has emerged as a promising therapeutic target. Cap-dependent translation initiation is a tightly regulated process that requires the assembly of the eIF4F translation initiation complex. The rate limiting component of the eIF4F complex is eIF4E, which binds to the 5' cap structure present in most mRNAs to initiate translation. Cap-dependent translation is kept in check by the translation suppressor 4E-BP1; in its active form, 4E-BP1 sequesters eIF4E to prevent translation initiation. 4E-BP1 is activated through dephosphorylation at multiple sites by protein phosphatase 2A (PP2A), a heterotrimeric phosphatase that requires a scaffold subunit, a catalytic subunit, and an interchangeable regulatory B subunit that gives the phosphatase broad targeting capabilities. Cancer cells use several mechanisms to bypass 4E-BP1 translational suppression, including inactivation of PP2A; aberrant mTOR-mediated phosphorylation and inactivation of 4E-BP1; and profound suppression of 4E-BP1 expression. We have found that the activity of 4E-BP1 can be restored in tumor cells using small molecule activators of PP2A, or SMAPs, a novel class of compounds that can reactivate selected PP2A heterotrimers and are being actively developed for use as anticancer agents in patients. Remarkably, we have also made the unexpected discovery that SMAPs/PP2A can upregulate 4E-BP1 expression, even in tumor cells that lack expression of the protein.

Significance of Problem: Inactivation or loss of 4E-BP1 is prevalent in most cancer types (e.g., colorectal, pancreatic, lung, breast), resulting in aberrant translation of oncogenic proteins and thereby contributing to tumor development and progression. Loss of 4E-BP1 function also plays a significant role in resistance to current targeted therapeutics such as ERK, mTOR, and MEK inhibitors. Thus, restoration of 4E-BP1's translation suppressive activity is anticipated to (a) promote anti-cancer activity (a) combat therapeutic resistance, and (c) introduce a novel anticancer therapeutic approach.

Hypothesis, Problem, or Question: This study is investigating the mechanism(s) by which SMAP compounds lead to restoration of the expression and activity of 4E-BP1 for inhibition of cap-dependent translation and tumor suppression.

Experimental Design: A panel of SMAP compounds (e.g., lead compound DT-061) is being used to restore PP2A activity in colorectal (CRC) and pancreatic cancer (PDAC) cell lines (e.g., HCT-116, SW-620, CAPAN-1, MiaPaCa2). SMAP effects on 4E-BP1 mRNA levels and 4E-BP1 transcription were measured using RT-qPCR and the Click-iT™ Nascent RNA Capture Kit (ThermoFisher Scientific), respectively. RT-qPCR and a transcription inhibitor (Actinomycin D) were used to test SMAP effects on ATF4 mRNA accumulation. Protein expression was analyzed via Western blotting and siRNA was used to test the involvement of candidate transcription factors in regulation of 4E-BP1 gene expression. Protein stability was determined using cycloheximide chase assays, proteasomal degradation was tested using the proteasome inhibitor MG-132, and the involvement of potential mediators (e.g., GSK-3β and PKD1) was tested using pharmacological inhibitors. The potential role of the deubiquitinase DUB3 is being tested with mutant constructs and Phos-tag Western Blotting.

Results/Data: We have found that SMAPs dephosphorylate and activate 4E-BP1, leading to suppression of cap-dependent translation. We have also determined that SMAPs restore the expression of 4E-BP1 mRNA and protein in 4E-BP1-deficient tumor cells by inducing transcription of the 4E-BP1 gene. We discovered that treatment of CRC and PDAC cells with SMAPs leads to upregulation of the transcription factor, ATF4, as well as loss of Snail/Slug, transcription factors that have been implicated in positive and negative control of 4E-BP1 gene expression, respectively. Based on these findings, follow up studies explored (a) the mechanisms underlying SMAP/PP2A induced ATF4 upregulation and Snail/Slug downregulation, and (b) the role of these factors in mediating SMAP-induced restoration of 4E-BP1 expression. RT-qPCR analysis revealed upregulation of ATF4 mRNA by SMAPs and use of the transcription inhibitor Actinomycin D pointed to a transcriptional mechanism. Snail downregulation was prevented with the proteasome inhibitor, MG132, indicating that SMAP-induced Snail loss is due to proteasomal degradation. Use of the GSK-3β inhibitors, LiCl and CHIR99021, and the PKD1 inhibitors, BIM and Gö6976, excluded canonical mechanisms of Snail degradation in the effects of SMAPs. Ongoing studies are exploring the role of inactivation of the Snail deubiquitinase, DUB3. We found that Snail loss does not fully explain the upregulation of 4E-BP1 by SMAPs, and siRNA knockdown experiments revealed that ATF4 plays a requisite role in 4E-BP1 upregulation by SMAPs/PP2A. We have also discovered that SMAPs dephosphorylate and activate TFE3 and TFEB, transcription factors that induce ATF4 transcription, and have shown that siRNA knockdown of TFE3 and TFEB blunts ATF4 and 4E-BP1 upregulation by SMAPs.

Conclusions: SMAPs activate select PP2A heterotrimers to upregulate the transcription factor ATF4 via a transcriptional mechanism involving TFE3/TFEB. SMAPs also downregulate Snail/Slug expression via a GSK3β and PKD1-independent proteasomal degradation mechanism that may involve inactivation of DUB3. Deficiency of ATF4 abrogates SMAP/PP2A-induced upregulation of 4E-BP1, pointing to a key role of this transcription factor in PP2A-mediated regulation of 4E-BP1 gene expression. Relief of transcriptional repression of 4E-BP1 by Snail/Slug may also contribute to the effect. Exploration of the role of these factors in transcriptional regulation of 4E-BP1 is ongoing using overexpression strategies. Studies are also ongoing to establish mechanisms of SMAP-induced Snail/Slug degradation. Together, the data support the use of SMAPs for restoration of 4E-BP1 function and translation control in tumors. We further anticipate that understanding of the mechanisms leading to restoration of 4E-BP1 expression and function will point to novel biomarkers of therapeutic efficacy.

TARGETING HOMOLOGOUS RECOMBINATION DEFICIENT CANCERS WITH SMALL MOLECULE INHIBITORS OF RPA:RAD52 PROTEIN-PROTEIN INTERACTION

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Background:
In day-to-day life DNA is exposed to various endogenous and exogenous insults that can cause different types of lesions. Of the several types of lesions, double-stranded breaks (DSBs) in DNA are very common and extremely toxic to cells. The cells are equipped with repair machinery to recognize and repair such breaks. There are two major repair pathways that are involved in repairing DSB, i.e., homologous recombination (HR) and non-homologous end joining. Of these two repair pathways, HR repair of DNA is the most accurate as it employs a sister chromatid to serve as a template to repair the break. When DSBs are recognized, they are resected and processed by exonucleases that result in a 3' overhang to which Replication protein A (RPA) binds and protects the single-stranded DNA (ssDNA). RPA needs to be displaced from the ssDNA for RAD51 to bind to the DNA. Rad51 binding and formation of a stable nucleofilament on the 3' overhang is a critical step for strand invasion and HR-mediated repair of DSB. This step of displacing RPA and loading RAD51 to the ssDNA is facilitated by the BRCA protein complex. HR-deficient cancers typically have a defect in one of the components of the BRCA protein complex. In that case, they tend to use an alternative (minor) pathway that utilizes RAD52 to displace RPA from ssDNA and repair DSB via HR.

Significance of Problem:
Mutations in genes involved in HR are drivers of tumor progression. Inaccurate repair of DSB will cause genomic instability and eventually lead to cell death. This presents a vulnerability of HR-deficient tumors that can be leveraged for strategic therapeutic targeting of these cancers. The alternative HR pathway is key for the survival of tumors with defective HR repair pathways. RAD52 addiction of such cancer cells can be exploited to induce cell death via synthetic lethality. RPA:RAD52 interaction is critical to displace RPA and allow the binding of RAD51 to the ssDNA. Inhibition of RPA:RAD52 interaction will impair alternative HR pathways in the HR-deficient cancer cells.

Hypothesis:
We hypothesize that inhibiting RPA:RAD52 protein-protein interaction will disrupt RAD52-mediated HR. This will result in genomic instability in cells, which would prove to be lethal to BRCA-deficient cancer cells.

Experimental Design:
We screened over 100,000 compounds from three different chemical libraries using a fluorescence-based protein-protein interaction assay (FluorIA) and identified eleven compounds as potential inhibitors of RPA:RAD52 interaction that were ranked based on their IC_{50} values. We tested the effects of the compounds on the viability of BRCA1/2 deficient cells using Presto Blue®. We are currently characterizing the potential inhibitors' interaction with RAD52 using differential scanning fluorimetry (DSF).

Results:
Of the 11 compounds that were identified in the FluorIA as potential inhibitors of RPA:RAD52 protein-protein interaction, three compounds were FDA-approved, and two out of these three showed decreased cell viability in BRCA-deficient cell lines (Al-Mugotir et al., 2021). Our findings suggest that the other eight hits have little or no effect on viability, but we continue to evaluate their synergistic effect with the three FDA-approved drugs. Our DSF experiments findings will demonstrate the drugs' binding to RAD52.

Conclusions:
Through a high-throughput screening assay, we identified potential inhibitors of the alternative HR pathways that HR-deficient tumors rely upon for their DSB repair. Our findings demonstrate that compounds inhibiting RPA:RAD52 interaction can induce cell death via synthetic lethality in BRCA-deficient cancers without affecting BRCA-proficient cells. We will further characterize the interaction of these inhibitors with RAD52 to optimize a drug's selectivity for RPA:RAD52 interaction and have an improved synergistic therapeutic outcome.

THE RE-CLASSIFICATION OF A *PMS2* VARIANT OF UNDETERMINED SIGNIFICANCE USING OUR FUNCTIONAL GENOMICS MISMATCH REPAIR *IN VITRO* MODEL.

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Background: Lynch syndrome (LS) is the most common hereditary cancer affecting an estimated 1 in 279 people. LS is caused by germline heterozygous mutations in mismatch repair (MMR) genes. While not directly cancer-causing, mutations in LS genes increase the number of somatic mutations over time, increasing cancer risk. *PMS2* is the most frequently mutated LS gene. However, the pathogenicity or functional effect of most missense *PMS2* variants is unknown leaving most classified as variants of uncertain significance (VOUSs).

Significance of Problem: There is a great need to re-classify *PMS2* VOUSs for improved clinical diagnostics and management as 90% of identified *PMS2* missense variants are classified as VOUSs. Our recent identification of a suspected hereditary cancer family carrying a missense variant in *PMS2* (c.86G>C; MAF: 0.000440 across all races) had conflicting interpretations of pathogenicity in ClinVar, prompting the need for an *in vitro* assay by which *PMS2* variants could be functionally assessed in a high-throughput way.

Hypothesis: We hypothesized that a portion of *PMS2* missense VOUSs have decreased MMR activity, which will increase cancer risk over time.

Experimental Design: For this study, we designed an *in vitro* assay by which *PMS2* variants could be functionally assessed in a fast and high-throughput way. CRISPR/Cas9 engineering was used to knockout *PMS2* expression in the HAP1 haploid cell line (MMR proficient) by targeting exon 7 of the canonical transcript. Single cell clones were isolated via limiting dilution and validated to confirm disruptive (frameshifting or nonsense) variants via Sanger sequencing and western blot. MMR function was quantified by cell viability after 72 hours of 6-thioguanine (6-TG) treatment. To test whether MMR function could be rescued in the *PMS2* KO line, full-length, wild-type (WT) human *PMS2* was cloned into the pCW57.1 Gateway vector and packaged into lentiviral particles. The *PMS2* KO lines were infected, selected with G418, and treated with doxycycline to induce WT *PMS2* expression. Using site-directed mutagenesis on the pCW57.1-WT *PMS2* plasmid, we generated one benign and pathogenic–confirmed in ClinVar–missense variant as well as the c.86G>C variant (VOUS) identified locally in 4 confirmed carriers of a suspected hereditary cancer family. Individual variants were again packaged into lentiviral particles and used to infect *PMS2* KO lines.

Results: The CRISPR-Cas9 knockout (KO) mutation in *PMS2* (insA) was confirmed by Sanger sequencing and western blot analysis. Regarding MMR function, two independent *PMS2* KO lines were significantly more resistant to 6-TG compared to the wild-type HAP1 parent line ($p<0.0001$; Tukey's test) indicating reduced MMR activity. Induced WT *PMS2* expression rescued 6-TG sensitivity ($p<0.0001$; Tukey's test) in *PMS2* KO cell lines. While the known pathogenic variant conferred significantly decreased MMR sensitivity ($p<0.0001$; Tukey's test), the VOUS was not significantly different from WT *PMS2* suggesting a benign classification.

Conclusions: In summary, we present a novel *in vitro* MMR assay for the high-throughput classification of human *PMS2* missense variants that can contribute to the re-classification of VOUSs identified in human patients—as seen with the *PMS2* c.86G>C variant.

MUC4 ALTERS THE COURSE OF PROGRESSION OF PDAC PRECURSOR LESIONS AND FAVORS IPMN-MEDIATED PDAC DEVELOPMENT

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Background & Significance: Pancreatic Ductal Adenocarcinoma (PDAC) is an incalcitrant malignancy with a median 5-year survival of only 12%, and targeting PDA precursors is an area of the highest priority. Amongst the most prevalent and easily identifiable high-risk individuals with PDAC precursors are patients with intraductal papillary mucinous neoplasm (IPMN). However, the tumor biology of IPMN and its mechanism of progression to invasive cancer and PDAC is grossly understudied. This gap in the field is an obstacle to adequately managing IPMN patients and developing therapeutic strategies. Currently, patients with IPMN are managed with active surveillance (AS) and surgery in case of high-risk attributes. IPMN and PDAC, along with other malignancies, are characterized by the aberrant expression, glycosylation, and localization of mucins as they develop from normal tissue to malignant cells. IPMN manifests as a massive dilation of the intra-pancreatic ducts due to the copious mucus (mucins) production, implicating mucin production as the hallmark of IPMN. Among all de novo expressed mucins, MUC4 is expressed significantly in the gastric IPMN (high-risk sub-type) and other subtypes with increasing levels as the degree of dysplasia advances. Our previous studies and literature demonstrated that MUC4 expression is critical in the progression of IPMN. Hence, investigating the role of MUC4 could provide a better understanding of the initiation of IPMN and the mechanistic action of IPMN-mediated PDAC.

Hypothesis: MUC4 is vital in developing IPMN and malignant progression of IPMN-mediated PDAC.

Experimental Design: We have generated and characterized human MUC4 transgenic (MUC4Tg: tetoMUC4;tTA; PdxCre) mice that conditionally express human MUC4 in the pancreas. In this Tet-off system, the expression of MUC4 can be switched off by adding tetracycline/doxycycline. The above mice were crossed with LSL$Kras^{G12D}$ mice to generate KC-MUC4 (tetoMUC4;tTA; $Kras^{G12D}$; PdxCre) mice. The pancreata of different age groups are harvested and further evaluated for MUC4 involvement in IPMN development and progression compared to PDAC mouse models: KC and KPC. The tissue samples, cell lines, and organoids derived from this model are used to characterize further and understand IPMN tumor biology.

Results: The expression of human MUC4 alone in MUC4 transgenic (MUC4Tg: tetoMUC4;tTA; PdxCre) mice did not affect the pancreas or other vital organs. However, Human MUC4 in the presence of KRAS mutation led to the formation of IPMN and PanIN lesions at five weeks of age, while none of the control mice developed IPMN lesions. There was no difference in overall tumor incidence in KC-MUC4 mice with and without doxycycline. Compared to PDAC mouse models, the pancreata of KC mice appeared normal, while KPC mice presented purely with PanIN lesions. The extent of precursor lesions (PanINs) in KC-MUC4 mice was comparable to that observed in KPC. Nonetheless, the lesions were histologically distinct in KPC and KC-MUC4 mice, as PanIN lesions in KC-MUC4 are of higher pathological grade. The pancreata tumor weight was higher across different age groups in KC-MUC4 mice compared to KC mice. KC-MUC4 tumors exhibited a significant increase in tumor cell proliferation and stroma compared to KC mice with no MUC4 expression. Further, MUC4 expression led to widespread metastasis in vital organs.

Conclusion: Our KC-MUC4 model shows the vital association of human MUC4 with the development and progression of IPMN lesions. The formation of IPMN in only KC-MUC4 mice suggests that MUC4 expression alters the progression of pancreatic precursor lesions to PDAC. This model system will allow us to conduct in-depth mechanistic studies of IPMN progression and use it as a pre-clinical model for IPMN.

NUCLEAR PHOSPHOINOSITIDE SIGNALING REGULATES LSD1'S ASSOCIATION WITH EPIGENETIC COMPLEXES

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Background

Phosphoinositides are lipid messengers regulating every aspect of cellular physiology. Contrary to dogmatic belief it has been reported that a significant fraction of phosphoinositides is found in the non-membranous nuclei since their initial discovery in the late 20th century, while cellular functions of nuclear phosphoinositides still remain largely enigmatic. Via proteomic screens, we identified several novel nuclear protein complexes that interact with nuclear-resident phosphoinositide metabolizing enzymes. Amongst, lysine specific demethylase 1 (LSD1, encoded by *KDM1A* gene) which is the first protein shown to remove methylations on histone residues was identified and validated to interact with a phosphoinositide generating kinase PIPKIα (encoded by *PIP5K1A*). Early studies revealed LSD1 as a repressive epigenetic writer, removing transcriptionally activating methylations such as on H3K4. Recent studies, by contrary, have discovered that LSD1 preferentially removes repressive methylations on histones such as on H3K9. The dual function of LSD1 (transcriptionally activating vs. repressing) is believed to be mediated by the epigenetic complexes LSD1 is associated with in the given cellular context. Overexpression of LSD1 is reported in many cancer types including breast, lung, and liver cancer. LSD1 is shown to regulate tumorigenesis such as proliferation, cancer stemness, drug resistance, tissue invasion, and metastasis, while exact molecular mechanisms of how LSD1 exerts tumor promoting functions remains to be elucidated.

Significance of Problem

Non-mutational epigenetic reprogramming is one of the emerging hallmarks of cancers. LSD1, as the first identified histone demethylase, plays a key role in chromosomal structural remodeling and tumorigenesis in several cancer types including breast cancer. Yet, our understanding of how LSD1 functions in tumorigenesis is incomplete. To this end, our identification of a novel interaction of LSD1 with PIPKIα is important. Our current data points out that a nuclear phosphoinositide species generated by PIPKIα serves as a cofactor facilitating LSD1 interactions with a transcriptionally activating epigenetic complex, promoting breast cancer tumorigenesis by regulating the expression of oncogenes such as *EGFR* which has a pivotal role in breast cancer. Our study provides key insight into the unprecedented roles of nuclear phosphoinositides in the regulation of LSD1-driven epigenetic gene expression in breast cancer.

Hypothesis

Considering the transcriptionally activating role of LSD1, we aim to identify novel interacting proteins of LSD1 and the underlying mechanisms of their interactions that cancers could be utilizing to activate oncogenic pathways. We hypothesize that phosphoinositides generated by nuclear phosphoinositide kinases, including PIPKIα, regulate the interactions between LSD1 and other epigenetic regulators such as ZMYND8. This phosphoinositide-driven epigenetic complex regulates aberrant oncogenic transcription programs in cancer cells.

Experimental Design

Our previous proteomic screens identified a novel interaction of PIPKIα with LSD1. The PIPKIα-LSD1 interaction was validated in triple negative breast cancer cells, MDA-MB-231 and MDA-MB-468, using co-immunoprecipitation, and *in vitro* binding assays. Agonist-stimulated changes of PIPKIα-LSD1 were monitored by co-immunoprecipitation and proximity ligation assay (PLA) imaged with confocal microscopy. In many instances, proteins that interact with phosphoinositide-metabolizing enzymes also bind to phosphoinositide species. Via sequence analyses the putative phosphoinositide binding region on LSD1 was identified. LSD1 and binding-region-deleted-LSD1 (generated by site-directed mutagenesis) binding to all seven phosphoinositide species were measured via *in vitro* binding assays. Cellular interactions and changes by agonist stimulation of LSD1 with phosphoinositides were monitored by PLA. Cellular functions of the PIPKIα-LSD1 nexus in breast cancer tumorigenesis were monitored by measuring known LSD1 transcription targets after depleting PIPKIα and LSD1 expression using RNAi. LSD1 has low substrate specificity *in vitro* and LSD1 is targeted to cellular substrates via interactions with other epigenetic regulators. The switches of LSD1 interactions with known LSD1 binding epigenetic regulators were analyzed upon agonist stimulation and PIPKIα depletion via co-immunoprecipitation and PLA.

Results/Data

Previous work by our lab identified LSD1 as a potential binding partner to PIPKIα via mass spectrometry. This was validated by co-immunoprecipitation of LSD1, and other known LSD1 interacting proteins, with PIPKIα. *In vitro* binding assay of recombinant LSD1 and PIPKIα demonstrated that the PIPKIα-LSD1 interaction is direct. The PIPKIα-LSD1 interaction and other known LSD1 interacting proteins' associations were increased upon agonist stimulation such as serum, EGF, and TNF-α in breast cancer cells. Also, *in vitro* binding assay of recombinant LSD1 to phosphoinositide conjugated beads showed that LSD1 can bind to the enzymatic product of PIPKIα, $PI4,5P_2$, and other phosphoinositide species $PI3,5P_2$ and $PI3,4,5P_3$. A polybasic motif (composed of a stretch of basic amino acids) within the intrinsically disordered region of LSD1 was identified as a binding site for these phosphoinositides. Cellular interactions of $PI4,5P_2$ (P=4.448E-9) and $PI3,4,5P_3$ (P=1.146E-6), but not $PI3,5P_2$, with LSD1 were altered in response to agonists. The $PI4,5P_2$-LSD1 interaction was significantly reduced (P=6.314E-7) by PIPKIα depletion, indicating that $PI4,5P_2$-generated by PIPKIα was responsible for $PI4,5P_2$ association with LSD1. The depletion of PIPKIα reduced LSD1 interaction with ZMYND8 (P=2.78E-26), leading to the attenuation of EGFR expression in breast cancer cells. The phosphoinositide binding-defective mutant LSD1 showed reduced interaction with ZMYND8, suggesting that the phosphoinositide binding controls LSD1 interaction with ZMYND8.

Conclusions

Given the data, we have identified a LSD1 containing epigenetic complex as a novel interactor of PIPKIα. LSD1 binds to $PI4,5P_2$ generated by PIPKIα in the LSD1-PIPKIα complex. The $PI4,5P_2$ binding of LSD1 controls the LSD1-ZMYND8 epigenetic complex formation to promote the expression of *EGFR* in breast cancer cells. Our study revels previously unknown roles of nuclear phosphoinositides in epigenetic gene expression in breast cancer.

TARGETING CYCLIN K ATTENUATES TUMOR GROWTH AND CONFERS THERAPEUTIC VULNERABILITY IN PANCREATIC CANCER

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Abstract:

Pancreatic cancer is the fourth leading cause of cancer death in the U.S., with a five-year survival rate at only 12%. Identifying critical molecular target underlying pancreatic cancer growth and drug resistance will provide therapeutic opportunities for this recalcitrant disease. Cyclins and cyclin-dependent kinases (CDKs) play versatile roles in promoting malignant attributes; therefore, they are actively targeted to improve cancer treatment. Currently, many inhibitors are developed against CDKs, of which four CDK4/6 inhibitors are approved by the FDA and many CDK inhibitors are examined in clinical trials. Importantly, the advancement of targeted protein degradation technologies makes it possible to target the previously “undruggable” cyclins. Despite the limited availabilities of degraders against cyclins, there are several Cyclin K-degrading drugs, indicating its promising druggability. Cyclin K is a transcriptional cyclin, and by forming complex with CDK12 or CDK13, regulates gene transcription. Studies have shown that Cyclin K is involved in essential biological processes, such as DNA damage response, mitosis, and pre-replicative complex assembly. Nevertheless, the function of Cyclin K in pancreatic cancer is unknown.

Here, we investigate the role of Cyclin K in cell growth (Aim 1) and therapeutic sensitivity (Aim 2) in pancreatic cancer. To examine how Cyclin K regulates pancreatic cancer growth, the Cyclin K-inducible knockdown and overexpression cell lines were established. The growth rate of pancreatic cancer cells was evaluated *in vitro* and *in vivo* by cell culture proliferation assay, immuno-deficient models, and immuno-competent mouse models. RT^2 cell cycler array and RNA-Seq were used to unravel the downstream targets of Cyclin K. To explore the translational value of Cyclin K-targeted combination therapies, different Cyclin K-degrading drugs, including PROTAC (*i.e.*, 7f and 9069), molecular glue (*i.e.*, HQ461 and NCT02), and Cyclin K-degrading inhibitor (*i.e.*, SR4835), were obtained. The efficiency of these drugs against Cyclin K was examined by western blotting in different pancreatic cancer cell lines. Of note, the drug effect of 7f and 9069 on tumors was monitored in immuno-competent mice. The synergism of Cyclin K depletion and chemotherapeutic insults, specifically GemTaxol (gemcitabine plus Taxol) and PARP inhibitors (PARPi, olaparib or niraparib), was assessed by examining the cell apoptosis markers cleaved PARP or cleaved caspase 3.

In Aim 1, our data show that Cyclin K knockdown impairs pancreatic cancer cell proliferation, whereas Cyclin K overexpression exerts an opposite effect. Loss of Cyclin K leads to tumor growth inhibition in mouse models and tumor regression/clearance in immuno-competent mice. Cell cycle RT^2 array screening identifies CDC20 as the target of Cyclin K mediating its role in cell proliferation. RNA-Seq results rank immune response among the top 40 altered gene ontology (GO) processes, with many immune-related genes listed as significantly changed genes by Cyclin K depletion. In Aim 2, we find that all these Cyclin K-degrading drugs trigger a marked Cyclin K degradation *in vitro*. Furthermore, 7f and 9069 are effective against Cyclin K and restrict tumor growth *in vivo*. Moreover, Cyclin K ablation, either by Cyclin K molecular glue (HQ461) or Cyclin K knockdown, renders pancreatic cancer cells more vulnerable to GemTaxol or PARPi treatment. Together, these findings demonstrate that Cyclin K is a powerful player controlling pancreatic cancer growth and therapeutic response, and in particular, a druggable target for pancreatic cancer treatment.

Keywords: pancreatic cancer; Cyclin K; degrader; CDC20; immune response; therapeutic sensitivity