56th ANNUAL MIDWEST STUDENT BIOMEDICAL RESEARCH FORUM

Saturday, March 8, 2025

ROOM 3040

10:00 a.m.	O-49	POST-TRANSCRIPTIONAL CONTROL OF SRSF9 AND EMT IN COLORECTAL CANCER Presenter: Priyanjali Mukherjee
10:15 a.m.	O-37	NOVEL ROLE OF SLC22A3 IN SEROTONIN TRANSPORT AND SUBSEQUENT REGULATION OF THE PANCREATIC CANCER STEMNESS MACHINERY Presenter: Nivedeta Krishna Kumar
10:30 a.m.	O-78	EXPANDING PROTAC SCOPE WHILE TARGETING FBXO21 FOR AML THERAPY Presenter: Suchita Vishwakarma
10:45 a.m.	O-76	GLYCOSYLTRANSFERASES AT THE HELM OF ORGAN SPECIFIC METASTASIS OF PANCREATIC DUCTAL ADENOCARCINOMA Presenter: Venkatesh Varadharaj
11:00 a.m.	O-72	LUTEINIZING HORMONE ACTIVATES HIPPO SIGNALING PATHWAY IN STEROIDOGENIC LUTEAL CELLS Presenter: Farzaneh Tamanaeifar
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11:30 a.m.	O-13	VALOSIN-CONTAINING PROTEIN P97 EXTRACTS CAPPING PROTEIN CP110 FROM THE MOTHER CENTRIOLE TO PROMOTE CILIOGENESIS Presenter: Rahit Dewanji
11:45 a.m.		**LUNCH**

POST-TRANSCRIPTIONAL CONTROL OF SRSF9 AND EMT IN COLORECTAL CANCER

Priyanjali Mukherjee, Chaitra Rao, Deepan Chatterjee, Dianna H. Huisman, Robert A. Svoboda, Siddesh Southekal, Heidi M. Vieira, Chittibabu Guda, Kurt W. Fisher, Olga A. Anczuków, Robert E. Lewis* (UNMC Omaha, NE)

Background, Significance and Hypothesis: Colorectal cancer (CRC) is the second-leading cause of cancer-related deaths in the United States. (Siegel RL., et al, CA Cancer J Clin., 2024) Approximately 43% of CRC cases involve KRAS mutations, which activate the RAS/MAPK pathway and are linked to significantly worse prognosis compared to wild-type KRAS. (McCall, J. L., et al, Molecular and Cellular Biology, 2016). Signaling through the Raf/MEK/ERK scaffold protein, KSR1 is critical in CRC tumor initiation, chemoresistance, and epithelial-to-mesenchymal transition (EMT). Prior analysis of EMT-related transcript translation revealed that Epithelial Stromal Interaction 1 (EPSTI1) is preferentially translated in a KSR1-dependent manner in CRC cells and that EPSTI1 is necessary and sufficient for E- to N-cadherin switching, playing a critical role in promoting tumor cell migration and invasion. The mechanism by which KSR1 drives TIC formation, facilitates the transition of TICs to DTPs and regulates post-transcriptional control of downstream effectors such as EPSTI1 remains unclear. A comprehensive understanding of KSR1-regulated RNA splicing and downstream effectors in RAS-mutated CRC cells could reveal novel vulnerabilities for therapeutic exploitation. **We hypothesize** that KSR1 governs RNA splicing by modulating RNA-binding proteins, a mechanism essential for driving EMT in CRC. Loss of KSR1 is expected to trigger widespread changes in RNA profiles, shedding light on previously unidentified regulators and pathways of alternative splicing that fuel colorectal cancer pathogenesis.

Experimental Design: To identify post-transcriptional regulators involved in KSR1-dependent transcriptional control of EMT, the PRADA tool (Prioritization of Regulatory Pathways based on Analysis of RNA Dynamic Alteration) (Yu et al. Cancer Discovery 2020) was employed on RNA sequences isolated from polysomes in HCT116 KSR1 knockdown (KD) cells and KD cells in which KSR1 expression had been restored through the expression of a KSR1 transgene. This analysis compared RNA-binding protein (RBP) profiles in the presence and absence of KSR1, identifying oncogenic RBPs and their roles in cellular dynamics. Using CRISPR/Cas9 system, KSR1 was knocked out (KO) in colorectal cancer cells, HCT116 and SW480 and the cells were subjected to Western blot analysis to assess the effect of KSR1 knockout on the levels of RBP SRSF9. To understand if SRSF9 phenocopies KSR1, siRNA-mediated SRSF9 KD in HCT116 and SW480 was followed by cell migration and invasion using an IncuCyte Zoom-based scratch assay and a Transwell assay with Matrigel coating, respectively. RNA was isolated from polysomes of both control and KSR1-depleted cells and analyzed using rMATS Turbo v4.1.0. To investigate the interaction between SRSF9 and EPSTI1, ribonucleoprotein immunoprecipitation (RIP) was performed on native ribonucleoprotein (RNP) complexes. RIP was carried out using anti-SRSF9 or rabbit IgG pre-coated protein A-Sepharose beads in control and SRSF9 KD HCT116 and SW480 CRC cells.

Data and Results: PRADA revealed that the binding site for eight RBPs were affected by KSR1 knockdown, amongst which the regulatory potential of the consensus binding sites for SRSF9 were markedly decreased upon KSR1 depletion. Western blot analysis revealed that SRSF9 expression levels were suppressed by KSR1 KO and that the expression levels were reinstated to normal when KSR1 was reintroduced with the help of a transgene. SRSF9 RNAi reduced cell migration by more than 50% and invasion through Matrigel by more than 75%. SRSF9 RNAi also decreased N-cadherin expression while increasing E-cadherin expression in HCT116 cells, underscoring its critical role in promoting CRC cell EMT, migration and invasion. rMATs based analysis of differentially spliced events (DSE) between total and polysome-bound mRNA in presence and absence of KSR1, revealed that KSR1 depletion resulted in the accumulation of differentially spliced transcripts in the polysomes of both cell lines (HCT116 and SW480s). Subsequent RIP experiments revealed that EPSTI1 mRNA immunoprecipates with SRSF9 in both CRC cell lines, HCT116 and SW480.

Conclusion and Future Directions: These findings identify a KSR1-dependent and SRSF9-regulated pathway in CRC cells that orchestrates pre-mRNA splicing and regulates migratory behavior, a key driver of cancer cell dissemination and metastasis. The data suggests that SRSF9 interacts with EPSTI1, an essential mediator of EMT in CRC cells and may be responsible for regulating EPSTI1 splicing. We hypothesize that SRSF9 functions as a key intermediary linking KSR1-dependent splicing with downstream mRNA translation in support of EMT. To study this we will express an alpha-tagged SRSF9 in KSR1 KO CRC cells and evaluate their migratory and invasive behavior. To determine the extent to which SRSF9 disruption mimics the effect of KSR1 KO on mRNA translational efficiency, we will perform long read RNA-seq on total and polysome bound RNA in KSR1 KO cells ± SRSF9 to assess the extent to which polysome-bound mRNAs are preferentially spliced in an SRSF9-dependent manner. Enhanced cross-linking immunoprecipitation combined with PCR (eCLIP-PCR) will identify polysome-bound mRNAs with SRSF9 binding sites, as candidate effectors of EMT.

NOVEL ROLE OF SLC22A3 IN SEROTONIN TRANSPORT AND SUBSEQUENT REGULATION OF THE PANCREATIC CANCER STEMNESS MACHINERY

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Background and Significance: Cancer stem cells (CSCs) are a small subpopulation of cells that can 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, the molecular mechanism for 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: *Embryonic and pancreatic progenitor stemness signatures emerge in pancreatic cancer to regulate and maintain the stemness potential and self-renewal; possibly being the origin of pancreatic cancer.*

Experimental Design/Methods and Results: By performing computational analyses of gene signatures shared between embryonic stem cells, CSCs, and PC cells using publicly available datasets, we identified several putative stemness-associated genes, including SLC22A3/OCT3. Our results reveal that SLC22A3/OCT3 is highly expressed in CSC sub-type population and pancreatic tumor tissues and organoids derived from Kras; PdxCre (KC) and Kras;p53; PdxCre (KPC) mouse models, while its expression is minimal or absent in normal pancreatic tissues. Functional studies demonstrated that knockdown (KD) of SLC22A3/OCT3 in PC cell lines significantly reduced spheroid size in serum-free 3D cultures, indicating a decline in the CSC population. Flow cytometry further confirmed a marked reduction in the side population (SP) fraction in SLC22A3/OCT3 KD clones compared to controls. *In vivo*, SLC22A3/OCT3 depletion led to reduced tumor burden and impaired oncogenic dedifferentiation, a hallmark of CSCs in PC. SLC22A3/OCT3, a transmembrane transporter, facilitates the uptake and efflux of organic cations, including monoamine neurotransmitters such as serotonin, dopamine, and norepinephrine. Interestingly, serotonin supplementation increased stemness potential in PC cells, but this effect was absent in SLC22A3/OCT3 KD clones, implicating serotonin transport as a key mechanism in CSC regulation.

Conclusions and Future Perspectives: Mechanistically, we propose that SLC22A3/OCT3 regulates serotonin transport in pancreatic CSCs, activating downstream pathways that enhance transcription factor binding and upregulate self-renewal and pluripotency-related genes. This process may involve serotonin-mediated epigenetic modifications, including serotonylation, to maintain CSC stemness and drive pancreatic tumor aggressiveness. Our findings highlight SLC22A3/OCT3 as a critical regulator of serotonin-driven CSC stemness in PC. *Understanding its role in CSC biology may provide novel insights into pancreatic cancer pathogenesis and open avenues for the development of targeted therapies aimed at disrupting CSC-mediated tumor progression.*

EXPANDING PROTAC SCOPE WHILE TARGETING FBXO21 FOR AML THERAPY

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Proteolysis-targeting chimeras (PROTACs) are small molecules that use the cellular ubiquitin-dependent proteolysis system to efficiently degrade targeted disease-causing proteins. PROTACs are heterobifunctional molecules with two ligands conjugated by a linker. The ligand at one end binds to the protein-of-interest (POI) while the other binds to an E3-ligase. In cells, PROTACs facilitate the formation of a POI:PROTAC:E3-ligase sandwich that drives POI ubiquitination by an E3-ligase. Although the genome codes for over 600 E3 ligases, only a few (CRBN or VHL-targeted ligands) are currently amenable to the PROTAC strategy, this limits the clinical opportunities and therefore there is a need to explore additional E3 ligases for therapeutic application. In this study, we present the discovery of a ligand that selectively binds to FBXO21, an E3 ligase. Exploring FBXO21 stems from its overexpression in Acute Myeloid Leukemia (AML) correlating with a significantly worse prognosis. We hypothesize that developing a binder for FBXO21 will not only add a new E3 ligase to the PROTAC toolbox but can also be potentially used in AML therapy. Currently, there are no known small-molecule modulators for FBXO21. Using cheminformatics tools, we identified peptide sequences that bind to the FBXO21 substrate binding site. We docked FBXO21 substrate (p85α/EID1)-derived peptides into the alpha fold FBXO21 structure using Schrödinger's GLIDE. Analysis revealed that the p85α/EID1 peptide's glutamic acid side chains interacted with FBXO21 YccV domain residues within 3Å distance. Moreover, these substrate peptides adopted an alpha-helical conformation. Borrowing the Hamilton lab strategy, we conducted iterative design and docking studies with substituted bi- or terphenyl backbones to mimic p85α/EID1 binding to FBXO21. We synthesized a set of analogs and evaluated them in *in vitro ubiquitination* assays. We identified 57-057, a substituted terphenyl analog that inhibited FBXO21-mediated ubiquitination of p85α but not FBXW7-mediated ubiquitination of Myc or FBXO9-mediated ubiquitination of DPPA5, thus demonstrating exquisite selectivity. Consistent with this observation MOLM13 cells treated with 57-057 showed a dose-dependent increase in p85α levels, suggesting the disruption of FBXO21-p85α interactions. Moreover, the FBXO21 binders inhibited the growth of AML (HL60 and MOLM13) cell lines with low-nM potencies. This preliminary investigation reports the discovery of a new E3 ligase binder. We are conducting structure-activity relationship studies to identify suitable positions on 57-057 to conjugate a linker and demonstrate proof-of-concept for expanding the PROTAC toolbox by developing CDK6 degraders using FBXO21 binders.

GLYCOSYLTRANSFERASES AT THE HELM OF ORGAN SPECIFIC METASTASIS OF PANCREATIC DUCTAL ADENOCARCINOMA

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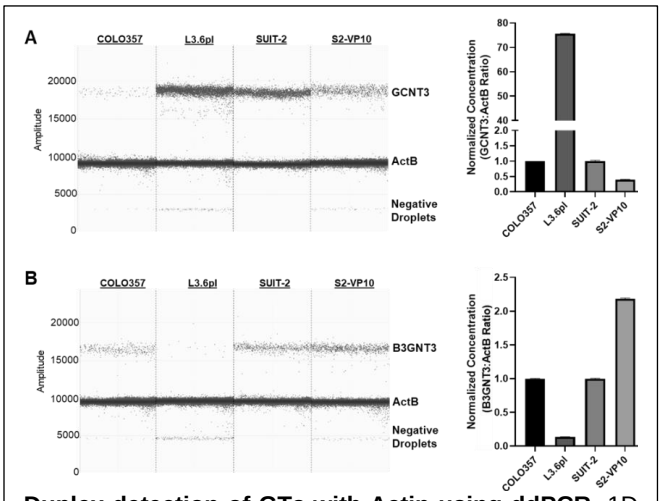
Background: The most common cause of mortality in cancer patients is the metastasis of neoplastic cells. Pancreatic cancer (PC) is not different in this trend, where patients majorly develop lung and liver metastasis, and an overall five-year survival rate is less than 14% despite advances in therapy. Glycosylation is one of the critical post-translational modifications in proteins, lipids, and even recently identified in RNA that orchestrate the addition of glycan structures using glycosyltransferase (GT). The expression of GTs is instrumental in modulating the phenotype of malignant cells and promoting cancer cell survival at the metastatic niche. Specifically, we found that upregulation of GCNT3 (glucosaminyl (N-acetyl) transferase 3) and B3GNT3 (beta-1,3-N-acetylglucosaminyltransferase 3) expression are key mediators in altering glycosylation patterns in PC cells and that enhance the metastatic proclivity towards liver and lung. Regardless of the specific proteins exhibiting aberrant glycan signatures, probing the pattern of glycosyltransferase expression is currently a potential avenue for cancer therapy by disrupting the molecular factors that support tumor malignancy and metastasis.

Significance of Problem: Malignant transformation of cancer cells is primarily associated with modifying tumor-cell glycosylation, a critical contributor to metastatic organotropism. However, the mechanistic underpinnings of glycosylation organ-specific metastasis remain poorly understood. Understanding the roles of glycosyltransferases in organotropism will provide valuable targets for cancer diagnosis, prognosis, and therapy.

Hypothesis: The unique expression of GCNT3 and B3GNT3 in pancreatic cancer cells drives liver and lung-specific metastasis.



Duplex detection of GTs with Actin using ddPCR: 1D droplet plots for A) GCNT3/ActB and B) B3GNT3/ActB. Error bars for 95% Poisson confidence interval.

Experimental Design: The COLO357 and SUIT-2 PC cells were used to develop organ-derived metastatic subline models like L3.6pl (Liver) and S2-VP10 (Lung) using orthotopic and tail vein injections of consecutive organ-enriched passages. We generated the second metastatic model by orthotopically injecting less number of PC cell line SUIT2 into the pancreas and harvested both lung and liver metastatic cells to avoid the discrepancy of different cell lines used for lung and liver metastasis in the first model. These two models replicate key aspects necessary to study the lung and liver-specific metastasis of PC. The RT^2 PCR array was used to analyze the gene expression of GTs in lung and liver metastatic models, providing insights into the altered GT expressions associated with metastasis. The qPCR analysis was performed on a selected panel of human GT genes involved in glycosylation modification, revealing varying GT expression levels, and focusing on two key GTs based on further validation assays. The droplet digital PCR **(figure)** and western blot analysis were used to confirm the differential expression of GTs at both the RNA and protein levels. The GCNT3 and B3GNT3 were knocked out using the CRISPR Cas9 system to observe the difference in migratory potential. Immunohistochemistry and Immunofluorescence experiments on the primary and metastatic PC tissues validated the specific expression of GTs in lung and liver metastatic tissues.

Results: We examined the expression patterns of GCNT3 and B3GNT3 at both the RNA and protein levels to assess their roles in lung and liver-specific metastasis. An unbiased transcriptional screen revealed a reciprocal >2-fold increase in GCNT3 and B3GNT3 expression in the L3.6pl and S2-VP10 metastatic cell lines, respectively. Further, dd-PCR results showed an increased number of DNA copies for GCNT3 (>100-fold) and B3GNT3 (>3.5-fold) in L3.6pl (liver) and S2-VP10 (lung) cells, respectively, compared to the PC controls. The western blot analyses confirmed that these metastatic liver and lung clones have distinct glycosyltransferase protein expression compared to the parental pancreatic cancer cells. The CRISPR KO of GCNT3 and B3GNT3 showed significant differences in the migratory potential of the liver and lung metastatic cells. These results were further validated in human tissue from the same patient's primary pancreatic tumor and metastatic lung and liver tissues collected through the UNMC Rapid Autopsy program (UNMC-RAP). Our IHC studies on RAP tissues validated that the GCNT3 was significantly overexpressed in liver metastatic tissues, and B3GNT3 was overexpressed in lung metastatic tissues. Further, Immunofluorescence studies reflected the same expressional pattern and highlighted the clinical significance of glycosyltransferase differential expression in the metastasis of pancreatic ductal adenocarcinoma.

Conclusion: Our data suggest that a clonal population of pancreatic cancer cells that highly express GCNT3 and B3GNT3 give rise to liver and lung metastasis, respectively, and highlights the correlation between glycosylation and organ-specific metastasis.

LUTEINIZING HORMONE ACTIVATES HIPPO SIGNALING PATHWAY IN STEROIDOGENIC LUTEAL CELLS

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Background, Significance, Hypothesis: Approximately 10-20% of human pregnancies end in miscarriage, with 80% occurring in the first trimester. A critical factor in early pregnancy success is the corpus luteum (CL), a transient endocrine gland formed from the ruptured follicle after ovulation. The CL produces progesterone, a hormone essential for embryo development, implantation, and maintenance of pregnancy. Insufficient CL function or progesterone production can lead to preterm pregnancy loss, posing significant challenges for both humans and livestock. Luteinizing hormone (LH), a gonadotropin from the pituitary gland, stimulates small luteal cells (SLCs) to initiate acute progesterone synthesis. Despite its critical role, the downstream mechanisms by which LH regulates steroidogenesis remain poorly understood, highlighting the need for further investigation. One potential mechanism involves the Hippo signaling pathway, a highly conserved regulator of cellular processes essential for ovarian homeostasis. The Hippo pathway includes kinases MST1/2, LATS1/2, and transcriptional co-activators YAP1 and TAZ. Activation of the Hippo pathway leads to the phosphorylation and cytoplasmic retention of YAP1 and TAZ, preventing them from entering the nucleus and inhibiting gene transcription. We hypothesized that LH activates Hippo signaling pathway in bovine steroidogenic SLCs and this activation is required for progesterone synthesis.

Experimental Design: To test this hypothesis, we cultured enriched populations of SLCs collected from mid-cycle bovine CLs and treated them with LH (10 ng/mL) for 1, 2, and 4 hours. Conditioned media was collected to measure progesterone concentrations, protein lysates were harvested to analyze post-translational modifications of Hippo signaling molecules, and RNA was extracted for sequencing analysis. Additionally, to determine the role of YAP1 and TAZ in steroidogenesis in bovine SLCs, we overexpressed mutant forms of YAP1 (YAPS127A) and TAZ (TAZS89A), which are resistant to LATS1/2 kinase phosphorylation and thus remain constitutively active, and subsequently measured progesterone concentrations.

Data and Results: We report that LH acutely stimulated progesterone synthesis and induced phosphorylation of LATS1, YAP1, and TAZ indicating Hippo pathway activation ($P<0.05$). RNA-seq analysis using IPA predicted inhibition of YAP1/TAZ nuclear localization as an early upstream event in LH signaling. Overexpression of constitutively active mutant YAPS127A and TAZS89A resulted in loss of LH-induced progesterone production ($P<0.05$).

Conclusion: Our findings provide novel insights into the molecular mechanisms by which LH regulates progesterone production in bovine SLCs. We demonstrated that LH stimulates the activation of the Hippo signaling pathway and that disrupting YAP1/TAZ function through overexpression of constitutively active mutant forms of YAP1 and TAZ impairs LH-induced progesterone production. These results highlight the critical role of Hippo signaling in luteal cell steroidogenesis. Together, our findings emphasize the importance of the Hippo signaling pathway in maintaining optimal progesterone synthesis and open potential therapeutic avenues for addressing luteal insufficiency.

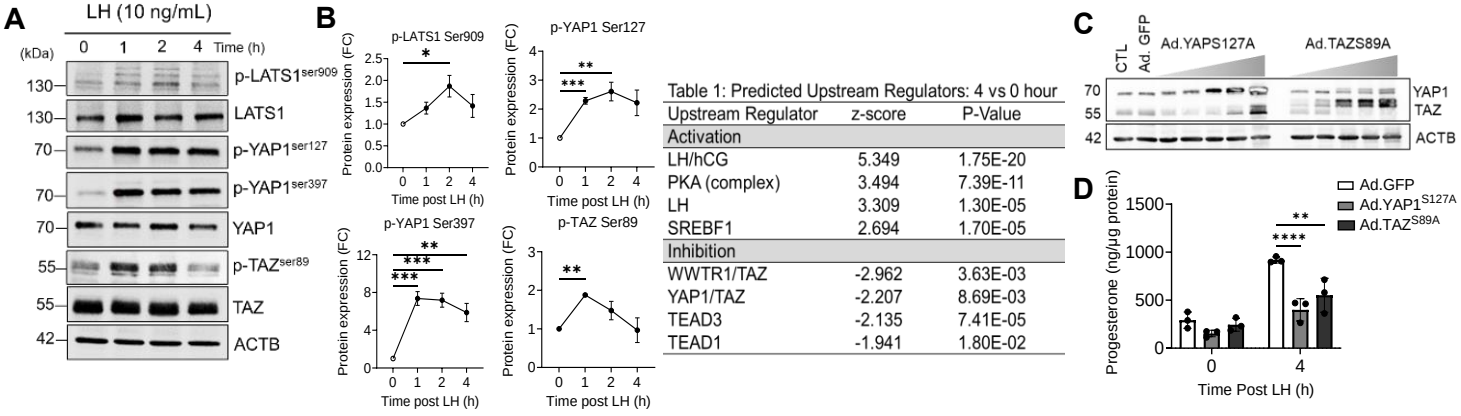

Table 1: Predicted Upstream Regulators: 4 vs 0 hour

Upstream Regulator	z-score	P-Value
Activation		
LH/hCG	5.349	1.75E-20
PKA (complex)	3.494	7.39E-11
LH	3.309	1.30E-05
SREBF1	2.694	1.70E-05
Inhibition		
WWTR1/TAZ	-2.962	3.63E-03
YAP1/TAZ	-2.207	8.69E-03
TEAD3	-2.135	7.41E-05
TEAD1	-1.941	1.80E-02

STRUCTURE-GUIDED DRUG DESIGN TARGETING GERANYLGERANYL DIPHOSPHATE SYNTHASE

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Background: Several incurable cancers are characterized by abnormal protein production and secretion. One prime example is multiple myeloma where monoclonal protein is excessively produced due to malignant plasma cell proliferation. Aberrant production and secretion of these cancer-related proteins lead to increased disease progression indicated by enhanced metastasis, tumor growth, and drug resistance. As MM is incurable with a high chance of developing drug resistance as the disease progresses, new treatments are highly desirable. One promising molecular target is geranylgeranyl diphosphate synthase (GGDPS) which synthesizes the geranylgeranyl diphosphate (GGDP) group. An important family of proteins which rely on geranylgeranylation are the intracellular trafficking Rab proteins. Studies have demonstrated that proper Rab geranylgeranylation is necessary for proper trafficking of these abnormal cancer proteins and continued cancer cell survival. By disrupting Rab geranylgeranylation we can disrupt monoclonal protein trafficking. This results in monoclonal protein buildup within the cancer cell, activation of the unfolded protein response, and apoptosis. In conjunction with a multi-disciplinary team, we are working to structurally characterize a pair of highly potent GGDPS inhibitors.

Significance of Problem: Multiple myeloma accounts for 10% of all hematological malignancies and is estimated to have affected over 34,000 individuals in the United States in 2021. Of these 34,000 people, over 1/3 are expected to die (12,000). While clinical advancements have been made in treating MM over the years, patients will eventually become either resistant or intolerant to current therapies. Therefore, there is a need to develop new therapeutic strategies targeting this malignancy. Additionally, GGDPS is continuing to be revealed as an important player in other disease models such as pancreatic ductal adenocarcinoma, prostate cancer, and Ewing sarcoma. Therefore, the development and characterization of GGDPS inhibitors has broad implications in cancer therapy.

Hypothesis: Our collaborators have synthesized new potent stereoisomeric inhibitors with high specificity for GGDPS deemed the homogeranyl (HG) and homoneryl (HN) compounds. Interestingly, each stereoisomer individually demonstrates impressive GGDPS IC50 values but exhibits even better inhibition when used as a mixture. We hypothesize that the stereoisomers bind preferential sites, one to the GGDP product site and the other to the FDP substrate site. We hope to use this knowledge to further assist in structure-based GGDPS drug design.

Experimental Design: To answer how these stereoisomers differentially bind GGDPS, we will use crystallography and cryo EM to solve the structure of our protein bound by these inhibitors. We first express and purify GGDPS using immobilized metal affinity chromatography and size exclusion chromatography. Crystallization conditions are initially screened using a 96-well block and are optimized until crystals can be reliably grown and diffract well. For cryo EM, our protein is frozen onto carbon grids where blot times, protein concentration, and grid types are optimized to ensure a variety of orientations and proper particle distribution.

Results: We were able to solve the most complete and highest resolution human GGDPS crystal structure to date (1.67 Å) with the endogenous GGDP product bound. Our structure contains the complete C-terminus and backbone of a flexible loop which has yet to be resolved in a human GGDPS structure. In addition, we have solved a yeast crystal structure and the first human GGDPS cryo EM structure with the HN drug bound. These structures show that HN binds the active site in a similar manner to the FDP substrate, which is inline with initial docking studies. Positively charged lysine and arginine residues are seen to interact with the negative bisphosphonate head, with negatively charged aspartic acids chelating magnesium ions. The hydrophobic tale of the HN compound is stabilized by hydrophobic leucines.

Conclusion: GGDPS co-purifies and crystallizes to high diffraction with its endogenous product. The HN compound binds both human and yeast GGDPS in the FDP substrate site. Important structure activity relationships include negatively charged aspartic acids which chelate magnesium. These magnesium ions stabilize the negatively charged bisphosphonate head along with lysine and arginine residues. Van der Waals interactions between a hydrophobic channel made of leucine residues help stabilize the hydrocarbon tail of the HN compound.

VALOSIN-CONTAINING PROTEIN P97 EXTRACTS CAPPING PROTEIN CP110 FROM THE MOTHER CENTRIOLE TO PROMOTE CILIOGENESIS

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Background: The primary cilium is a non-motile, microtubule-based extension from the plasma membrane that serves as a key signaling organelle and is generated in most mammalian cells. Aberrant primary ciliogenesis leads to a variety of developmental disorders known as ciliopathies. A key early step in primary ciliogenesis is the removal of the capping protein, CP110, a negative regulator, from the distal end of the mother centriole. Several dozen proteins have been implicated in CP110 removal, and recent studies highlight a requirement for its ubiquitination and subsequent proteasomal degradation for ciliogenesis to occur. A central unanswered mechanistic question is how ubiquitinated CP110 is extracted from the mother centriole to facilitate its proteasomal degradation. Valosin-containing protein (p97), a member of the ATPase Associated with diverse Activities (AAA) protein family has been recently implicated in the extraction and/or unfolding of ubiquitinated substrate proteins for their proteasomal degradation. p97 is involved in the regulation of a wide range of cellular processes including degradation, DNA damage responses and various forms of autophagy. Interaction with ubiquitinated client proteins is often mediated by a series of diverse adapters. To date, neither p97 nor any of its adapters have been implicated in the crucial step of CP110 extraction from the mother centriole, and its unfolding and degradation required for ciliogenesis. Our findings demonstrate a novel role for p97 in the process of primary ciliogenesis and support a mechanism by which ubiquitinated CP110 is degraded via p97-mediated unfolding and removal from the mother centriole.

Significance: The work elucidates how polyubiquitinated CP110 is extracted from the mother centriole, by implicating p97 in the unfolding and degradation of CP110. The identification of p97 as a critical mediator of CP110 removal provides a novel entry point for therapeutic interventions targeting ciliopathies. Furthermore, it opens new avenues for research into the interplay between AAA ATPase proteins, ubiquitination, and organelle assembly.

Hypothesis: We hypothesize that p97 is essential for the extraction, unfolding, and degradation of ubiquitinated CP110 at the mother centriole during ciliogenesis. Using serum-starved retinal pigmented epithelial (RPE-1) cells and fibroblasts, we show that ciliogenesis is impaired upon either p97 inhibition or depletion, resulting in CP110 accumulation at the centriole. Notably, CP110 depletion rescues ciliogenesis even when p97 is inhibited, indicating that p97 mediates ciliogenesis through CP110 removal. We also demonstrate that p97 inhibition enhances CP110 ubiquitination during serum starvation and identify a p97-CP110 interaction, supporting a role for p97 in CP110 proteasomal degradation. This study identifies p97 as a key regulator of CP110 turnover and provides mechanistic insight into the early steps of ciliogenesis, a process critical for cellular signaling and preventing ciliopathies caused by defective primary cilia biogenesis.

Experimental Approaches: To validate the role of endogenous p97 in primary ciliogenesis, we pharmacologically inhibited p97 with potent inhibitors NMS-873 and CB5083 in RPE-1 and mouse embryonic fibroblast (MEF) cells and used ARL13B as a marker to quantify ciliated cells. To further confirm p97's role in ciliogenesis, we used siRNA to deplete p97 levels in serum starved cells before quantifying the percentage of ciliated cells. Next, we demonstrated that CP110 depletion reverses the impaired ciliogenesis observed upon p97 inhibitor treatment, illustrating that p97 functions in tandem with CP110. We also assessed the ubiquitination of CP110 in serum starved RPE-1 cells. To determine whether endogenous CP110 and p97 interact, we performed coimmunoprecipitations in serum-starved RPE-1 cells.

Results: 1) Inhibiting p97 by NMS-873 and CB-5083 impeded cilia formation in RPE-1 and MEF cells. 2) p97 knock-down by siRNA decreased primary ciliogenesis in RPE-1 cells. 3) Knock-down of CP110 by siRNA rescued ciliogenesis upon p97 inhibition. 4) p97 inhibition enhanced CP110 ubiquitination in serum starved RPE-1 cells. 5) CP110 and p97 interacted in serum starved RPE-1 cells.

Conclusion: This study identifies p97/VCP as a key regulator of primary ciliogenesis, driving the extraction, unfolding, and proteasomal degradation of ubiquitinated CP110 at the mother centriole. Pharmacological inhibition or siRNA-mediated depletion of p97 impairs ciliogenesis by disrupting CP110 removal, a critical step in cilia formation. Remarkably, CP110 depletion rescues ciliogenesis upon p97 inhibition, underscoring its pivotal role as a substrate of p97. The observed interaction between p97 and CP110, coupled with enhanced CP110 ubiquitination upon p97 inhibition, highlights a mechanistic link between ubiquitin-proteasome system dynamics and ciliary assembly. These findings deepen our understanding of ciliogenesis and provide a foundation for developing therapeutic approaches targeting ciliopathies associated with defective cilia biogenesis.