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Qianlie Beixi Capsules treat diabetic induced erectile dysfunction by regulating the Hif-1α/VEGF pathway: an in vivo animal study

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Abstract

Background: Diabetes-induced erectile dysfunction (DIED) is a common complication of diabetes. Qianlie Beixi Capsules, a traditional Chinese medicine, has been used to treat it.

Materials and Methods: Streptozotocin (STZ) was used to induce diabetes in rats to establish the diabetic model, and apomorphine (APO) was used to screen for the erectile dysfunction model. Rats were divided into 6 groups: blank control group, model group, Qianlie Beixi Capsules low-dose group, Qianlie Beixi Capsules medium-dose group, Qianlie Beixi Capsules high-dose group, and HIF-1α inhibitor group. After two weeks, we measured the levels of T, LOX-1, and EMPs in rat serum, as well as bladder histology. Western blot and real-time fluorescent quantitative PCR (RT-qPCR) were used to detect the expression of HIF pathway-related proteins (mTOR, p70s6k, HIF-1α, VEGF, eNOS) in penile corpus cavernosum tissue.

Results: The rat penile erectile times was increased in rats in the QZ group and QG group ($p<0.05$). QZ group and QG group improved penile tissue pathology, increase the density of smooth muscle cells, reduce the amount of collagen fibers, blood sinus in a small amount of red blood cells. QZ group and QG group rat corpus cavernosum tissues HIF - 1 alpha and VEGF protein expression decreased ($p<0.05$). The expression of eNOS in the corpus cavernosum was increased in the QZ and QG groups ($p<0.05$). The expression of LOX-1 and EMPs in serum of rats in QZ and QG groups decreased ($p<0.05$).

Discussion and Conclusion: Qianlie Beixi Capsules can improve endothelial cell function in penile corpus cavernosum and inhibit penile corpus cavernosum endothelial fibrosis through the mTOR/HIF-1α/VEGF pathway, thus treating DIED.

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Extracellular Vesicle Delivery of CRISPR/Cas9 to Target Fatty Acid Metabolism in Prostate Cancer Cells

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Abstract

Background: Androgen deprivation therapy (ADT) is the first line of treatment for advanced prostate cancer (PCa). However, ADT can lead to the development of tumors that grow independently of androgen receptor (AR) pathway and become resistant to AR signaling inhibitors (ARSIs). AR-independent PCa cells exhibit elevated expression of ACSL4, which leads to dysregulation of fatty acid metabolism. This overexpression results in the enrichment of long-chain unsaturated fatty acids, promoting cell proliferation, migration, and invasion. Our previous research demonstrated that ACSL4 is a potential therapeutic target for AR-independent prostate cancer, as its knockdown significantly reduced cell proliferation both *in vitro* and *in vivo*. Utilizing CRISPR/Cas9 technology presents an opportunity to permanently modify the ACSL4 gene, targeting the dysregulated fatty acyl-CoA biosynthesis pathway in prostate cancer cells. However, CRISPR/Cas9 requires an effective delivery vehicle. We have established that extracellular vesicles (EVs) are capable of efficiently packaging CRISPR/Cas9 through N-myristoylation of Cas9 ribonucleoprotein (RNP).

Methods: Lentiviral vectors expressing myristoylated Cas9 (mCas9) were created by fusing an octapeptide derived from the N-terminus of Src kinase to the N-terminus of Cas9 gene. Three individual candidate single guide RNAs (sgRNAs) targeting different locations on the ACSL4 gene were bicistronically cloned into the lentiviral vector. The product was confirmed via Sanger sequencing. The knockout potential of the three different constructs were examined by transfection and lentiviral infection. Transduced cells were analyzed by western blotting, sequencing, and T7 analysis. EVs were produced by calcium chloride precipitation with mCas9-sgRNA-ACSL4 and VSV-G vectors. EVs were analyzed by transmission electron microscopy (TEM), particle tracking analysis, and western blotting. EVs were administered to recipient HEK-293T cells. Recipient cells were analyzed by next generation sequencing (NGS).

Results: Sequencing revealed that sgRNA-ACSL4 was successfully cloned into the mCas9 lentiviral vector. Transfected HEK-293T cells had a 58% knockout efficiency, infected HEK-293T cells obtained a 36% knockout efficiency of ACSL4. Knockout efficiencies were corroborated by decreased ACSL4 protein detected by western blotting. Produced EVs contained mCas9, VSV-G, and EV-specific proteins. EV-treated cells exhibited a 4.3% gene editing efficiency detected by NGS.

Conclusion and Future Directions: N-myristoylated Cas9/sgRNA ribonucleoprotein complex targeting ACSL4 produced an effective knockout. The EVs-mediated delivery of mCas9/sgRNA-ACSL4 successfully enabled gene editing of ACSL4. Future experiments will aim to enhance the efficiency of mCas9/sgRNA-ACSL4 delivery and will investigate targeting other oncogenes by EVs-mediated delivery.

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Prostatic Epithelial Cell Differentiation Lineage

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Prostate cancer (PCa) remains a leading cause of cancer-related deaths among men worldwide. The vast majority of prostate cancers are carcinomas of epithelial origin, underscoring the critical need for a deeper understanding of its biological underpinnings. Central to this endeavor is comprehending the differentiation and lineage of prostatic epithelial cells and identifying potential epithelial stem/progenitor cells within the prostate gland. Additionally, prostatic carcinogenesis often mirrors embryonic development, highlighting the importance of studying prostatic cell lineage differentiation. Understanding these processes can provide essential insights into the origins and progression of PCa, potentially leading to more effective preventive and treatments strategies.

The studies of prostatic stem/progenitor cells have led to several competing hypotheses regarding their identity and role within the prostate. One prominent theory suggests that basal cells are the stem cells of the prostate (1-4). These cells are characterized by their ability to differentiate into various cell types, a crucial feature of stem cells, and are essential for normal tissue regeneration and repair (1-4). Basal cells are thought to maintain the integrity of the prostate epithelium and play a pivotal role in its regeneration (1-4).

Another hypothesis posits that luminal cells, which are typically considered to be terminally differentiated and thus incapable of dividing, may also possess stem cell-like properties (5-7). Recent studies using sophisticated lineage-tracing techniques have shown that under certain conditions, luminal cells can indeed self-renew and generate new cells, suggesting a previously unrecognized plasticity and potential stem cell capacity (5-7).

Furthermore, there is growing evidence supporting the existence of co-expressing cells, which display markers of both basal and luminal cells (8-10). This finding suggests a more fluid, dynamic model of cell differentiation within the prostate, where certain cells may shift between states depending on the physiological or pathological context (8-10). These co-expressing cells could represent a pivotal element in the prostatic stem/progenitor cell differentiation pathway, adding another layer of complexity to the understanding of prostate cell biology (8–10).

Despite these advances, there is currently no consensus on which cell type serves as the definitive stem/progenitor cell of the prostate. One reason is that majority of the studies were carried out in different experimental models. The ongoing debate and research into prostatic progenitor cell identity underscore the need for more refined studies using advanced technologies such as single-cell

sequencing to analyze **innate** human fetus prostate samples and provide a high-resolution molecular atlas of human fetus prostate cells. Our results reveal distinct prostate cell clusters, each exhibiting unique transcriptional profiles and lineage-specific markers. Of particular interest is the identification of prostate progenitor cells, characterized by their highly embryonic stem-like and proliferative properties.

Our analyses suggest that in the human fetus prostate, there are no typical basal or luminal cells or embryonic stem cells; instead, the most undifferentiated cells are co-expressing cells, which give rise to both luminal and basal progenitor cells. The trajectory analysis, confirmed by basal and luminal cell differentiation scores, supports this finding. Furthermore, we demonstrated that the more differentiated cells guide less differentiated cells to undergo further differentiation based on our proposed prostatic epithelial differentiation cell lineage model.

Through trajectory analysis, we delineate the developmental progression of prostate epithelial cells, uncovering a lineage trajectory from progenitor cells to differentiated cell types. Integration analysis with adult prostate epithelial cells further elucidates the developmental continuum of prostate cell populations. Overall, our single-cell transcriptomics analysis provides a comprehensive molecular characterization of human fetus prostate cells, shedding light on their developmental dynamics and lineage relationships. These findings not only deepen our understanding of prostate development but also hold implications for prostate-related pathologies and therapeutic approaches. This approach could provide the resolution necessary to map the cellular lineage accurately and identify the true stem/progenitor cells in the prostate.

References:

1. Goldstein AS, Lawson DA, Cheng D, Sun W, Garraway IP, Witte ON. Trop2 identifies a subpopulation of murine and human prostate basal cells with stem cell characteristics. Proceedings of the National Academy of Sciences of the United States of America. 2008;105(52):20882-7.

2. Pignon JC, Grisanzio C, Geng Y, Song J, Shivdasani RA, Signoretti S. p63-expressing cells are the stem cells of developing prostate, bladder, and colorectal epithelia. Proceedings of the National Academy of Sciences of the United States of America. 2013;110(20):8105-10.

3. Ousset M, Van Keymeulen A, Bouvencourt G, Sharma N, Achouri Y, Simons BD, et al. Multipotent and unipotent progenitors contribute to prostate postnatal development. Nature cell biology. 2012;14(11):1131-8.

4. De Marzo AM, Nelson WG, Meeker AK, Coffey DS. Stem cell features of benign and malignant prostate epithelial cells. The Journal of urology. 1998;160(6 Pt 2):2381-92.

5. Leong KG, Wang BE, Johnson L, Gao WQ. Generation of a prostate from a single adult stem cell. Nature. 2008;456(7223):804-8.

6. Chua CW, Shibata M, Lei M, Toivanen R, Barlow LJ, Bergren SK, et al. Single luminal epithelial progenitors can generate prostate organoids in culture. Nature cell biology. 2014;16(10):951-61, 1-4.

7. Karthaus WR, Iaquinta PJ, Drost J, Gracanin A, van Boxtel R, Wongvipat J, et al. Identification of multipotent luminal progenitor cells in human prostate organoid cultures. Cell. 2014;159(1):163-75.

8. Wang Y, Hayward S, Cao M, Thayer K, Cunha G. Cell differentiation lineage in the prostate. Differentiation. 2001;68(4-5):270-9.

9. Xue Y, Smedts F, Debruyne FM, de la Rosette JJ, Schalken JA. Identification of intermediate cell types by keratin expression in the developing human prostate. The Prostate. 1998;34(4):292-301.

10. Hudson DL, Guy AT, Fry P, O'Hare MJ, Watt FM, Masters JR. Epithelial cell differentiation pathways in the human prostate: identification of intermediate phenotypes by keratin expression. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society. 2001;49(2):271-8.

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FXYD5 plays diverse roles in immune evasion and tumor progression in prostate cancer

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Abstract

Background:

FXYD5 is involved in various biological processes, including inflammation, tumor progression, drug resistance, and hypertension. It has been suggested as a potential biomarker for several cancers, with elevated levels found in ovarian and colon cancer. However, the role of FXYD5 in prostate cancer has not yet been elucidated.

Methods:

The expression levels of FXYD5 were detected by qPCR and western blot. CRISPR/Cas9 sgRNA and cDNA were used to generate FXYD5 knockout and overexpression prostate cancer cell lines, respectively. A CRISPRi screen was conducted to identify the Siglec-7 ligand. Prostate cancer cell proliferation was assessed using cell growth curves and colony formation assays. Cell migration was evaluated through Matrigel migration and scratch assays. Cell invasion was measured using a transwell migration assay. TMRE dye was used for evaluating mitochondrial membrane potential. Lactate and LDH assays were used to evaluate the lactate and LDH levels in cancer cells. A humanized mouse model was utilized to examine the role of FXYD5 in tumor growth and immune evasion. Single-cell RNA (sc-RNA) sequencing was used to assess FXYD5 transcript levels in tumor cells within prostate cancer tissues.

Results:

The expression levels of FXYD5 were higher in androgen receptor (AR)-negative prostate cancer cells compared to AR-positive prostate cancer cells. A CRISPRi screen identified FXYD5 as a potential Siglec-7 ligand. In a humanized mouse model, 22Rv1 tumor growth was inhibited with human immune cell modulation. However, when FXYD5 was overexpressed in 22Rv1 cells, tumor growth was not impacted by immune cell-based therapy. This was due to the interaction of FXYD5 on cancer cells with Siglec-7 on immune cells, thereby inducing immunosuppressive signals. Intrinsically, FXYD5 knockout promoted PC3 cell proliferation, migration, and invasion. *In vivo* studies showed that FXYD5 knockout promoted PC3 tumor growth, while overexpression of FXYD5 inhibited PC3 tumor progression. However, in AR-positive 22Rv1 cells, overexpression of FXYD5 promoted tumor progression. FXYD5 expression was detected in tumor cells by sc-RNA sequencing of human metastatic prostate tumor tissues. In vitro assays indicate that overexpression of FXYD5 in PC3, C42B, and 22Rv1 cells reduces mitochondrial membrane potential

and reactive oxygen species. Additionally, FXYD5 overexpression decreases lactate dehydrogenase and lactate levels in PC3 cells but does not impact these levels in 22Rv1 cells. In PC3 cells, FXYD5 overexpression leads to the formation of tight cell-cell interactions by downregulating membrane markers, while it does not affect cell-cell interactions in 22Rv1 cells.

Conclusion:

FXYD5 suppresses tumor growth in AR-negative prostate cancer cells and promotes tumor growth in AR-positive prostate cancer cells. Overexpression of FXYD5 inhibits immune cell-mediated tumor suppression via a Siglec-7-dependent mechanism. These findings suggest potential therapeutic strategies targeting FXYD5-based mechanisms.

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CD59 is a potential ligand for Siglec-9 in prostate cancer

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Abstract

Background:

Prostate cancer is a leading cause of death in men worldwide. Cancer immunotherapy has made tremendous progress in improving patients' quality and quantity of life. However, despite high immune cell infiltration in tumors, immune-checkpoint inhibitor-based therapy is largely ineffective for prostate cancer. Sialic acid-binding immunoglobulin-type lectins (Siglecs), which bind to sialic acids, are expressed in immune cells. Siglec-7 and Siglec-9, expressed in immune cells including dendritic cells and T cells, have been identified as potential immune checkpoints in various cancers. It is suggested that blocking the interactions between Siglec-7/9 and sialic acids can enhance immune responses and effectively inhibit tumor growth in several cancers. Siglec-9 ligands have been detected in prostate cancer tumor tissues. Despite these findings, the understanding of Siglec-7/9-sialic acid interactions in prostate cancer remains limited.

Methods:

Single-cell RNA (sc-RNA) sequencing was used to assess the Siglec-7/9 transcript levels in immune cells within prostate cancer tumor tissues. Flow cytometry was utilized to assess the expression levels of Siglec-7/9 on immune cells and their ligands on prostate cancer cells. T cell-mediated cytotoxicity was evaluated using a coculture system with GFP labeling on prostate cancer cells. Sialidase and anti-Siglec-7/9 antibodies were used to block the interactions between Siglec-7/9 and their ligands. A CRISPRi screen and mass spectrometry were used to identify Siglec-9 ligands. An in vivo humanized mouse model was further used to examine the inhibition of Siglec-7/9 and their ligand interactions. Immunohistochemistry and immunofluorescence were used to assess the expression levels of CD59 and immune cell markers. The CRISPR/Cas9 sgRNA system was employed to generate CD59 knockout prostate cancer cells.

Results:

High expression of Siglec-7 and Siglec-9 was found on myeloid cells, particularly macrophages, in human prostate tumors using sc-RNA sequencing. Elevated levels of Siglec-7 and Siglec-9 ligands were found in prostate cancer tissues compared to adjacent normal tissues. Further, high expression levels of these ligands and sialic acids were observed in prostate cancer cells. Immunofluorescence analysis confirmed the co-expression of Siglec-7 and Siglec-9 in macrophages within human metastatic prostate cancer bone tissues. Disrupting Siglec-7 and Siglec-9 interactions using anti-Siglec-7 and -9 antibodies inhibited

PC3 and 22Rv1 tumor growth in a humanized mouse model. Immunohistochemistry analysis showed reduced proliferation, increased apoptosis, and enhanced immune cell infiltration in tumors treated with anti-Siglec-7/9 antibodies. A genome-wide CRISPR screen and mass spectrometry suggested that CD59 is a potential Siglec-9 ligand candidate. The CD59 protein band was observed in prostate cancer cell protein lysates by Siglec-9 Fc pull-down assay. Knocking out CD59 reduced Siglec-9-Fc binding capacity and enhanced T cell-mediated cytotoxicity against prostate cancer cells. The expression of CD59 was detected in tumor cells through sc-RNA sequencing of metastatic prostate tumor tissues. Immunofluorescence analysis further validated CD59 expression levels in human prostate cancer tumor tissues.

Conclusion:

Disrupting the interactions between Siglec-7/9 and their ligands inhibits prostate cancer progression, and CD59 is a potential ligand for Siglec-9. These findings provide insight into targeting Siglec-7 and Siglec-9 immune checkpoints and pave the way for the development of novel immune checkpoint inhibitor-based drugs for prostate cancer.

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Tuning T Cell Receptor Immunotherapy Targeting Prostatic Acid Phosphatase for Advanced Prostate Cancer

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Abstract

Late-stage prostate cancer is an incurable disease with no effective therapy currently available. 20-30% of patients receiving local therapy will experience disease relapse. The rise in serum prostate-specific antigen (PSA) level in these patients is often described as biochemical recurrence. This stage of prostate cancer, when micro-metastasis has occurred and overall tumor burden is low, can be a critical time window for cell-mediated immunotherapy. We aim to develop T cell receptor (TCR) immunotherapy targeting prostatic acid phosphatase (PAP) to treat patients with chemically recurrent prostate cancer. Elevated expression of PAP is commonly observed in early and late stages of prostate cancer. PAP was previously used to develop the first FDA-approved cancer vaccine, Provenge, but the specific epitopes and cognate TCRs were not clearly defined. Our group has profiled the immunopeptidome of PAP on HLA-A*02:01 using a secreted MHC-based platform (ARTEMIS), and successfully isolated multiple TCRs reactive with PAP. Recent results have also demonstrated that further engineering with “catch bonds” on these candidate TCRs lead to dramatically improved cytotoxicity both in vitro and in vivo. This work demonstrated the feasibility of developing and enhancing TCRs targeting PAP for potential therapeutic usage.

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A cellular anatomy of healthy human male and female lower urinary tract.

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Abstract

Background: Benign and malignant disease of the bladder, ureter, urethra and prostate are common in aging. Fundamental questions regarding the disparate incidence and progression of lower urinary tract (LUT) disease in sex, age, and ethnicity require a deep understanding of normal. Our goal is to create a cellular reference atlas of normal adult male and female human lower urinary tract using single cell, spatial, and Xenium technologies.

Methods: Bladder, ureter, urethra, and prostate (from males) were collected fresh from surgery and enzymatically digested into a single cell suspension or frozen in OCT. Single-cell RNA sequencing and single nuclear RNA/ATAC sequencing were performed using the 10x Genomics Chromium platform. Data were integrated and analyzed using Seurat. For validation, RNAscope and immunostaining were performed on sections using anchor genes derived from single cell data to localize individual cell types. Spatial transcriptomics was performed using the 10X Genomics Xenium and Nanostring GeoMx platforms and analyzed with provided software.

Results: Several new cell types have been discovered in the male and female LUT. Some of these novel cell types appear to play central roles in various diseases. Each cell type is catalogued by transcriptional and anatomical profile into the Uberon and Cell Ontology databases and raw and processed data are made publicly available through the Human BioMolecular Anatomy Project and the GenitoUrinary Development Molecular Anatomy Project.

Conclusions: Several new cell types have been identified as central to LUT disease. The expansion and transcriptional alteration of an interstitial fibroblast in the periurethral area of the prostate is a new preclinical target in benign prostatic hyperplasia. The identification of antimicrobial club cells in the female urethra is a new preclinical target in urinary tract infections. The high incidence of tertiary lymphoid structures in healthy female vs. male bladder could help explain the 3-fold higher incidence of bladder cancer in men. Identification of novel fibroblasts could help explain the mechanism in which non-muscle invasive bladder cancer progresses into muscle invasive bladder cancer. The comparison of androgen signaling in seminal vesicle and prostate could help us understand only the prostate develops cancer. This healthy male and female LUT cellular atlas will be instrumental for understanding the molecular and cellular basis of disease incidence and progression.

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FOXA2 and ASCL1 Cooperate in Driving Neuronal Lineage Commitment in Prostate Cancer

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Abstract

Background: Potent androgen receptor (AR) pathway inhibitors (ARPIs) are highly effective in castration-resistant prostate cancer (CRPC). However, they play a role in the emergence of alternative lineage programs such as neuroendocrine prostate cancer (NEPC). ASCL1 has emerged as a central regulator of the NEPC phenotype, driving neuroendocrine differentiation. However, ASCL1's influence on neuronal lineage switching and maturation, as well as its partners in NEPC, remains largely unknown.

Methods: Chromatin immunoprecipitation sequencing (ChIP-seq) and RNA sequencing (RNA-seq) were performed to delineate the binding patterns and transcriptional impacts of ASCL1 and FOXA2. Functional assays, including gene knockdown and overexpression studies, were conducted to assess the effects on neuronal differentiation and tumor growth. Additionally, in vitro and in vivo models were used to evaluate the role of PROX1, a key target of ASCL1 and FOXA2.

Results: We provided insights into ASCL1's cistrome reprogramming in ARPI-induced versus terminal NEPC and showed that ASCL1 binding pattern tailors the subsequent expression of transcription factor combinations that underlie discrete terminal NEPC identity. We identified FOXA2 as a major co-factor of ASCL1 in terminal NEPC that it is highly expressed in ASCL1-driven NEPC. ASCL1 and FOXA2 were found to interact and co-regulate a subset of neuroendocrine-associated genes, including PROX1. In vivo studies showed that targeting PROX1 significantly decreased tumor growth, underscoring its role in maintaining the neuroendocrine phenotype.

Conclusion: FOXA2 and ASCL1 interact and work in concert to orchestrate terminal neuronal differentiation in prostate cancer and regulate key neuroendocrine-associated genes including PROX1. Our findings provide insights into the molecular conduit underlying the interplay between different lineage-determinant transcription factors to support the neuroendocrine identity in prostate cancer.

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The fate of renal units under conservative management for PUJO type of hydronephrosis.

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Abstract

The fate of renal units under conservative management for PUJO type of hydronephrosis.

Background - The management of the pelviureteric junction obstruction (PUJO) type of hydronephrosis is a significant and complex issue for pediatric urologists and pediatricians. The fate and natural history of this condition, particularly in symptomatic and asymptomatic children, is a topic of ongoing debate among medical professionals and parents. In this paper, we aim to shed light on this enigma through a systematic review of existing literature.

Material & Methods - The PRISMA Guidelines were used to search, retrieve and analyze the existing literature. Four databases, PUBMED, Embase, Scopus, and Web of Science, were searched with keywords “PUJO”, “Pelviureteric Junction Obstruction”, “Hydronephrosis”, and “Conservative management” with the combination of Boolean operations for relevant articles. These articles were screened by two reviewers, in the case of conflict the third reviewer decided about inclusion of the article. The original articles comparing the conservative management with surgical management for PUJO type of hydronephrosis were included and analyzed. Data specific to the children labelled as 'conservative management group' was collected, their demographic characteristics, renal functions, radiological findings and final outcome at the end of follow-up period was analysed.

Result - The preliminary query in various databases yielded 110 papers. Ninety-nine papers were excluded as they did fit the protocol. A total of 11 original articles were finally analyzed. Total 630 children were diagnosed with PUJO type of hydronephrosis and were not taken upfront for surgery as they did not qualify for surgical indication. The median follow-up duration was 55 months. 105 children underwent pyeloplasty during the course of follow-up for various indications for surgery which appeared during this period.

Conclusion - Given that 17% of children under conservative management eventually require surgical intervention during a median follow-up period of 55 months, it is imperative to re-examine the current indications for surgery in cases of PUJO (pelvi-ureteric junction obstruction) associated hydronephrosis. The criteria for conservative management versus surgical intervention demand rigorous re-evaluation to optimize patient outcomes and ensure evidence-based clinical decision-making.

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Patient-Derived Renal Cell Cancer Organoids as a Model to Assess Therapeutic Targets and Treatment Resistance Mechanisms

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Abstract

Background: Despite progress, response rate of renal cell carcinoma (RCC) to systemic therapy is ~30%. A critical need exists to develop novel therapeutic targets and personalized treatments. Patient derived organoids (PDOs),a 3D *ex-vivo* model system that recapitulate the histological and genomic landscape of parental tumors, represent a novel platform for individualized testing. We performed integrative molecular profiling of patient matched primary tumor tissue and RCC PDOs to identify predictive markers of drug response.

Methods: Tissue samples procured from normal and two separate tumor areas (T1/T2) from prospectively enrolled patients undergoing radical nephrectomy for RCC were molecularly profiled using bulk whole exome, whole transcriptome and single cell RNA sequencing (scRNAseq). This includes a patient undergoing metastasectomy of a peri-bronchial lymph node and a biopsy of a liver metastasis. PDO cultures were established to study responses to 26 clinically available drugs, targeting pathways implicated in RCC and other cancers. Dose response curves were obtained from IC50 experiments. PDO

scRNAseq analysis was performed before and after drug exposure to delineate response and resistance mechanisms.

Results: Eight patients with either clear cell RCC (ccRCC, n=6), papillary RCC (n=1) or chromophobe RCC (n=1) have been enrolled thus far. PDOs were successfully established for 9 normal and 9 tumor samples. Analysis of patient #5 with a ccRCC, revealed an associated copy loss of chromosome 3p region in both T1/T2 samples (**Figure 1**). ScRNAseq revealed high CA9, VEGFA and EGFR gene expression and two distinct tumor cell sub-populations (clusters 0 & 13). PDOs from patient #5 demonstrated sensitivity to Sunitinib targeting VEGFA-VEGFR axis and Neratinib targeting EGFR in both T1/T2. Treatment of PDOs with Neratinib led to loss of cluster-0 tumor cells but a concomitant expansion of cluster-13 tumor cells which notably had high interferon (IFN) gamma and IFN alpha response pathway transcriptomic signatures.

Conclusion: Integration of PDOs, a clinically relevant 3D *ex-vivo* model system, with multi-omics can enable data-driven development of personalized RCC therapies as well as insights into mechanisms of treatment resistance. Ongoing work will provide further insights into tumor heterogeneity, mechanisms of tumor evolution, and therapeutic resistance.

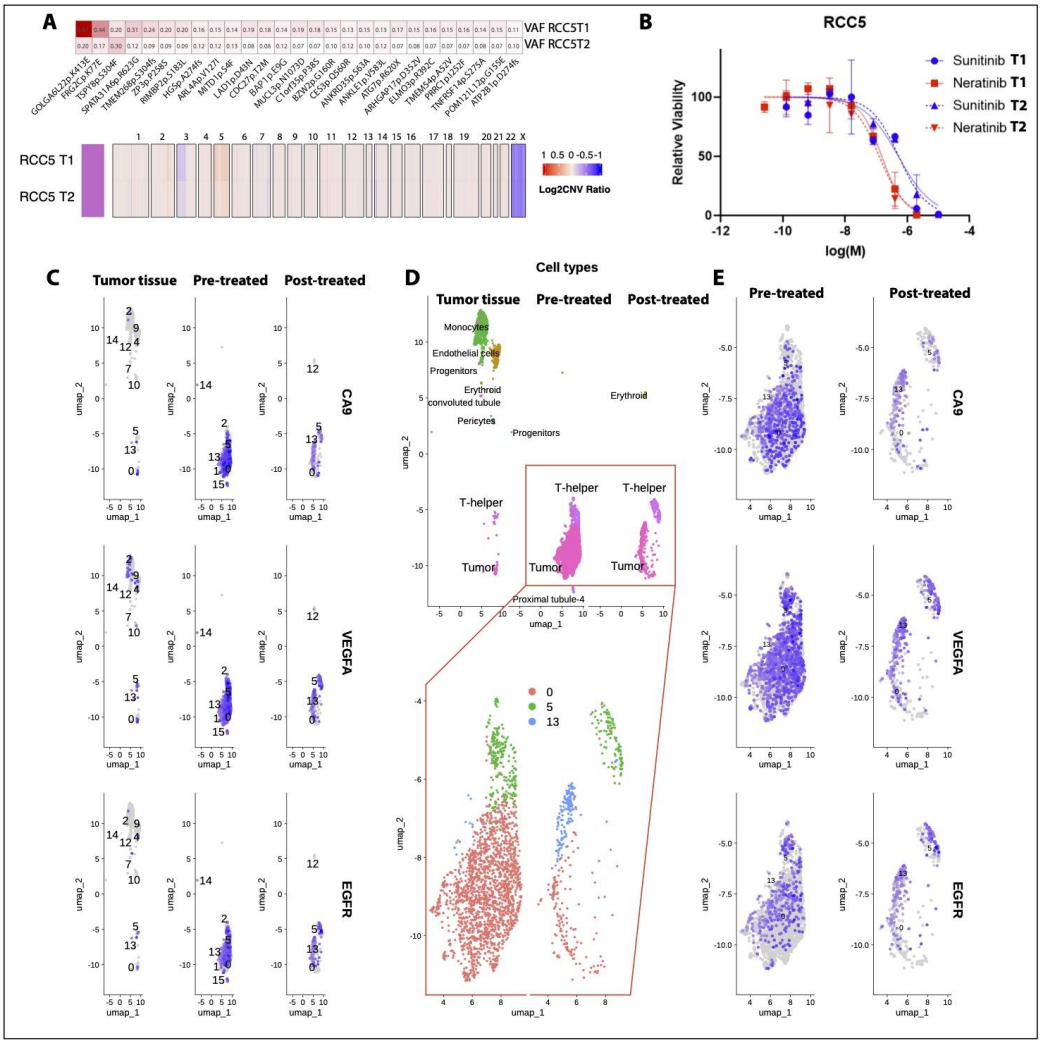

Figure 1: Multiplatform molecular profiling and patient derived organoids (PDOs) model system to personalize treatment for renal cell carcinoma and understand resistance mechanisms. A) Whole exome sequencing several mutations (top panel) and associated chromosome 3p copy loss (bottom panel) in both tumor regions (T1 and T2) from Patient #5 (Copy number variation plot shows genomic copy gains (red) and losses (blue). **B)** Dose response curve of PDOs to Sunitinib (blue) and Neratinib (red) in T1 (solid lines)/T2 (dashed lines). **C)** Cell type clusters from single-cell RNA seq (scRNAseq) data from primary tumor tissue (left panel) and PDOs pre- and post-neratinib treatment (middle and right panels). Tumor cells from both tissue and PDOs exhibited high transcript expression of carbonic anhydrase IX (*CA9*), vascular endothelial growth factor A (*VEGFA*), and epidermal growth factor receptor (*EGFR*) (dark blue = high expression). Numbers represents distinct cell types/populations with PDOs maintaining predominant tumor cell clusters identified in the primary tumor. **D)** Cell type assignment based on scRNAseq, in the order displayed in panel C (top panel). The bottom panel shows the Seurat clusters that correlated to T-helper and tumor cells in PDOs pre- and post-neratinib treatment, repectively. Predominant tumor cell populations (cluster 0 and 13) identified in the primary tumor and propagated in PDOs showed differential response to Neratinib, where cluster 0 responded positively to Neratinib and cluster 13 cells were resistant and expanded on Neratinib treatment. **E)** *CA9*, *VEGFA*, and *EGFR* gene expression in PDOs pre- and post-neratinib treatment with tumor cluster region from the bottom panel in D.

13

Establishment, characterization, and pre-clinical testing of a patient-derived xenograft model of human BPH

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Abstract

BACKGROUND: Benign prostatic hyperplasia (BPH), characterized by hyperplastic growth of stromal and epithelial cells, leads to lower urinary tract symptoms in a large percentage of aging men. Understanding of BPH and developing new mechanism-based therapies have been hampered by the lack of *in vitro* and *in vivo* model systems for this disease. Our objective was to develop and characterize a patient-derived xenograft (PDX) murine model for BPH.

METHODS: Paired BPH transition and normal (N) peripheral zone prostate tissue cores were obtained from 7 patients, precision-cut into slices, weighed, and implanted subrenally into $RAG2^{-/-}C^{-/-}$ male mice (n = 57) supplemented with testosterone. Tissue grafts were explanted after 1 week, 1 mo., 2 mo., or 3 mo., weighed and divided into fixed and frozen samples. Histopathological changes were visualized by H&E and immunohistochemistry using antibodies against Ki-67 and cleaved caspase-3. Proliferation and apoptosis were quantified with QuPath software and statistical differences were determined using a t-test or ANOVA. Serum prostate-specific antigen (PSA) was measured at the time of explant, and molecular analyses were performed using LC-MS.

RESULTS: BPH PDX weights showed striking increases in size compared to N PDXs derived from the same patient over time. Expansion of the epithelial layer and increased serum PSA concentrations were uniquely observed in the BPH PDXs. Proliferation in BPH PDXs was significantly higher than in N PDXs, particularly at 1 mo., and was characterized by an initial increase in stromal cells followed by a larger increase in epithelial cells. Apoptosis was significantly higher in BPH PDXs than N, particularly at 1 week. Molecular profiling revealed gene and protein expression patterns correlating with BPH pathophysiology such as an increased immune and stress response, followed by increased expression of proliferation and BPH-specific stromal signaling molecules (e.g. BMP5 and CXCL13). BPH PDXs showed a significant reduction in size after finasteride treatment. Preliminary experiments are investigating the effects of recombinant BMP5, a stromal signaling molecule implicated in BPH, and BMP inhibitors on N and BPH PDXs.

CONCLUSIONS: Systematic characterization of BPH and N PDXs showed differences in growth, proliferation, apoptosis, and molecular profiles. This PDX model retains features of human tissue and can be used to study BPH pathogenesis, understand the underlying molecular and cellular mechanisms, and identify potential targets for intervention.

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Macrophage-derived SPP1 induces epithelial-mesenchymal transition to a sarcomatoid state and increases PD-L1 expression in renal cell carcinoma

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Abstract

Background: Sarcomatoid dedifferentiation – thought to arise by an epithelial to mesenchymal transition (EMT) - may coexist in any histological subtype and stage of renal cell carcinoma (RCC) and is considered a poor prognostic factor. However, sarcomatoid RCC (sRCC) shows higher response rates to immune checkpoint inhibitors (ICI) than does the most common form of kidney cancer, clear cell RCC (ccRCC). The expression of PD-L1, a target of ICIs, is elevated in sRCC compared to ccRCC, providing a potential mechanism accounting for increased efficacy of ICIs in sRCC versus ccRCC. In this study, we investigated the changes in molecular mechanisms responsible for tumor progression associated with the transition from ccRCC to sRCC and explored potential therapeutic targets.

Methods: A sRCC nephrectomy specimen was probed for 1000 mRNA targets using single-cell spatial transcriptomics (NanoString CosMX) to assess gene expression changes between a total of 23 ccRCC and sRCC regions. RCC cell lines 786-O and UOK127 were used in in vitro assays. Human acute monocytic leukemia cell line THP-1 was differentiated into macrophage-like cells by addition of PMA and used for co-culture with RCC cell lines. Cellular mRNA and protein expression was assessed by q-PCR and Western blotting (WB).

Results: Spatial transcriptomic data showed dense M2-like macrophage infiltrate in histologically sarcomatoid areas. Macrophages in sRCC areas compared to ccRCC areas differentially expressed high levels of SPP1. The expression of EMT genes and PD-L1 was increased in RCC cell lines treated with human recombinant SPP1 (rhSPP1). Furthermore, rhSPP1 was confirmed to activate the Akt pathway and Erk1/2 pathway in RCC cell lines. THP-1-derived M2-like macrophages were confirmed to have M2 polarity and to express SPP1. RCC cell lines co-cultured with these macrophages showed increased expression of EMT-related proteins, suggesting enhanced metastatic potential. In addition, changes were observed in tumor-promoting factors such as CD44, PD-L1, and metallothionein. Knockdown of SPP1 in the macrophages diminished their ability to induce EMT and increase the tumor-promoting factors.

Conclusion: These findings indicate that macrophages in the tumor microenvironment promote EMT in RCC cells via SPP1. Similarly, increased expression of metallothionein and PD-L1 in RCC cells via SPP1 stimulation contributes to tumor progression and immune evasion. These findings suggest that SPP1 is a potential target for the treatment of sRCC.

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Targeting AXL in the bone microenvironment inhibits the growth and metastasis of lethal prostate cancer

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Abstract

Background: After failing primary and secondary hormonal therapy, castrate resistant and neuroendocrine prostate cancer metastatic to the bone is invariably lethal, although treatment with docetaxel and carboplatin can modestly improve survival. Therefore, agents targeting biologically relevant pathways in PCa and potentially synergizing with docetaxel and carboplatin in inhibiting bone metastasis growth are urgently needed.

Methods: Phosphorylated (activated) AXL expression in human bone metastases was assessed by immunohistochemical staining. We evaluated the effects of a novel soluble AXL signaling inhibitor, sAXL (batiraxcept or AVB-S6-500), on the growth of PCa patient derived xenograft models (PDX) injected intratibally and assessed lung metastases in these models. After injection of disaggregated LuCAP tumor cells into the tibiae, tumors were treated with sAXL and docetaxel alone or in combination and growth was monitored by microCT scanning. Tumor burden was quantified by human-specific antigen Ku70 staining, and metastasis to the lung was determined using qPCR of human-specific GAPDH. Transcriptomic profiling, western blotting and immunohistochemistry were performed to identify treatment-induced gene and protein expression changes.

Results: High AXL phosphorylation in human PCa bone metastases correlated with shortened survival time. sAXL as a single agent or in combination with docetaxel or carboplatin significantly suppressed intratibial tumor growth and suppressed metastasis to the lung through multiple mechanisms, including repression of cancer stemness genes (CD44, ALDH1A1, TACSTD2, ATXN1) and of the PI3K, JAK, MAPK, and E2F1/NUSAP1 signaling pathway.

Conclusions: Our study provides a robust preclinical rationale and mechanisms of action for using sAXL as a single agent or in combination with docetaxel or carboplatin to treat lethal mPCa.

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HSP70 Leverages STUB1 to Modulate N-Myc Protein Turnover in Lethal Prostate Cancer

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Abstract

Background: Imbalances in protein homeostasis (proteostasis) are associated with a wide range of diseases, including neurodegeneration and tumorigenesis. Prostate cancer has proven to be a key model for understanding how proteostasis regulates tumor progression. Neuroendocrine prostate cancer (NEPC) is the most aggressive type of prostate cancer with no effective treatment. The amplification of N-Myc (encoded by *MYCN*) is a key modification of NEPC. However, it has proven challenging to identify therapeutic strategies that reduce N-Myc transcriptional activity or levels owing to the presence of intrinsically disordered functional domains and lack of enzymatically active sites. In this study, we sought to take a different approach and identify the molecular chaperones involved in N-Myc proteostasis.

Methods: N-Myc was knocked down by siRNA. Cell proliferation and N-Myc expression were assessed using cell viability assays and Western blotting, respectively. Rapid immunoprecipitation pull-down assays followed by label-free mass spectrometry were used to identify N-Myc-binding proteins. Co-immunoprecipitation (Co-IP) and Proximity Ligation Assay (PLA) were performed to detect the interaction between HSP70 and STUB1 with N-Myc. In vitro ubiquitination assays were conducted to detect the ubiquitination of N-Myc. Site-directed mutagenesis (SDM) was used to construct N-Myc, STUB1, and ubiquitination deletion or point mutation plasmids. Cycloheximide (CHX) chase assays were employed to examine the half-life of N-Myc.

Results: Knockdown of N-Myc by siRNA significantly inhibited the growth of H660, UCDCaP-CR, and CWR22Rv1 cells. A total of 779 proteins were identified as N-Myc-binding proteins. Among them, several HSP70 family proteins, such as HSPA1B (HSP70), HSPA6, and HSPA8 (HSC70), were the most enriched. The HSP70/STUB1 complex interacted with N-Myc and regulated N-Myc protein turnover. This interaction appeared to occur at a degron 'SELILKR' in N-Myc. The K416 and K419 of N-Myc seem to be sites for ubiquitination by STUB1, where it added K11-linked polyubiquitin chains for further proteasome degradation.

Conclusions: The HSP70 interacts with N-Myc at a conserved binding motif and regulates N-Myc protein turnover through STUB1.

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Ep300 of urothelial basal stem cell programs cell proliferation and tumor micro-environment for tumorigenesis

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Abstract

Mutations of epigenetic factors are highly enriched in bladder cancer and most are tumor suppressors, therefore, it is lack of effective epigenetic targets. Here, we found that mutation of epigenetic factor *EP300* reversely correlates with patient prognosis while expression of *EP300* elevated in late-stage bladder cancer. Knockdown or knockout of *EP300* in bladder cancer cells SW780 and T24 attenuated cell proliferation. Strikingly, genetic deletion of Ep300 in adult *Shh*$^{+}$ rather than *Upk2*$^{+}$ cell lineage delayed the BBN-induced bladder cancer progression in mice. Single-cell transcriptome analysis revealed that *Ep300* deficiency reduced both *Myc* and *Zeb2* expression in urothelial cells; CUT&TAG analysis in SW780 confirmed that EP300 indeed binds to *MYC* and *ZEB2* promoter regions. Interestingly, we noted that wild type tumor contains a novel *Sox9*$^{+}$ cells which secreted Egf and targeted *Egfr*+ tumor cells while *Egfr*$^{+}$ tumor cells secreted Ngr1 to targeted *Sox9*$^{+}$ cells as a feedback. Cell communication analysis confirmed this interaction, in addition to intranet-interaction, the outran-interaction between tumor cells and cancer-associated fibroblasts also observed. Both interactions are significantly reduced in stem cell *Ep300* deletion mice. Although the deletion of *Ep300* prior to BBN exposure did not improve the survival curve of mice, yet deletion of *Ep300* at multiple time points during BBN exposure significantly improved the survival length of mice. Collectively, we provide multiple evidence supporting EP300 functions as an oncogene in bladder cancer progression and this target in urothelial stem cell could be a promising strategy for bladder cancer therapy.

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Shed Trop2 Extracellular Domain Signals through EGFR and Regulates Prostate Cancer Metastasis

Shed Trop2 Extracellular Domain Signals through EGFR and Regulates Prostate Cancer Metastasis

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Abstract

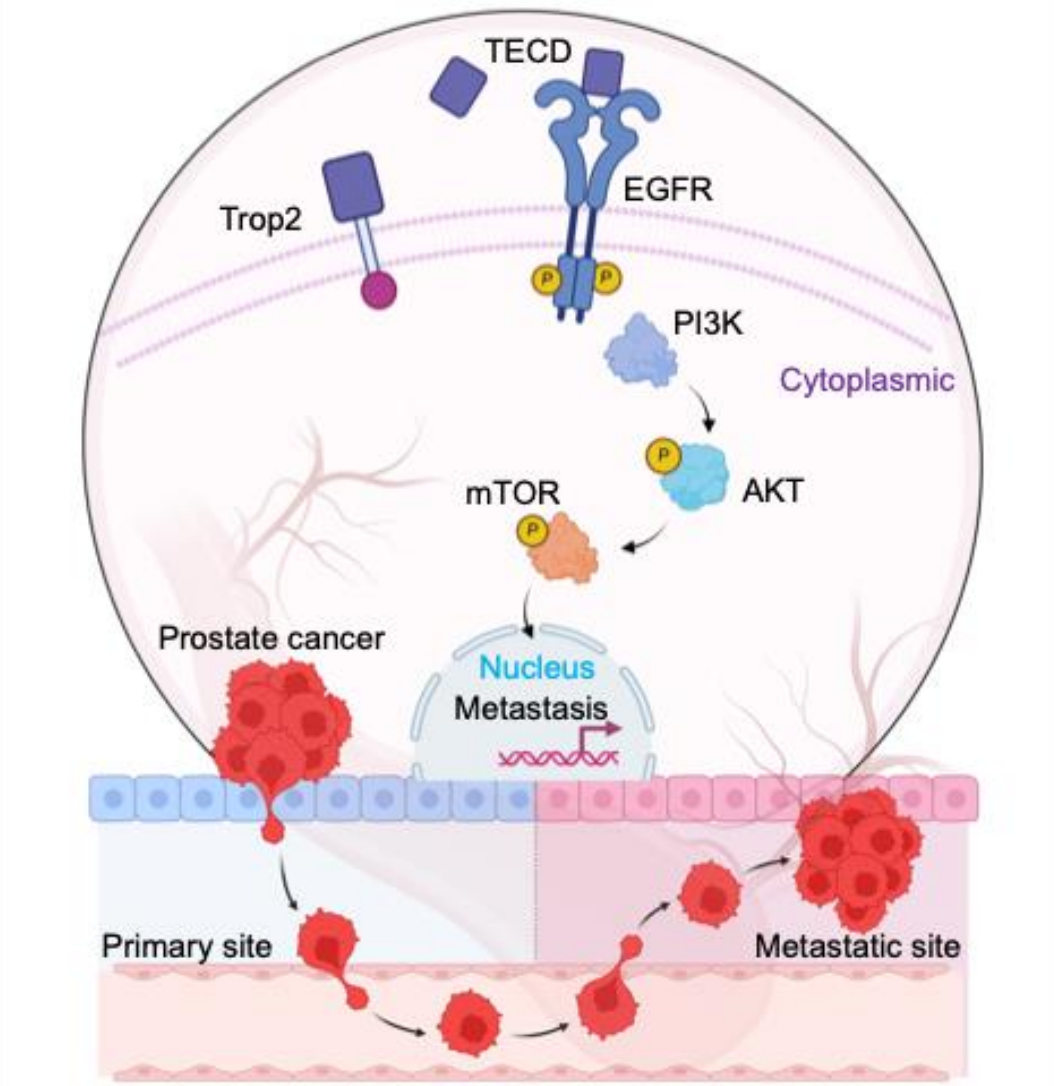
Background: Metastasis is the main cause of cancer-associated death in prostate cancer, highlighting urgent clinical needs to determine the mechanism underlying cancer progression. Trop2, an oncogenic transmembrane surface protein, is highly expressed in metastatic prostate cancer and is a prognostic biomarker for early detection of clinically significant prostate cancer. Here, we define the functional role of shedding Trop2 in tumor growth and metastasis in prostate cancer, and further identify the mechanisms underlying shedding Trop2 in driven prostate cancer metastasis.

Methods: Shed Trop2 extracellular domain (TECD) was accessed in prostate cancer cell culture media and serum from patients with normal vs prostate cancer by Western Blot and ELISA. Prostate cancer cell lines treated with vehicle or recombinant TECD or with overexpression of secreted TECD were accessed by migration, invasion, proliferation, and tumor sphere assays *in vitro*. To determine the effect of TECD on tumor growth *in vivo*, DU145 xenografts treated with veh or recombinant TECD (treated i.v. TECD 250ng/ml, every 7 days) were measured every five days. To determine the functional role of TECD in metastasis, DU145 and 22Rv1 cell lines were utilized to generate intracardiac injection metastatic model and spontaneous metastatic model. Mass spectrometry was performed on DU145 with overexpression of secreted TECD to define TECD targets. TECD target was validated by Immunofluorescence staining, immunoprecipitation, and proximity ligation assay.

Results: In this study, we report that TECD is detected in media from prostate cancer cells and serum from patients with clinically significant prostate cancer. Furthermore, our study reveals an important functional role of TECD in prostate cancer metastasis. We found that while shed TECD does not affect prostate cancer cell and tumor growth, it significantly increases cell migration, invasion, metastatic colonization, and spontaneous metastasis both *in vitro* and *in vivo*. TECD interactome studies and

proteomic studies reveal that TECD binds to epidermal growth factor receptor (EGFR) and shed TECD modulates a set of proteins associated with invasion, migration, mTOR signaling, and epithelial-to-mesenchymal transition. Furthermore, elevated shed TECD increases EGFR phosphorylation, resulting in the activation of the EGFR-PI3K-AKT-mTOR pathway in prostate cancer. EGFR inhibitors suppress the invasive ability of prostate cancer cells driven by TECD overexpression further supporting the key role of EGFR in TECD-mediated prostate cancer progression.

Conclusions: Our study suggests that TECD is a potential liquid biomarker for prostate cancer progression, reveals a new function of TECD in driving prostate cancer progression, and uncovers new mechanisms of TECD function through EGFR.



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Canonical AREs are tumor suppressive regulatory elements in the prostate

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Abstract

Background:

The androgen receptor (AR) is the central determinant of prostate tissue identity and differentiation, controlling normal, growth-suppressive prostate-specific gene expression. It is also a key driver of prostate tumorigenesis, becoming "hijacked" to drive oncogenic transcription. However, the regulatory elements determining the execution of the growth suppressive AR transcriptional program, and whether this can be reactivated in prostate cancer (PCa) cells remains unclear.

Methods:

A genome-wide approach was employed to manipulate regulatory elements containing canonical androgen response element (ARE) motifs, the traditional DNA binding site for AR. This method was designed to identify distinct AR-driven transcriptional programs.

Results:

Activation of AREs was found to be closely linked with differentiation and growth-suppressive transcription, and this activation can be reactivated to induce cell death in AR^+ PCa cells. In contrast, repression of AREs is well tolerated by PCa cells but has harmful effects on normal prostate cells. Finally, gene expression signatures driven by ARE activity are associated with improved prognosis and luminal phenotypes in human PCa patients.

Conclusions:

The study highlights that canonical AREs are responsible for a normal, growth-suppressive, lineage-specific transcriptional program, which can be reactivated in PCa cells for potential therapeutic benefit. Moreover, the genes regulated by this mechanism hold clinical significance for human PCa patients.

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Combined use of prostate cancer vaccine with immune checkpoint blockade synergizes to eliminate prostate cancer

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Abstract

Background: Immunotherapy has now become a standard of care treatment for various types of cancer. However, the clinical benefits of immunological interventions, such as tumor vaccines or the immune checkpoint blockade (ICB), have demonstrated only marginal effects in prostate cancer patients.

Methods: Our previous study demonstrated that an adenovirus-based bivalent prostate cancer vaccine (Ad-PS2) targeting two antigens was more effective than single antigen-based vaccines. To further improve its therapeutic impact and maximize the clinical benefit to prostate cancer patients, we tested the combination of Ad-PS2 bivalent vaccine and ICB (anti-CTLA4 and anti-PD1), analyzed immune T cell infiltrations in the tumors, and differential gene expression analysis on CD8+ T cells.

Results: Combining the Ad-PS2 vaccine with immune checkpoint blockade anti-CTLA4 resulted in complete tumor regression in mice, with all the treated mice becoming tumor-free. We also observed that the Ad-PS2 vaccine stimulated various functions in immune effector T cells with increased infiltration of CD8+ T cells in the tumor site. To understand the molecular mechanisms of tumor regression, we performed a global gene expression profile of splenic CD8+ T cells from mice with completely regressed tumors (responders) and those with growing tumors (non-responders) after receiving Ad-PS2 vaccine treatment. From the two sets of cDNA microarray data sets, we found constancy in 11 up-regulated and 777 down-regulated genes in non-responder mice. Gene network analysis revealed a coordinated expression of genes implicated in cell-mediated immune response, cell-to-cell signaling and interaction, and immune cell trafficking. The mRNA expression levels for the selected transcripts were analyzed using a qRT-PCR method and validated in purified CD8 T cells from naïve prostate cancer patients. Identifying these genes underscores the need for further validation in human samples, which could significantly enhance our understanding of immunotherapy response in prostate cancer patients.

Conclusions: Overall, our results indicate that combining the Ad-PS2 vaccine with anti-CTLA4 effectively eliminates tumors in mice, providing a strong rationale for further exploration and potential application in human patients.

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Small molecule inhibitor targeting EP300 attenuated bladder cancer progression

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Abstract

Enhancer-promoted tumorigenesis had been widely observed and enhancer targeting had been conceptually proven in multiple cancer types, yet the efficacy is still controversial. Here, we found that multiple small molecule inhibitors of EP300, a protein which is believed writing enhancer marks, significantly attenuated growth of bladder cancer cell SW780. In multiple bladder cancer cell lines test, A485 not only significantly reduced the acetylation level of both EP300 and histone H3 lysine 27 (H3K27), but also significantly inhibited 3D cell growth as well as cell migration. Bulk RNAseq and Cut-tag analysis in SW780 cell line revealed that A485 treatment blocked the transcriptional events of multiple oncogenes such as *MYC* and *CDK1* as well as EMT regulators *ZEB1* and *ZEB2*. Furthermore, SW780 CDX model confirmed that A485 significantly attenuated the tumor growth *in vivo*, in addition, long-term A485 treatment in BBN-induced mouse bladder cancer model significantly alleviated the progression of carcinoma *in situ* (CIS) to muscle-invasive carcinoma in histology analysis. Finally, in organoids model derived from both mouse and human bladder tumor, A485 significantly inhibited the growth of organoids as well as the expression of stem cell markers. Collectively, targeting enhancers through EP300 inhibition holds promise for bladder cancer therapy and warrants further investigation.

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Lysine methylation of FOXA1 alters its chromatin binding and represses E2F signaling

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Abstract

Lysine methylation of FOXA1 alters its chromatin binding and represses E2F signaling

Background: Prostate cancer (PCa) can circumvent conventional androgen deprivation therapies (ADTs) and progress to castration-resistant prostate cancer (CRPC). In recent years, second-generation androgen receptor (AR) signaling inhibitors (ARSi) have emerged as a principal treatment for CRPC. However, these treatments often fail as CRPC develops multiple mechanisms to sustain its progression. FOXA1 is a pioneer transcription factor crucial in CRPC by opening compact chromatin to facilitate the binding of other transcription factors, such as AR. Recent studies suggest that the chromatin binding of FOXA1 is altered and reprogrammed in CRPC, enhancing tumor progression and treatment resistance, but mechanisms driving this reprogramming remain unclear. Our recent studies have indicated that the histone modifiers, SETD7 and LSD1, can methylate and demethylate FOXA1 at lysine 270, regulating the dynamic chromatin binding of FOXA1 in prostate cancer cells. However, the precise role of methylated versus unmethylated FOXA1 in CRPC progression remains unclear.

Methods: In this project, we generated two doxycycline-inducible overexpressed FOXA1 stable lines in 22Rv1 cells (22Rv1-V5-FOXA1^{WT} and 22Rv1-V5-FOXA1^{K270R}), expressing wildtype and K270R mutant FOXA1 with silencing of endogenous FOXA1 to understand the role of K270 methylation in CRPC progression. Integrated analysis of ChIP-seq and RNA-seq was performed to assess the chromatin binding alteration and transcriptional reprogramming by the FOXA1 mutant. Furthermore, we assessed changes in treatment sensitivity in cells expressing this mutant.

Results: Our integrated analysis revealed that demethylation of FOXA1 significantly altered FOXA1 chromatin binding. Motif analysis further indicated that K270R FOXA1 mutant displayed enhanced enrichment for E2F and JUN motifs. GSEA analysis demonstrated that K270R mutant cells exhibit higher expression of E2F targets, with increased expression of E2F1. Additionally, the K270R mutant displayed resistance to enzalutamide and multiple epigenetic treatments, including inhibitors targeting EHMT1/2, a recently identified epigenetic activator of E2F1.

Conclusions: Collectively, our studies suggest that K270 methylation of FOXA1 functions to prevent oncogenic reprogramming and repress E2F activity. Therefore, increased demethylation of FOXA1 observed in CRPC reprograms FOXA1 chromatin binding and its transcription network, specifically activating E2F signaling and conferring resistance to epigenetic and other treatments.

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SRD5A2 Targets WNT5A to Regulate Prostate Growth

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Abstract

Background:

Steroid 5α-reductase 2 (SRD5A2) is a crucial enzyme involved in prostatic development and growth and is the primary target of 5α-reductase inhibitors, which are the main medical treatment for benign prostatic hyperplasia (BPH). However, the underlying mechanisms by which SRD5A2 regulates prostate growth remain largely unknown. We previously generated an SRD5A2-null (*Srd5a2*$^{-/-}$) mouse model, which exhibited approximately a 50% reduction in prostate volume. Using this animal model, along with human BPH tissue and cultured cell lines, we demonstrate that the absence of SRD5A2 leads to changes in cell-cell interactions between stromal and luminal cells, with WNT5A emerging as a critical modulator in this process.

Methods:

Bulk RNA sequencing (RNA-seq) and single-cell RNA sequencing (scRNA-seq) were performed on entire prostate tissues from *Srd5a2*$^{-/-}$ mice and their *Srd5a2*$^{+/+}$,*Srd5a2*$^{+/-}$ littermate controls. Significant ligand-receptor pairs were identified through heterotypic cell-cell communication analysis using Cellphone DB2. RNA fluorescence in situ hybridization (RNA FISH) was employed to visualize the spatial expression of WNT5A across all mouse prostate lobes. Additionally, human BPH surgical specimens (n=18) were analyzed to examine the expression of WNT5A and PTPRK transcripts and their correlation with SRD5A2 levels. A benign prostatic stromal cell line (BHPrS1) was transfected with the SRD5A2 gene to evaluate changes in WNT5A expression in cells overexpressing SRD5A2.

Results:

Bulk RNA-seq analysis revealed that myofibroblast and muscle contraction processes were significantly upregulated in *Srd5a2*$^{-/-}$ mice. scRNA-seq data indicated that stromal cells were among the most prolific interactors, with myofibroblasts being the most “outbound” cells. Additionally, the weighted value of ligands from fibroblasts showed a significant increase in *Srd5a2*$^{-/-}$ mice compared to controls (366 *vs*. 493). Notably, ligand receptor pairs such as WNT5A/PTPRK, TGFB2/TGFBR1, and NRP1/2/VEGFA were identified as highly significant interactions between fibroblasts/myofibroblasts and luminal epithelial cells.

In *Srd5a2*$^{-/-}$ mice, WNT5A expression was significantly lower in the periurethral lobe of the prostate (p=0.01) but higher in the anterior prostate lobe (p=0.05) compared to *Srd5a2*$^{+/-}$ and *Srd5a2*$^{+/+}$ littermate

control. In human BPH tissue, WNT5A and PTPRK transcript levels were both positively correlated with SRD5A2 expression (R=0.54, p=0.02; R=0.58, p=0.01, respectively). Furthermore, in vitro studies showed that overexpression of SRD5A2 in BHPrS1 cells significantly increased WNT5A expression (p=0.002).

Conclusion:

Our data suggest that alterations in SRD5A2 levels in the prostate are linked to changes in WNT5A expression. The stromal-specific WNT signaling pathway, particularly WNT5A, may represent a potential therapeutic target for patients who are unresponsive to 5α-reductase inhibitor (5ARI) therapy.

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SRD5A2 Expression Correlates with Prostate Volume

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Abstract

Background:

Steroid 5α reductase 2 (SRD5A2) is an essential enzyme that converts testosterone to dihydrotestosterone (DHT), which is a potent androgen involved in the development of the prostate. 5α-reductase inhibitors (5ARI) is the main medical therapy for benign prostatic hyperplasia (BPH), and it works by blocking SRD5A2 enzyme leading to DHT reduction shrinking of the prostate size. To further understand the function of SRD5A2 and the development/treatment of BPH, we sought to identify whether there is a correlation between SRD5A2 expression and prostate size.

Methods:

Sixty-one prostate biopsies from the Medical Therapy of Prostatic Symptoms (MTOPS) trial were obtained from NIDDK Central Repository. Clinical information including age, BMI, total prostate volume, transition zone volume, peripheral zone volume and serum DHT levels were used for correlation studies. SRD5A2 protein expression levels for each patient were scored by immunohistochemistry staining. Images were scored on a scale of 0-12 based on the staining intensity and percentage of positive staining.

Results:

SRD5A2 expression was positively correlated with total prostate volume (R=0.28 and p=0.03) and transition zone volume (R=0.31 and p=0.016). However, SRD5A2 expression was not significantly correlated with the peripheral zone volume or blood DHT levels.

Conclusion:

Our findings demonstrated that SRD5A2 expression in the prostate is directly linked with the prostate size, particularly in the transition zone where BPH develops. These findings underline the important role of SRD5A2 as an essential enzyme in the prostate and a key player in BPH.

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Early results of utility of contrast-enhanced ultrasound in assessing renal function in patients with unilateral ureteropelvic junction obstruction

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Abstract

Early results of utility of contrast-enhanced ultrasound in assessing renal function in patients with unilateral ureteropelvic junction obstruction

Background

B-mode ultrasonography (USG) is the main imaging modality in use; although it can give an idea about the degree of hydronephrosis (HDN) , it cannot differentiate obstructive from non-obstructive HDN. The commonly used modalities to diagnose obstruction are intravenous urography (IVU) and diuretic dynamic scintigraphy/renal dynamic study (RDS), although RDS is the more commonly used modality. The drawback of both these modalities is that they require exposure to ionizing radiation, which is a concern since these studies need to be repeated as multiple follow up studies. Studies have shown that contrast-enhanced USG is almost as sensitive as radioisotope renography in the quantitative diagnosis of chronic renal insufficiency, and correspondingly, contrast-enhanced USG could be a useful tool for the assessment of renal function in children with pelvi-ureteric junction obstruction (PUJO). The EFSUMB (European Federation of Societies for Ultrasound in Medicine and Biology) release guidelines state that intravenous CEUS is safe and effective in paediatric populations and can be used effectively to avoid ionizing radiation exposure.

Material & Methods

Sixteen patients with PUJO type of hydronephrosis were included for this cross-sectional study. The patients suspected of having HDN on the basis of clinical presentation first underwent USG KUB to assess whether HDN is present or not. On USG KUB, the renal parenchymal thickness and antero-posterior (AP) diameter of the pelvis were be measured. The diagnosis of PUJO was done on the results of RDS performed using Tc 99m LLEC with dose adjustment of the radionuclide as per Body Surface Area. The F-0 protocol was used, that is, the diuretic furosemide was administered in the dose of 0.5 mg/kg at the same time as the radionuclide. Sequential images were obtained up to 4 hours after injecting Tc-LLEC and furosemide. t ½, Split Renal Function (SRF) and the drainage curve were assessed to arrive at a diagnosis of PUJO. A t½ of greater than 20 minutes was suggestive of obstruction.

US and CEUS was performed with a standard abdominal convex probe 3.5 -5.0 MHz in older children and 5-12 MHz in smaller children using Scanner-Supersonic Imagine system (Aixplorer, France) by

experienced radiologist who was blinded to the patients' clinical and pathological information. Appropriate sized intra venous cannula as per age was used for the contrast injection. A bolus dose of 0.03 mL/kg of microbubbles containing sulphur hexafluoride (SonoVue) was injected intravenously, followed by a flush with 5 ml of normal saline. Raw images were observed and captured in real-time for 1 minute and then stored digitally in DICOM format. Then, all patients underwent 30 minutes of clinical observation at the end of procedure. Patients contralateral kidney was taken as control. The video clips were stored digitally for subsequent analysis. A third party software Vuebox (Bracco) was used to perform analysis. For the time intensity curve (TIC) data of the CEUS data, regions of interest was drawn in the renal cortex using the software. The regions of interest (ROIs) was placed as close to the same depth as possible in accordance with greyscale images and contrast images. The following perfusion parameters of the TIC was recorded, slope of the ascending curve (A), area under the curve (AUC), peak intensity (PI) and time to peak (TTP).

Results

Sixteen patients were recruited, and the median age was 6.5 years (range 2 years—11 years), with two females and 14 males. Eleven patients had left kidneys affected, and 5 patients had right kidneys affected. The median split renal function of the affected site was 44.5% with a range of 22- 58 percent. The median APD size was 29mm (range 20-43 mm). The image quality was sufficiently high for independent US and CEUS readings. No patients needed multiple applications of contrast agent. There were no serious adverse reactions to the contrast agent. There was no significant difference in the AUC or PI between the affected and contralateral kidneys. There was no significant correlation between AUC, PI and differential renal function. The ROC curves were drawn to differentiate DRF values of <40% and >40% using the TTP. The preliminary study shows time-intensity curve can be a predictor of renal damage.

Conclusion

This preliminary experience describes a new method for measuring renal function noninvasively using ultrasound. CEUS, a highly sensitive, rapid diagnostic imaging modality for detecting and monitoring renal function noninvasively, is almost as useful and accurate as diuretic renography in evaluating renal function while involving no radiation exposure.

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PLXND1 is a potential therapeutic target and prognostic marker for neuroendocrine prostate cancer

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Abstract

Background: Neuroendocrine prostate cancer (NEPC) is a highly aggressive subtype of prostate cancer, often associated with resistance to therapy and poor prognosis. Treatment-induced NEPC (t-NEPC) typically arises through lineage plasticity from adenocarcinoma in response to androgen receptor signaling inhibitors like enzalutamide. Despite its clinical significance, the mechanisms driving lineage plasticity and the transition to NEPC remain poorly understood. PLXND1, a receptor within the semaphorin family, plays a crucial role in regulating the cytoskeleton and cell adhesion. However, the involvement of PLXND1 in the development and progression of NEPC through lineage plasticity is not well defined.

Methods: We conducted transcriptomic analysis on cohorts of NEPC patients and enzalutamide-resistant prostate cancer cell lines. We assessed the correlation of PLXND1 with neuroendocrine feature genes and AR-targeting genes. The Kaplan-Meier method and Cox proportional hazards model were utilized to evaluate the association between PLXND1 expression levels and patient survival. Immunohistochemistry was employed to analyze PLXND1 expression in prostate adenocarcinoma and NEPC tissues. Cell viability assays were performed on C4-2B MDVR, CWR22Rv1, and H660 cells following PLXND1 knockdown using siRNA or PLXND1 knockout via CRISPR/Cas9. Additionally, the viability of patient-derived xenograft organoids was assessed after PLXND1 knockdown or knockout.

Results: Our analysis of various patient cohorts indicates that PLXND1 is highly expressed in NEPC patients, with expression levels positively correlated with neuroendocrine feature genes (Beltran cohort: CHGA [r=0.42, P=0.002], ENO2 [r=0.53, P<0.0001], SYP [r=0.59, P<0.0001], and NCAM1 [r=0.32, P=0.02]). Kaplan-Meier survival analysis reveals that high PLXND1 expression is significantly associated with poor prognosis in prostate cancer patients (TCGA: HR=1.80 [1.19-2.75], P=0.006; GSE21032: HR=3.23 [1.67-6.24], P<0.001). PLXND1 is overexpressed and negatively regulated by androgen receptor signaling in enzalutamide-resistant cells and NEPC patient cohorts (GSE126078: AR [r=-0.58, P<0.0001], KLK2 [r=-0.32, P=0.01], KLK3 [r=-0.39, P<0.0001], NKX3-1 [r=-0.41, P<0.0001]). Additionally, downregulation of PLXND1 expression inhibits NEPC cell proliferation and reduces the viability of PDX tumor organoids.

Conclusions: PLXND1 expression is elevated in NEPC and correlated with the poor survival of prostate cancer patients, suggesting it is a potential molecular indicator and therapy target for NEPC.

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Targeting Hypoxia-Inducible Factor-2 Alpha in Kidney Cancer

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Abstract

The transcription factor Hypoxia-Inducible Factor 2 Alpha (HIF2α) plays a crucial role in the development and progression of the most common type of kidney cancer, clear cell renal cell carcinoma (ccRCC). HIF2α allows cancer cells to adapt to hypoxic conditions, promoting angiogenesis and uncontrolled tumor growth. Targeting HIF2α through pharmacologic inhibition offers a therapeutic strategy for HIF2α-driven cancers. Using an in silico approach, we identified the novel Compound-c2 as a selective inhibitor that binds to the Per-Arnt-Sim-B (PAS-B) domain of HIF2α. Notably, Compound-c2 disrupts the interaction between HIF2α and the molecular chaperone Hsp70, leading to proteasomal degradation of HIF2α and the induction of apoptosis in ccRCC. This distinctive inhibitory mechanism sets Compound-c2 apart from previous HIF2α antagonists, positioning it as a promising alternative with potential applications in addressing drug resistance and providing a unique approach to inhibit HIF2α-related processes.

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Characterizing novel approaches to target receptor tyrosine kinase ROR2 in neuroendocrine prostate cancer

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Abstract

The evidence emanating from therapy-induced drug resistance indicates tumors can gain adaptation by transitioning to an alternative lineage state. In prostate cancer, the widespread clinical application of androgen receptor (AR) pathway inhibitors has led to the accretion of tumor relapsing with loss of AR signaling and a shift from a luminal state to an alternate program. Although different genomic and epigenomics aberrations are shown to correlate with this lineage reprogramming, the molecular and signaling mechanism orchestrating the development of lineage plasticity under the pressure of AR-targeted therapies still needs to be fully understood. Studies have shown that receptor tyrosine kinases (RTKs) can rewire the transcriptome, promoting survival and growth in the absence of AR activity. However, which RTK feeds into lineage plasticity and de-differentiation following AR pathway inhibition is still obscure. Here a survey of RTKs identified ROR2 as the top-upregulated RTK following AR pathway inhibition, which feeds into lineage plasticity and de-differentiation by promoting stem cell-like and neuronal networks. Mechanistically, we showed that ROR2 activates MAPK/ERK/CREB signaling pathway to facilitate the expression of the lineage commitment transcription factor ASCL1 and support lineage plasticity and treatment resistance. The expression pattern of ROR2, combined with its role in regulating the pro-neuronal commitment transcription factor ASCL1 and influencing lineage plasticity, highlights its promise as a therapeutic target. However, the development of ROR2-specific agents is impeded by a scarcity of high-affinity binders that can selectively recognize its extracellular domains. To overcome this challenge, we have harnessed Variable New Antigen Receptors (VNARs) derived from shark immune systems. Notable for their small size and distinctive structural attributes, VNARs provide unparalleled advantages for crafting next-generation antibody therapies. Their extended hypervariable loops facilitate binding to epitopes that traditional antibodies cannot reach, thus offering enhanced targeting capabilities for ROR2. We immunized nurse sharks with peptides mirroring distinct extracellular domains of ROR2, using established immunization protocols. Afterward, B cell mRNA was isolated to construct a VNAR library. From this library, we identified four VNARs that exhibit high specificity and affinity for ROR2. These VNARs are under evaluation for their potential in ROR2 VNAR-drug conjugates, aiming to advance a novel class of ROR2-targeted agents that could significantly impact the treatment of AR-independent resistance in prostate tumors.

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Infiltrating lipid-rich macrophage subpopulations identified as a regulator of increasing prostate size in human benign prostatic hyperplasia

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Abstract

Background: Macrophages exhibit marked phenotypic heterogeneity within and across disease states, with lipid metabolic reprogramming contributing to macrophage activation and heterogeneity. Chronic inflammation has been observed in human benign prostatic hyperplasia (BPH) tissues, however macrophage activation states and their contributions to this hyperplastic disease have not been defined. We postulated that a shift in macrophage phenotypes with increasing prostate size could involve metabolic alterations resulting in prostatic epithelial or stromal hyperplasia.

Methods: Single-cell RNA-seq of $CD45^+$ transition zone leukocytes from 10 large (>90 grams) and 10 small (<40 grams) human prostates was conducted. Macrophage subpopulations were defined using marker genes.

Results: BPH macrophages do not distinctly categorize into M1 and M2 phenotypes. Instead, macrophages with neither polarization signature preferentially accumulate in large versus small prostates. Specifically, macrophage subpopulations with altered lipid metabolism pathways, demarcated by *TREM2* and *MARCO* expression, significantly accumulate with increased prostate volume. *TREM2*$^+$ and *MARCO*$^+$ macrophage abundance positively correlates with patient body mass index and urinary symptom scores. $TREM2^+$ macrophages have significantly higher neutral lipid than $TREM2^-$ macrophages from BPH tissues. Lipid-rich macrophages were observed to localize within the stroma in BPH tissues. *In vitro* studies indicate that lipid-loaded macrophages increase prostate epithelial and stromal cell proliferation compared to control macrophages.

Conclusions: These data define two new BPH immune subpopulations, $TREM2^+$ and $MARCO^+$ macrophages, and suggest that lipid-rich macrophages may exacerbate lower urinary tract symptoms in patients with large prostates. Further investigation is needed to evaluate the therapeutic benefit of targeting these cells in BPH.

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Comparing the Chromatin Accessibility Landscape in White and Black Patients with Localized Prostate Cancer

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Abstract

Background: The most significant cancer disparity in the US has been observed in prostate cancer (PCa), with Black men facing the highest incidence and being twice more likely to die from their disease. Although socioeconomic factors, access to care and treatments play an important role, in a military population these factors are often even, but the disproportionate impact on Black men persists. Consequently, important biological factors including genetics, epigenetics, and tumor microenvironment are likely contributors to this disparity. While certain genomics factors are associated with higher prevalence among Black men, how stemness and phenotypic plasticity impact PCa progression and aggressiveness based on race are not well understood.

Methods: This study utilized spatial ATACseq using prostatectomy frozen sections from White (n=3) and Black (n=3) patients treated at the Center for Prostate Disease Research at the Walter-Reed National Military Medical Center. All samples were age and Gleason-grade matched, as it is currently not known if these variables would influence our overall results.

Results: Our current analysis demonstrates a total of 19,445 and 12,101 peaks in Black and White men respectively. Consistent with earlier studies, pseudo-bulk analysis of differentially accessible chromatin regions (DARs) revealed an overall increase in chromatin accessibility of interferon response genes and lipids metabolism in Black men. Moreover, 10 defined cell types including epithelial, immune, and stroma cells were identified. Gene ontology analysis demonstrated heterogenous cellular states including luminal epithelial cells enriched in Black men. In addition, White patients presented enrichment of a previously described mixed basal-luminal epithelial cell identity. Transcription factor (TF) motif analysis revealed that White men had enrichment of motifs associated with the androgen receptor signaling pathway and chromatin architecture organizers, whereas Black men enriched for TFs associated with neuroendocrine PCa. Moreover, while accessibility of *DNMT1* was increased in White men, *EZH2* was higher in Black men, corroborating previous findings suggesting that *EZH2* overexpression is a prognostic marker in Black men.

Conclusions: Our preliminary data provides a view of different chromatin accessibility profiles between Black and White men with localized PCa, which likely underlie divergent transcriptional responses that can determine therapeutic resistance and tumor progression.

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Umbrella cell maintains the mechanical force barrier in urinary bladder via intermediate filament Krt20

Student Jun Jiang Master[1], Student Yongjia Bao master[1], Student Tao Huang Bachelor[1], Student Hanbo Zhang Bachelor[1], Postdoctoral fellow Jiehui Chen Doctor[2], Dr. Congcong Dong Doctor[3], Professor Chunming Guo Doctor[1]

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Abstract

Bladder is a dynamic organ responsible for storing and expelling urine as needed. As the primary barrier, umbrella cells adapt to frequent distention and shrinking dynamics. However, the role of umbrella cells in maintaining mechanical forces within the bladder remains largely unknown. Here, we found that an intermediate-filament cytokeratin 20 has an unprecedented role upon bladder compliance and mechanical fibrosis. Interestingly, keratin 20 is conserved across species, from fish to mammals. In mice, keratin 20 specifically exists upon the apical umbrella cells, forming a mesh-like structure that interacts with other fibers. When keratin 20 is knocked out in mice, several effects occur. First, the umbrella cells become larger. Second, there is a higher frequency of distended mega bladders, indicating increased compliance. Surprisingly, this knockout has minimal impact on weight and lifespan. Additionally, mutant mice exhibit larger total voiding volumes, and staining reveals fibrosis. RNA sequencing analysis revealed that the Tgfb1 signaling and Hippo signaling are activated, indeed, yap nuclear translocation is elevated in the mutant bladder. These changes likely result from exposure to mechanical distention forces. Tgfb1 inhibitor significantly reduced fibrosis yet did not rescue overload of mechanical force pressure in mutant. Intriguingly, keratin 20 knockout also affects the rhythm of ureter contraction and delays physiological urine reflux in the right kidney. This delay, in turn, affects the pathology of the right kidney during bacterial infections. Collectively, umbrella-specific keratin 20 acts as a barrier, preventing impairment from mechanical force dynamics during the voiding cycle.

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Urothelial deletion of Arid1a promotes BBN-induced bladder cancer progression partially via retinoic acid signaling reduction

Student Chunyue Wu Master[1], Student Xianbin Duan Bachelor[1], Student Chenglong Sang Bachelor[1], Student Lu Li Master[1], Student Qun Wnag Master[1], Associate Professor Shi Fu Doctor[2], Professor Cheng Peng Doctor[1], Professor Haifeng Wang Doctor[2], Professor Chunming Guo Doctor[1]

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Abstract

Epigenetic factors are highly enriched in cancer, and many of them function as tumor suppressors. However, their precise mechanisms and effective treatments remain elusive. In our study, we discovered that mutations in the epigenetic factor ARID1A positively correlate with patient prognosis, while its expression decreases in late-stage bladder cancer. Notably, knocking down or knocking out *ARID1A* in SW780 bladder cancer cells accelerates cell proliferation and organoid growth, whereas overexpressing *ARID1A* slows down cell growth. Furthermore, genetic deletion of Arid1a in either Upk3a+ or Krt20+ cell lineages significantly accelerates BBN-induced bladder cancer progression, leading to a worsened survival curve in a dose-dependent manner. Bulk RNAseq analysis revealed that the retinoic acid-associated signaling pathway is attenuated in *ARID1A*-mutated cells. Remarkably, replacing retinoic acid partially rescues ARID1A-mutated cell lines and improves the survival curve of Arid1a cKO mice. Collectively, our findings indicate that ARID1A functions as a tumor suppressor in bladder cancer, partially by modulating retinoic acid signaling. Targeting Arid1a-mutated bladder cancer with retinoic acid replacement could be a promising precision treatment strategy.

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Targeting the Antioxidant Capacity of the Xc-GPX4 System Suppresses ARID1A-Deficient Bladder Cancer

Student Mengxue Zhao Master's degree[1], Student Xuefeng Wang Bachelors'degree[1], Student Rui Guo Bachelors'degree[1], Student Xianbin Duan Bachelors'degree[1], Student Jie Zhou Bachelors'degree[1], Student Tao Huang Bachelors'degree[1], Associate professor Shi Fu Doctor's degree[2], Professor Haifeng Wang Doctor's degree[2], Professor Cheng Peng Doctor's degree[1], Professor Chunming Guo Doctor's degree[1]

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Abstract

Chromatin modifiers are often protective from tumor and frequently mutated in bladder cancer, yet the precise treatment for those mutations remains largely unknown. In our study, we focused on *ARID1A*, which encodes a critical component of the switch/sucrose nonfermentable (SWI/SNF) complex. *ARID1A* mutations account for 26% of bladder cancer cases and are associated with worsened prognosis. To investigate vulnerability, we generated multiple *ARID1A*-deficient bladder cancer cell lines and screened for susceptibility. Interestingly, *ARID1A*-deficient bladder cancer cells were specifically vulnerable to inhibitors of the antioxidant glutathione (GSH), leading to ferroptosis. Notably, T24 *ARID1A*-deficient cells exhibited over 100-fold increased sensitivity to RSL-3 compared to wild-type cells. In a CDX model, RSL-3 efficiently inhibited SW780 *ARID1A*-deficient tumor growth. Remarkably, RSL-3 also significantly extended survival in *Arid1a* cKO mice in a BBN-induced bladder cancer model. Mechanistically, *ARID1A* deficiency enhanced the nuclear translocation of YAP1 and its transcriptional activity, promoting an epithelial-mesenchymal transition (EMT) that contributes to vulnerability. Additionally, *ARID1A*-deficiency impaired the expression of SLC7A11, a gene encoding a cystine transporter, resulting in reduced GSH levels and further contributing to vulnerability. Cut-tag analysis confirmed that ARID1A is located in the promoter regions of *NFE2L2* and regulates SLC7A11 through NFE2L2. Finally, RSL-3 significantly induced the death of human cancer organoids especially in late stage bladder cancer. Collectively, *ARID1A*-deficient bladder cancers could be precisely targeted via ferroptosis inducers.

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Thalidomide targets prostatic fibrosis and smooth muscle to alleviate lower urinary tract dysfunction.

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Abstract

Background: Benign Prostatic Hyperplasia (BPH) is a prevalent condition characterized by prostatic tissue overgrowth and smooth muscle dysfunction, leading to urethral narrowing and chronic urinary flow obstruction. It is often accompanied by lower urinary tract symptoms (LUTS) such as urinary frequency, urgency, and nocturia. Emerging research indicates a potential role of fibrosis in BPH development and treatment resistance. However, no targeted therapies for prostatic fibrosis are available, underscoring the necessity for additional research. This study aims to assess the effects of thalidomide, an antifibrotic, on lower urinary tract dysfunction in an aged mouse model of BPH/LUTS.

Methods: The effects of thalidomide on gene expression in human prostate stromal cells (BHPrS1) were assessed using a multiplex nucleic acid hybridization assay. For *in vivo* studies, aged (24-month-old) C57BL/6J mice with lower urinary tract dysfunction, identified using the void spot assay (VSA), received daily i.p. injections of 10 mg/kg thalidomide for 6 weeks, with weekly VSA monitoring. After treatment, mouse urogenital tracts were harvested for fibrosis analysis using histopathology and CTFire/CurveAlign software. Thalidomide's impact on smooth muscle was evaluated using immunohistochemistry and *ex vivo* bladder and prostate contractility assays.

Results: Thalidomide significantly decreased fibrosis-associated gene expression in human prostate stromal cells and improved lower urinary tract dysfunction in aged mice. Picrosirius red (PSR) staining showed a downward trend in total collagen within the dorsal and lateral prostate (DLP) in treated mice. Moreover, treated mice exhibited a significant decrease in thicker collagen fibers in the DLP ($p<0.01$) and anterior prostate ($p<0.05$). PSR analysis of the prostatic urethra revealed a significant reduction in total collagen ($p<0.05$) and in thicker collagen fibers ($p<0.001$) in thalidomide-treated mice. Collagen fibers around the prostatic urethra of treated mice had overall less alignment ($p<0.01$). Analysis of smooth muscle function showed that thalidomide relaxes urogenital smooth muscle via neurogenic modulation.

Conclusions: Thalidomide attenuates lower urinary tract dysfunction in aged mice by acting as an antifibrotic and modulating urogenital smooth muscle tone. This warrants further investigation of thalidomide as a multimodal therapy for BPH/LUTS in aging men. 5U54DK104310, 5R01DK131175, 5R01DK127081, T32GM141013.

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Enhancing Visibility and Engagement: The Impact of the #CAIRIBUatAUA Social Media Campaign at the 2024 American Urological Association Annual Meeting

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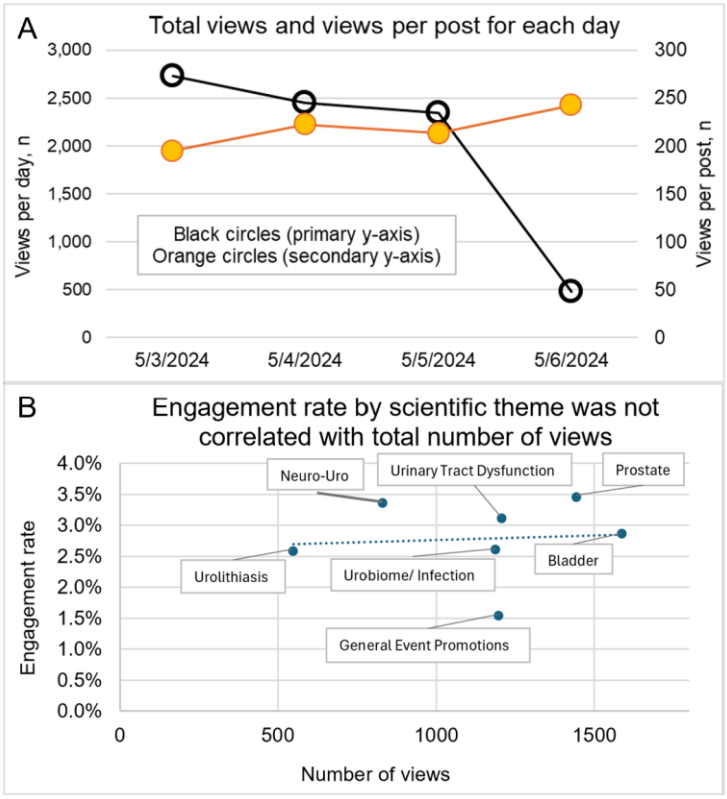
Abstract

BACKGROUND: The CAIRIBU Interactions Core (CIC) promotes CAIRIBU-affiliated investigators at Urology conferences. We piloted a social media campaign to highlight CAIRIBU investigators presenting at the 2024 American Urological Association (AUA) meeting.

METHODS: Volunteers from the CAIRIBU community were recruited, assigned to specific AUA sessions, and asked to post live on X using #CAIRIBUatAUA. Posts from May 3-6 were aggregated, with metrics collected for total posts, tagged accounts, and scientific themes. Outcomes included views (times a post appears on a user's screen), engagements (likes + retweets + comments), and engagement rates *(engagements/views)*100.*

RESULTS: 4 volunteers (all women; 1 student, 2 early-stage investigators, 1 established investigator) posted from their accounts, with additional posts from the official CAIRIBU X account. Total followers of all accounts was 760 (152±120 per account). Volunteers posted 9.5±5.2 times daily (total of 38 posts), tagging a total of 22 unique investigators/institutions across their posts. Posts generated 8,009 views, 229 engagements, and a 2.6±1.2% engagement rate. While the number of posts declined each day, views remained consistent (Figure, panel A) as did engagement rates (data not shown). Bladder posts had the most views (1,588), but posts on prostate and neuro-urological disorders had the highest engagement rate (3.4%). There was no correlation between views and engagement rate (Figure, panel B), nor between engagement rates and the authors' numbers of X followers.

CONCLUSION: The pilot campaign at the AUA meeting achieved significant reach and engagement, underscoring the potential of targeted social media campaigns to enhance professional visibility, promote knowledge sharing, and foster connections during live events. Our results suggest that authors more established in their fields vs. those with higher numbers of followers may be key in maximizing social media reach. Future campaigns will involve more investigators and leverage those with expertise and strong engagement within their research communities.


A
Total views and views per post for each day
Views per day, n
Views per post, n
3,000
2,500
2,000
1,500
1,000
500
0
300
250
200
150
100
50
0
Black circles (primary y-axis)
Orange circles (secondary y-axis)
5/3/2024
5/4/2024
5/5/2024
5/6/2024
B
Engagement rate by scientific theme was not correlated with total number of views
Engagement rate
4.0%
3.5%
3.0%
2.5%
2.0%
1.5%
1.0%
0.5%
0.0%
Neuro-Uro
Urinary Tract Dysfunction
Prostate
Urolithiasis
Urobiome/ Infection
Bladder
General Event Promotions
0
500
1000
1500
Number of views

36

Catalyzing Connections to Advance Benign Urology Research

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Abstract

BACKGROUND: The CAIRIBU U24 Interactions Core (CIC; CAIRIBU = Collaborating for the Advancement of Interdisciplinary Research in Benign Urology) was funded by the National Institute of Diabetes and Digestive and Kidney Diseases in 2020. A primary aim of the CIC is to foster interdisciplinary research. We assessed the short-term impacts of a 1½-day "collaborative summit" to stimulate collaborations among early-stage investigators (ESIs).

METHODS: Though the event was promoted broadly, it was built around CAIRIBU-affiliated ESIs. We designed activities in which they would introduce themselves, describe their research, and interact with other attendees in breakout groups related to specific research interest areas: lower urinary tract dysfunction, infection/urobiome, and neuro-urological and pain processes in the urinary tract. Within strategic networking sessions, participants were led through a "Speed of Light Thinking" activity during which research ideas were generated privately, then shared and compiled. Groups further refined ideas using affinity mapping and prioritization.

RESULTS: Of 32 invited ESIs, 20 accepted - 55% women, 50% K12 Scholars (current or prior), 25% CIC Collaboration Award recipients, and 25% CAIRIBU urobiome investigators; several also received Urology O'Brien Center Opportunity Pool awards. Overall, 119 participants from 53 unique institutions participated in-person or virtually and were established investigators (34%) or ESIs (30%), students (23% - graduate and undergraduate), and other (13%). Research needs and priorities emerged; several collaborations were planned (**Table**).

CONCLUSION: We created a platform for vigorous discussion around 3 major research areas of interest within the CAIRIBU Community. The strategic networking sessions proved to be an effective mechanism for identifying key knowledge gaps, generating potentially high-impact research questions, and encouraging collaboration. The summit engaged a diverse cross-section of students, ESIs, and established investigators from the urology research community and was successful in enabling deep knowledge exchange and networking.

TABLE. ***Research priorities and survey responses.*** Data show some of the research priorities identified by participants through facilitated networking sessions within research breakout groups (top half) and preliminary event feedback (bottom half). Percentages in columns do not add up to 100% as only those citing "strongly agree" or "agree" were included.

RESEARCH TOPIC	PRIORITIES
Lower urinary tract dysfunction	• Develop "universal language" to describe dysfunction, pathologies, cell types, etc. • Promote and incentivize sharing of data and research resources • Characterize differences on anatomic and molecular levels between humans and animal models currently in use
Infection and urobiome	• Collect and share data from global and population-level studies • Develop workable model systems to test hypotheses and therapeutics • Understand microbe-microbe interactions, host-microbe interactions
Neuro-urological and pain processes in the urinary tract	• Explore impacts on pathologic processes and symptoms of environment, geographic location, socioeconomic status, demographics, diet, and history • Create tissue bank to study specific cell types, gene expression, biomarkers • Generate models to study brain/bladder/urethra crosstalk mechanisms, effects of stress, etc.

SURVEY ITEM	STRONGLY AGREE	AGREE
Participant gained new knowledge	62%	38%
Participant expanded their research network	50%	45%
Participant interested in follow-on discussions and/or collaborative initiatives	72%	22%

ANONYMIZED COMMENTS FROM KEY EARLY-STAGE INVESTIGATOR PARTICIPANTS WHO DESCRIBED A NEW OR POTENTIAL COLLABORATION MADE DURING THE SUMMIT
Will work with 2 groups on a new project
Will collaborate on a pilot grant to look at environmental chemicals in urine of individuals with LUTS
Multiple collaborations planned, one on a neuro-uro project and another on infection
Will collaborate with an ESI investigator who will share primate urethra specimens
Potential new ideas to work with another ESI on trials and creating a study database
Will collaborate on a metabolomics project
Will collaborate with another ESI to assess expression in our mice for some genes of interest to us

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Nucleosome remodeling factor (NURF) shapes nuclear receptor (NR) signaling in African American prostate cancer.

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Abstract

Background: African American (AA) men face greater prostate cancer (PCa) aggressiveness than European Americans (EA). This study explored how SMARCA5 in the NURF complex distorts the epigenome and NR signaling in AA PCa.

Methods: H3K27ac and AR cistromes (DHT, 10nM/6h) were measured in non-malignant prostate (HPr1AR [EA], RC43N [AA]) and PCa cell lines (LNCaP [EA], RC43T, RC77T [AA]). SMARCA5 was elevated in LNCaP, RC43T, and RC77T cells using dCas9-activator and chromatin accessibility (ATAC-Seq) and gene expression (RNA-Seq) measured ($1,25(OH)_2D_3$, 100nM/6h). $1,25(OH)_2D_3$ regulation of miRNA (Nanostring) was assessed in EA and AA cells, and serum $25(OH)D_3$ associations with PCa measured in Nigerian men.

Results: The epigenome differed between AA and EA PCa. Basal H3K27ac cistromes (~15-20k sites) significantly overlapped between RC43N and HPr1AR, less so between RC43T and LNCaP, but converged with DHT treatment. H3K27ac cistrome motif enrichment was significantly different between AA and EA cells: Helix-Loop-Helix motifs (e.g., TWIST2) were enriched in RC43N; STAT motifs (e.g., STAT1) were enriched in RC43T, and similarly NR motifs were distinct with SF-1, VDR, and orphan receptors (e.g., RORG) enriched in RC43T. Basal AR cistromes differed but converged with DHT treatment. Public AR cistromes from primary AA tumors were enriched for STAT (e.g., STAT1) and homeobox (e.g., Goosecoid) motifs.

We examined SMARCA5's impact on the epigenome and transcriptome. SMARCA5 regulated ~1200 genes between RC43T and RC77T, enriched for interferon, inflammation responses, and distinct NR signaling. The transcriptome was accentuated in SMARCA5 + $1,25(OH)_2D_3$ related to tamoxifen, and progesterone signaling. Ongoing RNA-Seq in CRISPR-activated LNCaP and ATAC-Seq in all CRISPR models are defining how nucleosome positioning drives DEGs. Translationally, AA PCa-specific $1,25(OH)_2D_3$-regulated miRNA (e.g., miR-25) also regulated by SMARCA5 and involved in circadian rhythm. Low serum $25(OH)D_3$ in Nigerian men was significantly associated with aggressive PCa.

Conclusion: The epigenome is shaped by genomic ancestry. The epigenomic-regulator SMARCA5 is significantly downregulated in AA PCa and impacts NRs, including VDR signaling. Clinical correlates

include low serum 25(OH)D_3 levels in Nigerian PCa patients, and distinct miRNA regulation. These findings suggest that African ancestry shapes SMARCA5 functions, NR signaling, and tumor outcomes.

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Function of Protein Arginine Methyltransferase 5 (PRMT5) in the Development of Neuroendocrine Prostate Cancer

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Abstract

Background: Neuroendocrine prostate cancer (NEPC) is a highly aggressive form of prostate cancer that accounts for about 20-30% of deaths in patients with castration-resistant prostate cancer (CRPC). NEPC can develop spontaneously, but most cases arise as treatment-induced NEPC (tNEPC). Our research identifies the protein arginine methyltransferase 5 (PRMT5) as a crucial epigenetic factor in NEPC and suggests it could serve as a potential biomarker for predicting tNEPC.

Methods: We created both in vitro cell culture and mouse models to replicate conditions seen in patients undergoing androgen signaling inhibitor (ASI) or androgen deprivation therapy (ADT). By using doxycycline-inducible shRNAs and catalytic inhibitors, we investigated the role of PRMT5 and its cofactor methylosome protein 50 (MEP50) in these models. We assessed cell viability, neurite growth, and changes in neuroendocrine-related gene transcription. Pro-NEPC development was evaluated through immunofluorescence, immunohistochemistry, and western blotting. PRMT5 methyltransferase activity was further validated by examining histone deposition with western blotting and ChIP-qPCR. We analyzed PRMT5 and MEP50 expression levels in prostate tissue samples from CRPC and NEPC patients for clinical relevance.

Results: Our analyses revealed higher levels of PRMT5 expression in NEPC. Overexpression of these proteins in prostate cancer cells induced neuroendocrine differentiation (NED). Depleting PRMT5 and MEP50 prevented NED and restored sensitivity to ASI/ADT. PRMT5 and MEP50 promote chromatin changes through the dimethylation of H4R3, which is closely linked to the NED phenotype. Additionally, overexpressing PRMT5 and MEP50 in mice led to NEPC development, while reducing their expression could prevent NED in xenograft models. High levels of PRMT5 and MEP50 were associated with increased recurrence in patients undergoing ADT and their likelihood of recurrence post-treatment.

Conclusion: Our findings establish PRMT5 as a significant epigenetic regulator in NEPC development and highlight its potential as a predictive biomarker for tNEPC.

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Lin28b promotes neuroendocrine prostate cancer progression through regulating RNA compartmentalization and selecting translational reprogramming

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Abstract

Background

Therapy-induced neuroendocrine prostate cancer (t-NEPC) is a highly aggressive subtype of prostate cancer. Tumor progression to t-NEPC is frequently accompanied by cancer cells acquiring stemness phenotypes. We have previously shown that Lin28b regulates a stemness gene network to promote t-NEPC development. However, Lin28b is sequestered in stress granules (SGs) under stress conditions. It remains unclear whether Lin28b regulates RNA compartmentalization to control a translational reprogramming that can contribute to t-NEPC development.

Methods and Results

Using immunofluorescence microscopy, we observed that Lin28b knockout in the t-NEPC cell model of DuNE cells reduced the stress granule (SG) formation rate under arsenite (ARS) treatment from 59% to 20%. Consistently, Lin15, a novel Lin28b-specific inhibitor developed through computer-aided drug design, showed strong inhibition of SG formation in DuNE cells. We applied a sucrose gradient polysome fractionation (SGPF) assay to enrich polysomes and soybean peroxidase-based proximity labeling (APEX) to enrich SGs, followed by RNA-seq analyses. We found that under ARS treatment Lin28b promotes the enrichment of stemness genes and neuroendocrine markers (e.g., NRCAM, SRRM4, HMGA2, SALL4, CHGA, and NPTX1) in the polysomes, and these mRNAs were excluded from SGs by Lin28b. We further confirmed that these polysome-enriched genes are highly expressed at the protein level, consistent with their enrichment in polysomes. Furthermore, we found that Lin28b forms a protein complex with SALL4, a transcription factor that regulates a panel of stemness genes. There is a functional synergy between Lin28b and SALL4 that stimulates prostate cancer cell survival and enhances cancer cell stemness, allowing them to endure stress conditions.

Conclusions

These results indicate that under stress conditions, Lin28b promotes NEPC progression by sequestering specific mRNAs into SGs and increasing cancer stem cell (CSC) gene transcriptional activity by selectively enriching them in polysomes.

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Characterization of PARP Inhibitor Response in Prostate Tumor Cells Reveals Drug Tolerant Persistent Phenotype

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Abstract

Background

PARP inhibitors (PARPi) have improved management of prostate cancer, but acquired resistance and progression remain inevitable. Understanding treatment failure will improve outcomes. Drug tolerant persistence (DTP) is characterized by a small population of tumor cells which survive treatment through transient acquisition of insensitivity. DTP cells are not intrinsically resistant but may slowly proliferate, allowing for mutation accumulation, development of resistance, and progression. DTP cells may also increase mutability, further driving this process. Characterization of drug tolerant persistence and mechanisms enabling a DTP state under PARP inhibition remain unknown in prostate cancer.

Methods

Viability assays determined efficacious doses of olaparib and rucaparib in PARPi sensitive C4-2B metastatic castration-resistant prostate cancer cells and the C4-2B abiraterone-resistant derivative cells, AbiR. Phase contrast microscopy, western blots, beta-galactosidase activity assays, and flow cytometry characterized response to olaparib and rucaparib. RNA-seq determined the transcriptomic profile of PARPi DTP cells.

Results

C4-2B and AbiR response to olaparib and rucaparib is heterogeneous, characterized by cell death and cytostasis. Despite prolonged exposure to high dose PARPi, we observe a small minority of cells which persist, display varying altered morphologies, and altered cell cycle dynamics. While most of these cells appear arrested, we consistently note the presence of a subset which form colonies. C4-2B and AbiR cells exposed to high PARPi dosing for 9 days followed by drug holiday regain normal, parental cell morphology and become re-sensitized to treatment in line with acquisition of a DTP phenotype. RNA-seq reveals transcriptomic characteristics of drug tolerant persistence including evidence for increased mutability, altered metabolism, and cell identity changes.

Conclusions

Our data suggest that transient, drug tolerant persistence may mediate survival of a minority of tumor cells. These persisters may acquire resistance and fuel progression. Future efforts will characterize this phenotype further and investigate vulnerabilities that target survivors to improve treatment efficacy.

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Correlation of different pathological features in benign prostatic hyperplasia

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Abstract

Background:

Benign Prostatic Hyperplasia (BPH) is a prevalent condition causing deteriorating lower urinary tract symptoms in aging men. It is recognized as a disease with multiple pathological features, including cellular proliferation, smooth muscle dysfunction, inflammation, and fibrosis. Most recently, we identified lipid accumulation as a novel pathological component. However, the correlation between collagen accumulation, immune cell infiltration, and alterations in lipid metabolism in BPH has not been previously investigated. This study aims to clarify how various pathological aspects of BPH are interrelated, offering insights into the underlying mechanisms of the disease and potentially leading to more effective treatment strategies.

Methods:

The prostate tissues were collected from BPH patients who underwent Holmium Laser Enucleation of the Prostate (HoLEP) surgery (n=8). Immune cell infiltration was assessed using immunohistochemistry (IHC) for CD45, a marker of immune cells. The degree of fibrosis, manifested as accumulation of collagen, was quantified with Picrosirius Red staining and further analyzed by fluorescence microscopy. Lipid droplets were detected by Oil Red O staining. Percentage of $CD45^+$ cells, lipid/area and fluorescence intensity were quantified using Inform software and correlated to prostate size.

Results:

Significant correlations were found between $CD45^+$ cells and collagen ($r = 0.856$, $p = 0.0067$) and prostate size and collagen ($r = -0.858$, $p = 0.0064$). There was a negative correlation between $CD45^+$ cells and lipid droplets ($r = -0.726$, $p = 0.0412$). Additionally, collagen and lipid droplets were negatively correlated ($r = -0.711$, $p = 0.0481$). Although not statistically significant, there was a positive correlation between prostate size and lipid droplets ($r = 0.487$, $p = 0.2214$). The correlation between $CD45^+$ and prostate size was moderate and negative ($r = -0.600$, $p = 0.1157$), but not statistically significant.

Conclusions:

The study revealed that, in BPH, there are considerable correlations among the pathological characteristics, with a very strong relationship between inflammation and fibrosis, and an inverse correlation between prostate size and fibrosis. In addition, lipid content negatively correlated with fibrosis and inflammation. These suggest the presence of a smaller, more fibrotic prostate subgroup and a potentially larger, lipid-rich phenotype. Our future experiments will increase the sample size to perform association studies of treatment type, symptoms and other BPH patient correlates.

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The Effect of Sleep Fragmentation on Prostate Inflammation

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Abstract

Benign prostatic hyperplasia (BPH) is the enlargement of the epithelium and stroma in the transitional zone of the prostate gland. It is common in men over 50, with prevalence exceeding 90% by age 70. Prostate enlargement can lead to urethral obstruction, causing lower urinary tract symptoms (LUTS) the most burdensome of which is nocturia, which results in sleep fragmentation (SF). BPH often features inflammation, though the pathogenesis is uncertain and likely multifactorial, involving factors such as infection, diet, and hormonal changes. SF is known to cause inflammation in cardiovascular and neuroinflammatory diseases, suggesting it may also cause inflammation in BPH. Thus, the aim of our study was to determine if SF results in prostatic inflammation, and to correlate prostatic immune cell infiltration with nocturia in BPH patients.

Three-month-old black 6 (C57BL/6J) mice were housed in a SF chamber, where an automated moving rod interrupted their sleep every two minutes, from 6am-6pm, for three months. Following euthanasia, all four lobes of the prostate (ventral, anterior, lateral, and dorsal) were harvested. Prostate tissue was collected from BPH patients undergoing Holmium Laser Enucleation of the Prostate (HoLEP) who also completed the international prostate symptom score questionnaire to determine the nightly number of nocturia events. Inflammatory level was established using immunohistochemistry for CD45. Images were taken on a Mantra Quantitative Pathology Workstation and $CD45^+$ cells were counted manually (mouse) or with Inform software. Differences between SF and control mice were determined using a Mann-Whitney t-test. A linear regression analysis was performed to assess the correlation between nocturia events and $CD45^+$ cells.

Increases in $CD45^+$ cells were observed in the ventral (2.6 fold increase, p=0.0020), anterior (2.5 fold increase, p=0.0127), lateral (1.7 fold increase, p=0.0127), and dorsal (1.8 fold increase, p=0.0047) prostate lobes between SF (n=5) compared to controls (n=10) in mice. $CD45^+$ cells from BPH patients showed a trend for positive correlation with nocturia events (r= 0.2569, p=0.2306, n=6).

Our findings in a mouse SF model suggest that SF drives pro-inflammatory changes in the prostate, providing novel insights into the relationship between sleep quality and prostate health. Our pilot

human study, involving only 6 patients, also highlighted a potential correlation between prostatic inflammation and nocturia events. Our future studies will focus on increasing sample number and establish immune cell types and processes.

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Receptor Tyrosine Kinase Transcriptional Regulation via the Pro-neural Factor ASCL1 in Neuroendocrine Prostate Cancer

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Abstract

Background: Tumor resistance to second-generation hormonal therapies, like enzalutamide and abiraterone acetate, often result in androgen receptor indifferent phenotypes, like an aggressive disease variant, neuroendocrine prostate cancer (NEPC). With limited therapeutic options and poor prognosis, it is important to study the molecular basis of NEPC and identify new therapeutic targets. The pro-neural transcription factor, ASCL1, is a driver of NEPC and regulates expression of druggable target genes, such as the cell surface marker DLL3. Here, we aim to identify additional key cell surface markers that can be used as biomarkers or druggable targets in ASCL+ NEPC.

Methods and results: We analyzed RNA-seq data from parental and ASCL1 knockdown NCIH660 NEPC cells and nominated two receptor tyrosine kinases (RTKs), KIT and RET, with RET as the top cell surface gene strongly correlated to ASCL1. RET is an RTK expressed in neuronal tissues and is implicated in NEPC survival and growth. Here, we show that RET gene expression strongly correlates to ASCL1 but not NEUROD1 in various NEPC patient sample and mouse model datasets. This data is corroborated by scRNA-seq data in CRPC-NEPC samples, with no correlation between RET and ASCL1 in the CRPC-Adenocarcinoma (AdCa) samples. We also saw that RET gene expression mirrors ASCL1 gene expression in a mouse xenograft model as both genes increase in tandem and temporally as the tumor progresses towards a neuroendocrine phenotype. Informatics modeling of whole exome sequencing data from patient samples shows that RET and ASCL1 have substantially similar gene network signatures in NEPC as compared to AdCa, implying that the genes reside in the same gene ecosystem in NEPC. Remarkably, we do not see any correlation between RET and NEUROD1 gene networks in either subtype. We also saw increased ASCL1 recruitment and H3K27ac marks at RET transcription sites in ASCL1+ PDXs, but not in CRPC-AdCa or NEUROD1+ PDXs, indicating the direct transcriptional regulation of RET by ASCL1. We also demonstrated a similar relationship between RET and ASCL1 in small cell lung cancer (SCLC), a neuroendocrine cancer, using publicly available bulk RNA-seq, scRNA-seq, and ChIP-seq data. We also see a reduction of RET protein with ASCL1 knockdown, and induction of RET protein with ASCL1 overexpression.

Conclusion: These results highlight the critical role of ASCL1 in mediating RET signaling in NEPC and will be crucial to identify this subtype when stratifying patients for RET or other novel therapeutic interventions.

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Identifying Germline Variants Associated with Metastatic Prostate Cancer Through an Extreme Phenotype Study

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Abstract

Background: Studies of germline variants in cancer genomics have been mainly focused on their connections to cancer predisposition, however an understanding of how heritable factors contribute to cancer progression and metastasis remain limited.

Objective: To identify uncommon to rare germline nonsynonymous variants that increase the risk of metastatic prostate cancer once the cancers develop and to validate any functional effects.

Design: We assembled an extreme phenotype cohort of 52 patients who had been diagnosed with high-grade PCa with > 7 years of follow-up. Localized treatment naïve tumors were available for all. In half of the cases, the tumor had metastasized to bone; in half it had not. Tumor far adjacent normal DNA

samples were exome sequenced and analyzed for germline variants with allelic frequencies ≤ 2% in gnomAD v2.1.1. The findings were validated using 2 independent cohorts including a closely matched Australian cohort of 53 aggressive PCa patient germlines and 973 patient germlines from the Pan Prostate Cancer Group. A KDM6B variant was identified and engineered into the prostate cancer cell line LNCaP to test the hypothesis that a subset of identified variants can confer metastatic phenotypes.

Results: Variants in 25 DNA Damage Repair (DDR) genes were significantly enriched in the metastatic arm (p-value=4.57e-06) whereas the prevalence of synonymous variants at minor allele frequencies of ≤2% were similar between the metastatic and nonmetastatic arms. The predictive power of variants in 53 non-DDR genes was validated in an Australian cohort (p=0.0165) and correlated with high-risk PCa in the Pan Prostate Cancer Group germline cohort. A KDM6B variant discovered in our extreme phenotype cohort showed functional significance in this context, despite being annotated as benign in ClinVar.

Conclusions: We identified a series of germline variants that were enriched among prostate cancer patients who developed metastases relative to prostate cancer patients whose tumors did not metastasize. Moreover, we showed that one of these variants confers a metastatic phenotype. Our findings show that germline testing at diagnosis may improve treatment stratification in PCa. Extreme phenotype studies such as this may advance prostate cancer biology.

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Identification of Aggressive Subtypes of AR-Dependent CRPC and Their Drivers Through an Analysis of Super-Enhancer Atlas

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Abstract

Identification of Aggressive Subtypes of AR-Dependent CRPC and Their Drivers Through an Analysis of Super-Enhancer Atlas

Background: Metastatic castration-resistant prostate cancer (mCRPC) is a heterogeneous disease, characterized by diverse drivers of progression and mechanisms of therapeutic resistance. Super-Enhancers (SEs) are large clusters of enhancers that can robustly drive transcription and have been identified at key oncogenic drivers. In this study, we evaluated SEs using public ChIP-seq data from patient-derived xenografts (PDX) and CRPC cell lines to determine whether CRPC can be categorized by distinct SE patterns.

Methods: We performed Rank Ordering of Super-Enhancers (ROSE) analysis to identify SEs from public H3K27ac ChIP-seq data in the LuCaP and MURAL PDX series. To create a Super-Enhancer (SE) Atlas, we combined reproducible SE regions found in at least two samples. Molecular subtypes were determined through consensus clustering of these SE regions. Subtype-specific SEs were identified by comparing each subtype against the others. We integrated matched RNA-seq data to identify the top-ranked transcriptional drivers for each subtype, which were evaluated in representative cell line models.

Results: We identified five distinct SE programs in mCRPC. Three of these corresponded to androgen receptor (AR)-dependent CRPC, designated as the AR-1, AR-2, and AR-3 subtypes. The remaining two programs were aligned with neuroendocrine (NEPC) and double-negative (DNPC) subtypes. For each subtype, we identified top SE-driven genes that exhibited higher expression levels compared to other subtypes. Within the AR subtypes, AR-1 and AR-2 were identified as particularly aggressive, with strong enrichment in hypoxia pathway and epithelial-mesenchymal transition, respectively. Notably, we identified HNF1A, TWIST1, and TBX10 as the top SE-driven transcriptional drivers in AR-1, AR-2, and AR-3, respectively. Analysis of public CRPC patient datasets revealed distinct $HNF1A^{+}$, $TWIST1^{+}$, and $TBX10^{+}$ tumor subsets within the AR-dependent subgroup. Among these, HNF1A is recognized as a master regulator of the gastrointestinal-lineage transcriptome in prostate cancer. Furthermore, HNF1A knockdown in 22Rv1 cells significantly decreased HIF-1α activation, suggesting its potential role in hypoxia regulation.

Conclusions: Our study indicates that the SE Atlas in mCRPC is associated with tumor progression, therapy resistance, and lineage plasticity. The molecular classifications defined by SEs could potentially guide therapeutic decisions and aid in identifying key oncogenic drivers in each CRPC subtype.

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Transcriptomic Landscape of Tumor Microenvironment in Testicular Seminoma Reveals Novel Molecular Insights into Pathogenesis

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Abstract

Background: Seminomas is a malignant testicular germ cell tumor (TGCT) affecting male adults and is characterized by low somatic mutation burden and histological homogeneity. Though seminoma is highly treatable with high survival rate, the mechanisms driving tumor development and progression remain poorly understood. Tumor/immune microenvironment (TME) has a vital role in cancer progression with implications in cell death resistance and immune escape. Herein, we aimed to unravel the transcriptomic TME landscape of seminomas and better understand seminoma pathogenesis.

Methods: We performed single cell RNA-sequencing (scRNA-seq; 10X-Illumina platform) on pure seminomas from four patients undergoing orchiectomy. A total of ~24,000 single cells were analyzed using Seurat/Bioconductor R packages and standard means for hierarchical clustering, cell annotation and data visualization. Key findings were validated by in situ hybridization RNA analysis (chromogenic RNAscope) and Visium spatial transcriptomics (10X, spots, resolution of 1-10 cells/spot) in an independent set of seminoma samples.

Results: ScRNA-seq analysis revealed 10 major cell types with T-cells and Natural-Killer cells being predominantly enriched, where tumor cells consisted of a distinct subpopulation with limited presence (1%). A unique set of signature genes (e.g., *POU5F1, KHDC3L, DPPA5, PCSK1N, NANOS3*) was identified, including novel markers, highly expressed in seminoma cells and associated with pivotal cellular processes e.g., developmental pluripotency and germ cell development. Notably, this geneset was also highly differentially expressed (DE) in The Cancer Genome Atlas (TGCA) seminomas (n=73) compared to TCGA non-seminomas (n=38) (FDR<0.01). Amongst top marker genes, *KHDC3L*, also higher expressed in seminomas compared to normal testis, was validated by RNA-scope. Notably, KHDC3L demonstrated the

strongest positive correlation with DPPA5 (Spearman/Pearson; R2=0.8, p<0.0001), top #3 ranked DE gene as well as with other top 20 DE genes identified by scRNA-seq. Remarkably, similar strong correlations among other top DE genes were also found in the testicular TGCA cohort.

Conclusions: Our analyses revealed novel marker genes, uniquely expressed in seminomas which consist of a homogeneous cell population across all tumors analyzed, clearly distinct from other cell types identified. Strong correlations among top DE genes including master transcription factors (e.g., POU5F1, NANOG, SOX4, MYBL2) reveal genes involved in the same protein pathway/networks likely suggesting synergic effect/involvement in the same regulatory circuitry.

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PPARG expression regulates tumor initiation in mouse models of bladder cancer

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Abstract

Bladder cancer ranks among the most common diagnosed malignancies worldwide affecting both men and women. Despite favorable 5-year survival rates for early non-muscle invasive cases, high recurrence rates and limited therapeutic options complicate disease management. Advancements in novel therapeutic strategies will rely on the use of relevant disease models. Recently, we published our method to create de novo bladder cancer models by lentiviral transduction of primary mouse urothelial cells. By exposing urothelial cells to a pool of barcoded lentiviral vectors, common overexpression and loss-of-function events found in human bladder cancer can be introduced. We've shown that this method can generate heterogenous tumors with distinct histological regions that recapitulate those seen in patients. Barcode enrichment analysis revealed that overexpression of NECTIN4, YWHAZ, PPARG, and activating mutation PIK3CA-E545K, were commonly found across the multiple histological subtypes produced. These findings emphasize an opportunity to study bladder cancer tumorigenesis using a model that can induct specific genetic modulations. PPARG stands out as a strong candidate for further oncogenic evaluation for both its common transduction event enrichment and as an important regulator of urothelial cell differentiation. To discern its functional contribution, a four-vector lentiviral pool model that consists of NECTIN4, YWHAZ, PIK3CA-E545K along with either constitutively active VP16-PPARG, wild-type PPARG, and dominant negative L466A-PPARG isoforms were produced. We found that all mice transduced with lentiviral pools containing WT-PPARG produced tumors (6/6 mice) while those transduced with VP16-PPARG and L466A-PPARG produced fewer tumors (4/6 mice and 2/6 mice respectively). Interestingly, mice that contained cells transduced without any of the PPARG isogenic variants and only the other three factors produced no tumors (0/6 mice). This pilot experiment highlights a possible oncogenic mechanism for PPARG in which it may be regulating efficient bladder cancer initiation in mice. It also suggests that a unique regulatory network may drive this effect given that constitutively active VP16-PPARG was not as efficient in producing tumors and may not allow for discrete modulation of this transcription factor by other cancer promoting cofactors. Investigation into the driving oncogenic mechanisms of bladder cancer would open the window for targeted treatment earlier in non-invasive cases where many patients categorize and where therapeutic treatment is optimal for survival and reducing invasive progression.

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Genomic and transcriptomic aberrations differentiating teratomas at metastatic sites from primary germ cell tumors of testis

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Abstract

Background: Testicular germ cell tumors (TGCTs) are the most common malignancy occurring in young men between the ages of 20-40 years and include various histologically diverse cancer subtypes. Amongst all TGCTs, mature teratomas present the most advanced differentiation state and can lead to secondary malignant transformation into non-TGCT cancer types (such as sarcoma and adenocarcinoma) in various organs and late relapse with worse clinical outcome. Currently, there are no effective systemic therapies in the teratoma cases of unresectability, incomplete resection, or multifocal metastases and teratoma patients often demonstrate resistance to chemotherapy. Both primary and metastatic teratomas share several morphological similarities. However, they inhabit distinct microenvironments, potentially driven by different molecular alterations and transcriptomic vulnerabilities, which are currently limitedly studied.

Methods: We performed whole exome sequencing in 16 primary TGCTs and 17 matched post-chemotherapy pure metastatic teratomas collected from retroperitoneal lymph nodes undergoing orchiectomy). Most primary tumors (n=11) had mixed histological components, seven of them had dominant embryonal carcinoma component (>50%), two were 99-100% teratomas, one was a pure testicular seminoma. Recurrent cases were treated with one cycle of bleomycin, etoposide and/or? cisplatin chemotherapy followed by surgical resection of residual tumor. Tumors removed from two different metastatic sites were analyzed for two patients. Tumor samples were compared to matching normal samples (blood) to distinguish germline and somatic genetic alterations. Bulk RNA-sequencing was performed in 13 residual teratomas removed from lymph nodes after chemotherapy (metastatic teratomas) and 12 primary testicular teratomas. Key findings were validated at spatial level

by 10X Visium transcriptomics (Illumina platform) in one mixed TGCT sample, predominantly enriched in teratoma (95%).

Results: A total of 583 single nucleotide variants (SNVs) were identified across all 33 tumors analyzed, with the majority being missense alterations (82%). The most frequently mutated genes were associated with RTK/RAS signaling, with KRAS gain-of-function alterations being the predominant oncogenic event. Metastatic teratomas had median tumor mutation burden (TMB) of 0.65 and matched primary tumors had a lower median TMB of 0.47. GISTIC analysis revealed recurrent chromosomal 3p11.1 gain in metastatic site teratoma comparison to two primary TGCT datasets (internal and The Cancer Genome Atlas/TCGA cohort) (p-value = 0.0001). Five loci including chr10q26.3, 16p11.2, 19p13.2, 19q12 and 22q12 showed recurrent loss events in metastatic teratomas compared to primary tumors. In addition, gene set enrichment analysis of bulk RNA-sequencing data revealed significantly enriched, clinically relevant pathways e.g., collagen network formation, cell cycle checkpoint, T-cell immune response activation amongst others in metastatic compared to primary TGCTs (FDR/q-value < 0.01).

Conclusions: Both primary TGCT and metastatic teratomas demonstrate a very low TMB with no significant differences. Clinically relevant/druggable pathways were identified including T-cell activation and differentiation as well as several genes related to collagen modification were overexpressed in metastatic teratomas compared to primary tumors. Overall, we identified distinct genomic and transcriptomic features that may contribute to TGCT tumorigenesis and malignant transformation to teratomas.

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TSC1 mutant bladder cancer requires TFE3, identifying a potential therapeutic vulnerability

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Abstract

Background: Bladder cancer (BLCA), both non-muscle invasive and muscle-invasive, frequently (6-10%) has biallelic inactivation of the tumor suppressor gene *TSC1*. However the mechanism by which *TSC1* loss promotes BLCA development beyond mTORC1 activation is uncertain. Currently, treatment with rapalogs/other mTOR inhibitors has shown limited effectiveness against *TSC1* mutant BLCA.

Materials: We performed differential gene expression and gene set enrichment analyses (GSEA) using The Cancer Genome Atlas (TCGA) RNA-sequencing data for *TSC1*-mutated BLCA (*TSC1*mt-BLCA, n=26) and *TSC1* wild-type tumors (*TSC1*wt-BLCA, n=382) and compared them to an internal cohort of other *TSC1*/*TSC2*-mutant tumors (n = 48) seen in patients with Tuberous Sclerosis Complex (TSC). Integrative multi-omic transcriptomic, epigenetic, and functional analyses were performed using *TSC1*-mutant/addback BLCA cell lines. Validation studies were performed in a *TSC1*mt-BLCA patient derived xenograft model.

Results: Comparison of *TSC1*mt-BLCA and *TSC1*wt-BLCA tumors revealed a conserved TSC-associated gene expression signature in *TSC1*mt-BLCA that was strikingly similar to that observed in syndromic TSC tumors. GPNMB had the highest increase (median fold change 9.8). GSEA and DESeq2 indicated both mTORC1 hyperactivation and enhanced lysosomal gene expression in *TSC1*mt-BLCA. Immunohistochemistry confirmed these expression changes in a separate cohort of BLCA, including elevated nuclear-localized TFE3 in TSC1mt-BLCA, a known regulator of lysosomal gene expression. TSC1-mutant BLCA cell lines (HCV29, RT4) recapitulated the human BLCA expression phenotype, and showed TFE3 nuclear localization, which was reversed by rapamycin. TFE3 knockout in HCV29 cells had major effects, reducing growth and lysosomal gene expression, and inducing inflammatory and senescence-associated gene expression, effects not seen in TSC1-addback HCV29. TFE3 CUT&RUN analysis of HCV29 cells confirmed TFE3's direct regulation of lysosomal and other gene pathways. Further, *TSC1*mt-BLCA patient derived xenografts retain the TFE3-driven expression phenotype.

Conclusions: Our data reveals *TSC1*mt-BLCA to be a distinct molecular subtype of bladder cancer, and show that human *TSC1*mt-BLCA tumors and cell lines have a transcriptional signature similar to that of TSC syndromic tumors, characterized by both mTORC1 activation and TFE3-driven gene expression. The

essential role of TFE3 expression in TSC1mt-BLCA development suggests that it may represent a therapeutic vulnerability.

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Targeting Wnt5A/ROR1 Signaling as Therapy for Metastatic Prostate Cancer.

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Abstract

BACKGROUND -- Wnt5A and its non-canonical Wnt receptor, ROR1, have emerged as a promising new target pathway for lethal, metastatic prostate cancer. Wnt5A is a significant marker of poor prognosis in circulating tumor cells (CTCs) in bone metastatic castration resistant prostate cancer (mCRPC) patients. ROR1 is expressed in lethal types of mCRPC including neuroendocrine prostate cancer (NEPC). A therapeutic ROR1 antibody, Zilovertamab, is in been clinical trials in other cancers. We investigated ROR1 targeting strategies and therapies in pre-clinical models of metastatic prostate cancer.

METHODS -- ROR1 expression was determined using RNASeq, qRT-PCR, FACS, IHC and Western blotting. ROR1 signaling was blocked using the anti-ROR1 therapeutic antibody, Zilovertamab, or CRISPR/Cas9 ROR1 knock out. Cell growth measured in 2D using an Incucyte and in 3D using time lapse confocal imaging. Cell cycle analysis was performed in live cells in 2D cultures and 3D organoids using the ***Fucci2BL*** bicistronic **F**luorescent, **U**biquitination-based **C**ell **C**ycle **I**ndicator reporter system. Tumor growth of the PDX, PCSD13, was measured via calipers and IVIS. RNASeq was performed on PC and PDX cells in 2D and 3D cultures and in xenograft tumors.

RESULTS -- We showed that ROR1 was expressed at high levels in the mCRPC cell lines, PC3, DU145, and in the bone metastatic prostate cancer PDX, PCSD13. CRISPR/Cas9 Knock out of ROR1 in PC3 and DU145 cells showed increased sensitivity to docetaxel inhibition of proliferation in vitro in 2D real time Incucyte proliferation assays and in 3D organoids. Strikingly, 3D Organoid formation was significantly reduced in ROR1 KO cells indicating reduced organoid/tumor initiating activity. Gene expression analysis revealed loss of Wnt5A expression in ROR1 KO cells in 3D organoids. GSEA showed that ROR1KO cells had decreased interferon pathways but increased integrin pathways and alterations in cancer stem cell

pathways. Treatment of PCSD13 PDX in vivo with Zilovertamab plus docetaxel synergistically increased tumor growth inhibition in vivo and modulated cancer stem cell and cell cycle expression profiles.

CONCLUSIONS -- Therapeutic targeting of these tumor initiating stem/progenitor cells may prevent the evolutionary diversification of a tumor and overcome a critical clinical barrier to cancer treatment. We showed synergistic response in PDX and PCs cell line models to Zilovertamab plus docetaxel. We are conducting a phase 1b clinical trial with zilovertamab plus docetaxel in patients with metastatic CRPC (CirmD, NCT05156905, PI R Mckay).

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The role of the small GTPase RhoA in the human urinary bladder

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Abstract

Overactive bladder (OAB) is a common clinical condition with a prevalence of 16 % and affects the patients' quality of life significantly. Currently, antimuscarinic drugs are the first-line medical therapy for the management of OAB. However, their application is limited due to their several side effects (e.g. dry mouth, obstipation). According to the literature the RhoA-ROCK signaling is linked to the pathogenesis of urinary bladder dysfunctions. Moreover, our previous results show that ROCK plays a pivotal role in the regulation of micturition and contractions induced by carbamoylcholine (CCh) and bradykinin (BK) in murine and human urinary bladder (UB).

Thus, we aimed to further analyze the role of the small GTPase RhoA in the intracellular signaling of the human urinary bladder smooth muscle (UBSM) contraction. Our final goal is to provide a theoretical basis for the development of a more specific medication for OAB with fewer adverse effects.

Experiments were performed on human UBs which were surgically removed due to bladder malignancy. UBSM strips were dissected from a macroscopically tumor-free area, approved by a pathologist, then they were mounted on a myograph system. After that, the strips were challenged with one single dose of CCh or BK. RhoA activity was measured in the human UBSM. Pharmacological pretreatment was applied depending on the experiment.

Our results show that the muscarinic receptor agonist CCh induced a steep increase in RhoA activity in the human UBSM compared to that of its vehicle. The CCh-induced RhoA activity was almost completely abolished in detrusor strips treated with the M_2 receptor antagonist AF-DX 16. CCh-induced RhoA activity was not altered in the presence of the M_3 receptor inhibitor 4-DAMP suggesting that the CCh-induced RhoA activation is regulated mostly by the M_2 receptors in human UB. Next, we wanted to investigate the role of the inflammatory mediator BK in RhoA activation in human UB. BK evoked an increase in RhoA activity compared to that of its vehicle. The BK-induced RhoA activation was diminished in human UBSM tissues treated with the B_2 receptor inhibitor HOE-140 demonstrating the pivotal role of the B_2 receptor in RhoA activation.

We concluded that the RhoA-ROCK pathway plays an essential role in the human UBSM. Moreover, CCh-induced RhoA activation is mediated mostly by the M_2 muscarinic receptors. In addition, RhoA

activation induced by BK seems to be mediated mostly by the B_2 receptors. The detailed understanding of the RhoA-ROCK pathway in the human UB may offer a novel, more specific target in the treatment of OAB.

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ERBB3 overexpression is enriched in racially diverse populations of patients with localized prostate cancer and is associated with a unique AR activity signature

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Abstract

Background

Despite successful clinical management of localized prostate cancer (PrCa), the 5-year survival rate for men with castration-resistant prostate cancer (CRPC) is only 32%. Verified risk factors for advancement to CRPC include age, family history, and race. Strikingly, Black/African American (AA) men are two to four times more likely to die of PrCa than those in other racial/ethnic groups in the US. Identification of molecular expression and activity patterns that vary by racial group may provide a key to addressing outcomes related to survival disparities, as current treatments have not been developed with attention to the inclusion of diverse patient populations. Additionally, developing a biomarker to stratify patients who will progress on standard-of-care therapy could guide intelligent intensification of therapeutic strategies and improve survival, particularly in high-risk patient populations.

Methods

Targeted deep sequencing was performed as routine care for treatment naïve patients in the UI cohort (n=30), and immunostaining was performed in racially diverse tissue microarray (n=149). Bioinformatics analyses were utilized to identify signaling pathways associated with biomarker overexpression in the UI cohort, consolidated publicly-available RNA-seq datasets (n=664), and a racially diverse Gene Expression Omnibus dataset (n=68).

Results

We identified increased incidence of ERBB3 overexpression (OE) in racially diverse PrCa patient populations, where it was associated with advanced disease at diagnosis. At the protein level, HER3 expression was negatively correlated with intraprostatic androgen in Black/AA men. Bioinformatics analyses showed a positive correlation between ERBB3 expression and the Androgen Response pathway despite low intraprostatic androgen and stable expression of androgen receptor (AR) transcript in Black/AA men. Clinically, ERBB3 OE was associated with high pre-treatment serum PSA in Black/AA men and was positively correlated with a clinically adaptable AR signature score. Preliminary outcomes data suggest Black/AA men with ERBB3 OE have a shorter time to disease progression.

Conclusions

In diverse PrCa patient populations, ERBB3 OE was associated with high AR signaling and serum PSA despite low intraprostatic androgen, suggesting ERBB3 OE as a prospective biomarker for ligand-

independent AR activation. In this context, ERBB3 OE could stratify patients for intensification of therapy in castration-sensitive disease and emphasizes the importance of biomarker-directed clinical trials with diverse populations.

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Notch Regulates Uroplakin Expression in Renal Urothelium

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Abstract

Background. Childhood urinary tract obstruction (**UTO**) is met with limited treatment options and represents a leading cause of chronic and end stage kidney disease in children. Our lab discovered that UTO triggers a renoprotective adaptation that includes increased uroplakin (**UPK**) expression in the renal urothelium. A prior study predicted NOTCH as a transcriptional regulator during peak UPK expression in the developing renal urothelium, but the significance of NOTCH in this tissue is unclear. Our unpublished findings show that KRT5 renal urothelium progenitors express activated NOTCH following UTO. We **hypothesize** that NOTCH regulates UPK expression in the renal urothelium.

Methods. *Hoxb7*^{Cre};*RBPJ*$^{fl/fl}$ (***RBPJ***$^{\mathbf{UB\text{-}KO}}$) and *RBPJ*$^{fl/fl}$ control mice were used to conditionally disrupt NOTCH signaling during renal urothelium development. Kidneys were collected at embryonic (E17.5, E18.5), neonatal (P1, P7), juvenile (P14, P21) and adult (P42+) time points. NOTCH abrogation was confirmed using RNAscope, and urothelium was evaluated using immunofluorescent assays (**IF**). *Krt5*^{iCre};*RBPJ*$^{fl/fl}$ (***RBPJ***$^{\mathbf{K5\text{-}KO}}$) and *RBPJ*$^{fl/fl}$ control mice were used to inducibly disrupt NOTCH during UTO. UTO was modeled using unilateral ureteral obstruction (**UUO**) and kidneys were collected at post operative day (**POD**)7, and urothelium was evaluated using IF. Urothelium organoids were used to validate the role of NOTCH using pharmacologic (**DBZ**) and genetic (*RBPJ*$^{K5\text{-}KO}$) NOTCH loss of function strategies, and organoids were evaluated using Western blot.

Results. RBPJ deletion was specific and efficient in *RBPJ*$^{UB\text{-}KO}$ renal urothelium (P<0.0001). NOTCH targets, *Hes1* and *Hes6*, were significantly decreased in *RBPJ*$^{UB\text{-}KO}$ renal urothelium compared to *RBPJ*$^{fl/fl}$ controls (P<0.0001, and P=0.0307, respectively). UPK was significantly decreased in *RBPJ*$^{UB\text{-}KO}$ kidneys at all time points (P<0.0001). At POD7, UPK was significantly reduced in *RBPJ*$^{K5\text{-}KO}$ compared to *Krt5*^{iCre};*R26*$^{tdT/+}$ mice. Pharmacologic (DBZ) and genetic disruption of NOTCH resulted in decreased UPK in urothelium organoids.

Conclusions. NOTCH promotes UPK expression in the renal urothelium during development and UTO. Future studies will interrogate the specific NOTCH ligands and receptors that promote UPK expression in renal urothelium. Additional studies are underway to determine whether NOTCH gain of function increases UPK and exerts a renoprotective effect in preclinical UTO models.

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PIEZO1 regulates UPK expression in the renal urothelium during UTO, and in a novel renal urothelium organoid model

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Abstract

Background. Urinary tract obstruction (**UTO**) is met with limited treatment options and is a leading cause of pediatric chronic and end-stage kidney disease (CKD/ESKD). Not all patients develop CKD/ESKD and renoprotective adaptations may explain positive outcomes. We discovered that increased uroplakin (UPK) expression represents a renoprotective adaptation to UTO, but the regulation of UPK expression in the renal urothelium is unclear. UTO exerts mechanical forces on the renal urothelium. The mechanoreceptor PIEZO1 is expressed by renal urothelium, but its role during UTO has not been investigated. We **hypothesize** that PIEZO1 activation promotes UPK expression during UTO.

Methods. PIEZO1 was conditionally deleted using *Hoxb7*^{Cre};*Piezo1*$^{fl/fl}$ (*Piezo1*^{cKO}) and *Piezo1*$^{fl/fl}$ mice served as controls. Tissue-specific *Piezo1* deletion was evaluated using RNAscope. UTO was induced using unilateral ureteral obstruction and kidneys were collected at post operative day (POD)7. We developed a novel renal urothelium organoid model and used Yoda1 to activate PIEZO1 *in vitro*. Immunofluorescence and Western blot were used to evaluate urothelial protein expression.

Results. We confirmed deletion of *Piezo1* in the renal urothelium of *Piezo1*^{cKO} compared to *Piezo1*$^{fl/fl}$ mice. We detected a significant decrease in UPK in the renal urothelium of *Piezo1*^{cKO} compared to *Piezo1*$^{fl/fl}$ kidneys at POD7. *Piezo1*^{cKO} mice also exhibited significantly worse parenchymal thinning that coincided with increased suburothelial collagen deposition at POD7. Next, compared to carrier-treated controls, Yoda1 significantly increased UPK in our novel renal urothelium organoid model system. Importantly, UPK was not increased in Yoda1-treated organoids derived from *Piezo1*^{cKO} mice.

Conclusions. This study 1) demonstrates critical roles for PIEZO1 in UTO-induced renal urothelium remodeling, 2) validates the significance of UPK expression in parenchymal integrity during UTO, 3) showcases the first-ever renal urothelium organoid model system, and 4) validates that PIEZO1 regulates renal UPK expression in an in vitro model. These findings advance our understanding of renoprotective adaptations to UTO, and mandate additional mechanistic evaluation of how PIEZO1 regulates UPK expression.

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Evaluating the Role of MEIS1 in HOXB13-Dependent Prostate Cancer Progression and AR Signaling

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Abstract

Background: Prostate Cancer (PrCa) is one of the leading causes of cancer-related death in men. Our lab has identified the HOX protein cofactor MEIS1 as a potential tumor suppressor in prostate biology. We have demonstrated that loss of MEIS1 protein expression strongly correlates with a higher likelihood of biochemical recurrence and metastatic progression (Bhanvadia et al., Clin Can Res 2018). Further, MEIS1 suppresses proliferation and cancer progression in a HOXB13-dependent manner (Van Opstall, ELife 2021). Androgen Receptor (AR), a key driver of prostate cancer, has been documented to interact with HOXB13 to promote prostate cancer progression. Despite the evidence that MEIS proteins are likely tumor-suppressive in PrCa, the interaction and functional impact between MEIS1, HOXB13, and AR has not been clearly defined. We hypothesize that increased MEIS1 expression reduces oncogenic AR-HOXB13 interactions and decreases oncogenic AR signaling.

Methods: Our laboratory derived cell lines overexpressing lentiviral constructs of MEIS1 and/or CRISPR-generated knockouts of HOXB13 in both castration-resistant CWR-22Rv1 and androgen-sensitive LAPC4 cell lines. The nuclear interaction between MEIS1 and AR was evaluated with co-immunoprecipitations and proximity ligation assays. IncuCyte Live-Cell Analysis Systems were used to elucidate the *in vitro* growth and migration phenotype of these prostate cancer cells under AR pathway modulation.

Results: Proliferation assays demonstrate that MEIS1 expression is dependent upon HOXB13 to decrease the proliferation of prostate cancer cells. Under AR-stimulated conditions, MEIS1 overexpression without HOXB13 results in a significantly higher growth rate compared to control and overexpressing MEIS1 cells alone.

Conclusions: These data support our hypothesis that MEIS1 has a HOXB13-dependent tumor suppressive role in prostate cancer cell lines. However, the functional role of MEIS1 proteins and how the MEIS1-AR complex regulates transcription in the prostate is not clear. Filling such knowledge gaps has the potential to reveal novel mechanisms of MEIS1 proteins to implement new biomarkers to predict prostate cancer progression and new targets to therapeutically block cancer metastasis.

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PPARγ Promotes Uroplakin Expression *via* GRHL3 During Development and Disease

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Abstract

Background. Urinary tract obstruction (**UTO**) is a leading cause of pediatric chronic and end-stage kidney disease (**CKD/ESKD**). Patients with a history of UTO face increased risk of CKD progression, but renoprotective adaptations may explain positive outcomes. Increased uroplakin (**UPK**) expression in the renal urothelium protects the kidney during UTO. High expression of UPK occurs during embryogenesis but is limited in the normal adult renal urothelium. PPARγ signaling promotes UPK expression but has not been investigated in the renal urothelium. We **hypothesize** that PPARγ promotes UPK expression and that PPARγ activation will limit parenchymal injury during UTO.

Methods. *Upk2*^{iCre} mice were used to drive PPARγ loss of function (**PPARγ-LOF**, using *Pparg*$^{fl/fl}$) or gain of function (**PPARγ-GOF**, using VP16-*Pparg*^{fl}). Unilateral ureteral obstruction (**UUO**) or Sham surgery was performed, and kidneys were collected at postoperative day (**POD**)7. Ultrasound was used to evaluate renal parenchyma and histologic, immunofluorescence (**IF**), and western blot (**WB**) assays were used to evaluate renal urothelium. The ChEA3 web application was used to predict transcription factors (**TFs**) that govern UPK expression during development and UTO. Candidate TFs were validated at E18.5 and POD7.

Results. PPARγ-LOF exhibited reduced UPK while PPARγ-GOF mice had increased UPK at POD7. PPARγ-LOF resulted in tissue injury and parenchymal thinning while PPARγ-GOF resulted in parenchymal preservation at POD7. ChEA3 predicted PPARγ and its upstream (KLF5) and downstream (GRHL3, and FOXA1) regulators at E18.5 and during UUO. IF validation confirmed PPARγ, KLF5, GRHL3, and FOXA1 expression at these stages. KLF5 was highly expressed at E18.5 and at POD7, but not in the renal urothelium of Sham-operated mice. Finally, GRHL3 was altered by genetic manipulation of PPARγ at E18.5 and at POD7, while FOXA1 was unchanged.

Conclusions. Activation of PPARγ promotes UPK following UTO and recapitulates a normal developmental program. PPARγ activation in the renal urothelium represents a renoprotective mechanism during UTO and likely signals through GRHL3 to regulate UPK expression in the renal urothelium. Pharmacologic activation of PPARγ represents an attractive therapy for UTO that requires additional preclinical investigation.

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Exercise alleviates the onset of abnormal voiding behavior in steroid hormone BPH mouse model

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Abstract

Background: Benign prostatic hyperplasia (BPH) with lower urinary tract symptoms (LUTS) is a disease of aging men that leads to a substantial decrease in quality of life, increased morbidity. Risk factors for LUTS/BPH include obesity, frailty, and a sedentary lifestyle, all of which increase with age. Exercise intervention trials demonstrate reversal of several LUTS/BPH risk factors, suggesting that exercise could be an effective first-line therapy. Recently, we demonstrated that voluntary wheel running in aged mice led to a significant reduction in urinary frequency often associated with LUTS/BPH. In this study, we examine the effect of exercise on the development of abnormal voiding behavior using a steroid hormone model of LUTS/BPH and accelerated aging. We hypothesized that exercise intervention would delay the onset of urinary frequency compared to sedentary animals.

Methods: Using eight-week-old C57Bl/6J mice, we implanted 2.5mg 17β-estradiol (E2) and 25mg testosterone (T) as compressed pellets subcutaneously. Animals were allowed to recover for 1-week post-implantation prior to having access to a voluntary running wheel. We collected tissue samples after 4- and 8-weeks of exercise.

Results: Notably, mice given access to running wheels showed a significant delay in the onset of urinary frequency compared to sedentary controls following steroid hormone implantation. Exercise also increased postsurgical grip strength in steroid hormone implanted animals. Additionally, exercise increased mobility but did not alter anxiety-type behavior compared to sedentary mice.

Conclusions: Steroid hormone implantation recapitulates behavioral aspects of aging, and aerobic exercise delayed the onset of steroid hormone mediated urinary dysfunction. While these findings suggest that exercise may be a potent non-invasive intervention for BPH/LUTS, the testosterone in the model complicates the behavioral interpretation of the data.

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Potential Role for Retinoic Acid Signaling in Renal Urothelial Remodeling During Congenital and Acquired Urinary Tract Obstruction

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Abstract

Background: Urinary tract obstruction (UTO) is a leading cause of chronic and end-stage kidney disease (CKD/ESKD) in children. Increased Uroplakin (UPK) expression and urothelial plaque formation protects the kidney from obstructive injury. The purpose of this study was to unravel mechanisms responsible for renal urothelial remodeling during UTO.

Methods: Megabladder (*Mgb*$^{-/-}$) mice were used to model congenital UTO. Incorporation of a *Upk2*^{Cre};*R26*^{zsGreen} reporter enabled efficient labeling of renal UPK+ cells, which were isolated by FACS and subjected to RNAseq. Unilateral ureteral obstruction (UUO) served as an acquired model of UTO and kidneys were collected on postoperative days (POD) 2 and 7. Urothelial lineage markers and retinoid signaling molecules were evaluated by immunofluorescence (IF) microscopy.

Results: We identified 88 differentially expressed genes in UPK+ cells from *Mgb*$^{-/-}$ vs. control kidneys by RNAseq. Pathway analysis indicated activation of retinol and retinoid metabolic processes, based on upregulation of *Cyp1b1*, Adh7, and *Rbp4* expression. IF confirmed that CYP1B1 and ADH7, along with other proteins implicated in retinoic acid signaling, RBP1 and ALDH1A1, are enriched in *Mgb-/-* and UUO renal urothelium. We localized ADH7, CYP1B1, and RBP1 to superficial and intermediate urothelial cells, and ALDH1A1 to intermediate and basal cells.

Conclusion: Congenital and acquired UTO trigger activation of retinoic acid signaling in the renal urothelium. Additional studies are required to functionally interrogate the role of retinoic acid signaling in UTO-induced renal urothelium remodeling.

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Exploiting ERBB3 Overexpression in Prostate Cancer to Modulate Enzalutamide Sensitivity

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Abstract

Background

ERBB3(HER3*) i*s a member of the ERBB family of receptor tyrosine kinases (RTKs), which includes *ERBB1*(EGFR), *ERBB2*(HER2), and *ERBB4*(HER4)(Mujoo et al., 2014). HER3 is the only member of this family which does not have kinase activity(Lyu et al., 2018). Despite the lack of intrinsic kinase activity HER3 is frequently implicated in cancer and is a known mechanism of resistance to several therapies(Mishra et al., 2018). Recently*, ERBB3* overexpression was found to be associated with increased androgen response in Black/African American patients with castration-sensitive prostate cancer and promoted Enzalutamide resistance in prostate cancer cell lines(Vellky et al., 2024). Despite these observations, the functional significance of *ERBB3* overexpression is unknown and warrants further investigation.

Methods

ERBB3 (HER3) over-expressing prostate cancer cells and enzalutamide-resistant cells were utilized for growth assays. Growth assays were performed using Incucyte live cell imaging and cell titer glo assays and validated via Western blot.

Results

We have identified HER3 as a potential therapeutic target that can prevent enzalutamide resistance or resensitize prostate cancer cells to enzalutamide. HER3 phosphorylation and cell proliferation is decreased when targeting HER2 (trastuzumab) and EGFR (erlotinib) in combination with enzalutamide treatment. Additionally, pan-AKT inhibitors decrease growth in HER3 overexpressing cells more than control cells. Enzalutamide-resistant cells can also be resensitized to enzalutamide when treated with ERBB family targeting drugs.

Conclusions

HER3 overexpression was associated with increased response to ERBB family targeting therapeutics in combination with enzalutamide and AKT downstream inhibitors as well as ERBB family targeting increasing sensitivity to enzalutamide. These results support *ERBB3* expression as a biomarker and

pharmacologic target for treatment to prevent and overcome enzalutamide resistance and increase progression-free survival in patients with castration-sensitive prostate cancer.

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Urinary cytokine levels post neoadjuvant chemotherapy in patients with muscle-invasive bladder cancer

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Abstract

Background: Standard-of-care treatment for muscle invasive bladder cancer (MIBC) includes neoadjuvant cisplatin based chemotherapy followed by surgical removal of the bladder (radical cystectomy). Persistence of disease after neoadjuvant chemotherapy (NAC) portends worse outcomes. Non-invasive biomarkers that can predict response to therapy or disease persistence could improve treatment algorithms. Prior research indicates urinary cytokines are substantially elevated in patients with bladder cancer (BCa). Moreover, elevated cytokine levels in urine are predictive of response to therapy in patients receiving Bacillus-Calmette-Guerin. The purpose of this study was to determine if urinary cytokine levels would be beneficial for detection of disease or prediction of future recurrence in patients receiving neoadjuvant cisplatin based therapy prior to radical cystectomy.

Methods: Urine specimens were obtained at the time of cystectomy from patients with MIBC who received cisplatin based NAC (n=73) and healthy controls (n=6) under the Kansas University Medical Center Bladder Cancer Longitudinal Biorepository for Development of Novel Therapeutics/Biomarkers (NCT03413982). A novel panel of ten cytokines (CXCL1, IL-1ß, IL-1RA, IL-6, IL-8, IL-18, CXCL10, MCP-1, MIP-1ß and SDF-1α) previously demonstrated to be associated with BCa was generated for multiplex cytokine analysis (Procartaplex, Thermo Scientific). Each sample was run on three plates and averaged for a final value. Values were corrected to urinary creatinine levels as assessed by a commercially available kit (Cayman Chemical). Response to therapy was assessed via pathological downstaging at the point of cystectomy.

Results: All cytokines tested, except for SDF-1α, were significantly elevated in urine of BCa patients at the time of cystectomy relative to controls. Receiver operating characteristic (ROC) analysis indicated multiple cytokines were tightly associated with BCa when compared to control samples. Reductions in urinary cytokine levels at the point of cystectomy did not correlate with response to therapy in this cohort though.

Conclusion: Urinary cytokine levels are elevated in patients with BCa at the point of radical cystectomy. Larger cohorts and studies assessing both overall levels and relative changes in cytokine levels before and after neoadjuvant chemotherapy are needed to better assess the use of urinary cytokines as biomarkers.

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POU2F3 and OCA-T1 Drive Lineage Plasticity Towards a Stem-Like Identity Through Chromatin Remodeling and Epigenetic Reprogramming in Advanced Prostate Cancer

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Abstract

Background: The development of neuroendocrine prostate cancer (NEPC) from castration-resistant prostate cancer (CRPC) is driven by several molecular events, including epigenetic changes. However, it remains unclear whether this transition requires cells to assume a stem-like intermediate state and what factors are responsible for initiating this transformation. Using a genetically engineered mouse model (GEMM) of prostate cancer, we identified that a previously unrecognized transcription factor (POU2F3) and its binding partner (OCA-T1) were expressed in a subpopulation of tumor cells which appeared to be transcriptomically intermediate between CRPC and NEPC. While these findings suggest that POU2F3 and OCA-T1 are expressed during the transition to NEPC, the mechanism by which these factors regulate lineage fidelity remains unknown.

Methods: We performed scRNA-seq and scATAC-seq in *Pten*$^{f/f}$;*Rb1*$^{f/f}$;*MYCN*$^{+}$ (*PRN*) GEMM to identify subpopulations of tumor cells. We created LNCaP cells harboring N-Myc overexpression and CRISPR-mediated deletion of *RB1* along with a doxycycline-inducible construct to temporally express POU2F3 and OCA-T1. We performed androgen deprivation, determined transcriptional changes by RNA-seq, identified POU2F3/OCA-T1 binding sites by ChIP-seq, and assessed changes to chromatin accessibility and enhancer activation by ATAC-seq and H3K27ac CUT&RUN.

Results: Data from *PRN* GEMMs revealed the *Pou2f3*$^{+}$ population to be enriched with stem- and neural-like transcriptional programs. Expression of POU2F3 and OCA-T1 in LNCaP cells led to a suppression of androgen signaling and a gain of stem and NEPC marker gene expression as well as an enrichment of NEPC and stem-cell gene expression signatures derived from clinical datasets. ATAC-seq and H3K27ac CUT&RUN revealed that POU2F3/OCA-T1 expression led to an expansion of open chromatin sites, predominantly at intergenic loci consistent with distal enhancers. These newly-gained accessible chromatin sites overlapped significantly with known superenhancers (SE) from clinically relevant NEPC patient-derived models while motif analysis of ChIP-seq implicated POU2F3 and OCA-T1 binding at these newly-opened SE-like elements.

Conclusions: We have demonstrated that POU2F3 and OCA-T1 are associated with the acquisition of a stem-like state in both *in vivo* murine and *in vitro* human models. This state is characterized by an expansion of open chromatin surrounding known SE in NEPC. Future studies will determine how the newly identified downstream targets of POU2F3/OCA-T1 contribute to NEPC progression and reveal the potential to target them therapeutically.

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Morphologic evaluation of the C57Bl/6 Aire$^{-/-}$ mouse as a model of benign prostatic hyperplasia-associated inflammation

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Abstract

Morphologic evaluation of the C57Bl/6 Aire$^{-/-}$ mouse as a model of benign prostatic hyperplasia-associated inflammation

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Background: Inflammation is commonly observed in benign prostatic hyperplasia (BPH), yet the impact of immune cells in BPH is not fully understood. Several rodent models have been utilized in BPH research; however, differences in prostate anatomy and immune responses between humans and rodents have presented challenges. Previous studies describe relatively mild inflammation in C57Bl/6 Autoimmune Regulator (Aire)-deficient mice. Here we compare prostate inflammation of Aire$^{-/-}$, Aire$^{+/-}$, Aire$^{+/+}$, and the Prostate Ovalbumin Expressing Transgenic (POET)-3 mouse model of inducible prostatitis to human BPH.

Methods: 8-12 week-old C57Bl/6 Aire$^{-/-}$, Aire$^{+/-}$, and Aire$^{+/+}$ mice were immunized with prostate homogenate and Freund's Complete Adjuvant and boosted with prostate homogenate and Freund's Incomplete Adjuvant after 10 days. Prostatic inflammation was induced in 8-12 week-old POET-3 mice via T cell adoptive transfer. Prostates and major organs were collected at various ages for histology, immunofluorescence, and flow cytometry. Histologic features were compared between Aire mice, POET-3 mice, and human BPH tissues.

Results: Aire$^{+/-}$, Aire$^{+/+}$, and naïve POET-3 (Aire$^{+/+}$) prostates were morphologically similar, lacking significant inflammation in the prostate or in other organs. In previous studies, induced prostatic inflammation in POET-3 mice peaks at 6-7 days post-T cell adoptive transfer and is largely resolved at 1 month. Aire$^{-/-}$ inflammation was confined to the prostate and was predominantly lymphoid with a small myeloid component. Lymphoid cells were arranged in loose aggregates and organizing lymphoid structures with germinal centers, which were not seen in POET-3. Immune cells were significantly higher in Aire$^{-/-}$ prostates up to 18 months of age but not significantly different at ≥24 months.

Conclusion: In contrast to the relatively rapid resolving inflammation of the POET-3, inflammation persists in Aire$^{-/-}$ mouse prostates for several months before declining with age and frequently develops into organized lymphoid structures. Overall, the Aire$^{-/-}$ mouse model shares many morphologic features with the persistent inflammation associated with human BPH.

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miR-199a is highly expressed in prostate stromal and a biomarker of aggressive disease in the serum

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Abstract

Title: miR-199a is highly expressed in prostate stromal and a biomarker of aggressive disease in the serum

Background: One in eight American men will be diagnosed with prostate cancer (PCa) during their lifetime, representing the second highest mortality cancer in the US. Multifactorial causes, including socioeconomic, environmental, and genetic factors cause significantly higher incidence and mortality of PCa in African-American (AA) patients relative to European-American (EA) patients. Many men from both populations have indolent disease, necessitating new biomarkers capable of identifying aggressive PCa. Although PCa arises from the glandular epithelium, paracrine actions from the surrounding fibromuscular stromal cells support tumor growth during PCa. In particular, microRNAs, a class of small noncoding RNAs capable of downregulating specific target transcripts, can regulate gene expression in distant cells via packaging into extracellular vesicles.

Methods: To investigate miRNAs correlated with prostate cancer aggressiveness, miRNA-containing extracellular vesicles were drawn from patient serum and patient-derived cultures. Nextgen sequencing was correlated with Gleason grade to identify miRNAs associated with adverse pathology.

Results: Interestingly, miR-199a was largely expressed by tumor stroma and identified in the serum. miR-199a expression negatively correlated with aggressive disease in African-American patients, but not in European-American men. Immunohistochemistry likewise showed that miR-199a expression decreased in stromal cells near cancerous regions and increased proximal to benign epithelia, implying significant crosstalk between cell types.

Conclusions: Ongoing work will characterize mechanisms associated with miR-199a in the prostate microenvironment, including regulation of miR-199a expression. These data will give important new insights into crosstalk between stromal and epithelial cells in the tumor microenvironment. Similarly, we will investigate miR-199a levels in serial blood draws to determine relationships between miR-199a levels and the course of disease in African-American patients. Characterizing miR-199a-associated crosstalk between stromal and epithelial cells may both better inform its use as a biomarker of aggressive disease in the serum and reveal novel mechanisms in the stroma informing disparities in PCa outcomes.

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myCAF-derived myokines promote prostate tumor growth via angiogenesis and immune suppression

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Abstract

Background:

A prominent side effect of androgen deprivation therapy (ADT), the mainstay treatment for advanced prostate cancer (PrCa), is an obese frailty syndrome that includes fat gain and sarcopenia, a progressive loss of skeletal muscle mass and strength. ADT induces catabolic TGFβ family myokines in muscle, and blocking myokine signaling prevents ADT-induced sarcopenia. We discovered that myokines are expressed in tumor in addition to muscle, and that circulating tumor derived myokines accelerate both muscle mass loss and strength loss in the PTEN null PrCa mouse model.

Methods:

Pb-Cre4 x $Pten^{fl/fl}$ mouse prostate tumor volume changes were serially quantified during myokine blockade or anti-angiogenic therapy using high-resolution high-frequency ultrasound (HFUS) imaging. Myokine and VEGF protein levels were assayed in cell lysates and conditioned media using ELISA, and microvessels in tumors quantitated using CD31 IHC. RNAseq of myokine-treated cells was analyzed using CAMERA to agnostically identify affected Hallmark pathways. Gene expression in mouse prostate tumors were dissected using scRNAseq analysis of >200k cells. Immune cell populations were examined transcriptomically and using IHC.

Results:

Myokine blockade therapies administered to prevent ADT-induced sarcopenia unexpectedly decreased tumor volume markedly in both sham and castrated Pten PrCa mice. This suggests both constitutive and ADT-induced myokines drive tumor growth. Mechanistically, myokines induced angiogenic factors including VEGF from PrCa epithelial cells, and blockade damaged tumor endothelium, suggesting tumor growth control is mediated via the microenvironment. Growth of tumor in Pten PrCa mice treated with anti-VEGFR2 mAb was arrested. Tumor conditioned media increased myofibroblast secretion of myokines. Myofibroblast-like cancer-associated fibroblasts are the primary source of myokines in tumors of Pten PrCa mice. A subset of PrCa patient tumors show myokine amplification, which is further increased in CRPC. In our clinical trial, ADT increased circulating active myokines in most men. Immune pathways mice were altered by myokine blockade in tumors of Pten PrCa.

Conclusions:

Catabolic TGFß-family myokines regulate tumor growth by increasing two distinct pro-growth activities. One is an angiogenic factor secreted into the tumor microenvironment that signals via

VEGFR2. However, VEGF blockade only arrests tumor growth. Blockade of a second myokine induced activity results in frank tumor regression.

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Bradykinin-induced detrusor muscle contraction activates both $G\alpha_{q/11}$- and $G\alpha_{12/13}$-coupled signaling

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Abstract

Background: Urinary bladder dysfunctins are getting more prevalent due to the aging population. The exact pathomechanisms of these disorders are often unclear, although there is accumulating evidence on the prominent role of inflammatory mediators. Accordingly, the nonapeptid bradykinin (BK) has been proposed in the regulation of bladder tone and implicated in the pathophysiology of detrusor overactivity. Our study aimed to elucidate the signaling pathways of BK-induced detrusor muscle contraction with the goal of identifying potential novel therapeutic targets of its disorders.

Methods: Experiments have been carried out on bladders isolated from wild-type or genetically modified [smooth muscle-specific inducible knockout (KO): $G\alpha_{q/11}$-KO, $G\alpha_{12/13}$-KO and constitutive KO: thromboxane prostanoid (TP) receptor-KO, cyclooxygenase-1 (COX-1)-KO] mice and on human bladder samples. Contractions of detrusor strips were measured by myography.

Results: BK induced concentration-dependent contractions in both murine and human bladders, which were independent of secondary release of acetylcholine, ATP, or prostanoid mediators. The B_2 receptor antagonist HOE-140 markedly diminished, whereas the B_1 receptor antagonist R-715 did not alter BK's contractile effect in both species. Consistently with these findings, introducing the agonist of B_2 but not B_1 receptors induced contractions of the same magnitude as BK. Interestingly, both $G\alpha_{q/11}$- and $G\alpha_{12/13}$-KO murine bladders showed reduced response to BK, indicating that simultaneous activation of both pathways is required for the contraction. Furthermore, the Rho-kinase (ROCK) inhibitor Y-27632 markedly decreased contractions in both murine and human bladders.

Conclusion: These results indicate that BK evokes contractions in murine and human bladder smooth muscle, acting primarily on B_2 receptors. Our data gained from the smooth muscle-specific $G\alpha_{q/11}$-KO and $G\alpha_{12/13}$-KO mouse bladders proved that $G\alpha_{q/11}$-coupled and $G\alpha_{12/13}$-RhoA-ROCK signaling appear to mediate the BK-induced contractions simultaneously. Furthermore, inhibition of ROCK reduces the contractions in both species, identifying this enzyme, together with B_2 receptor, as potential targets for treating voiding disorders associated with enhanced BK release.

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Disruption of bone-tumor interplay in metastatic prostate cancer

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Abstract

Background

Radium-223 dichloride (Ra-223) is an alpha-emitting radiopharmaceutical used for treating bone metastases in castration-resistant prostate cancer (CRPC). This study investigates the effects of Ra-223 on bone and gene expression in tumor, tumor-bearing and non-tumor-bearing bones.

Methods

We apply patient-derived xenografts, MDA PCa PDX, that our group has pioneered their establishment and use for translational research. When applied in vivo, many of our models address the unique marked dependence of prostate cancer in the microenvironment, by mirroring the hallmark characteristics of bone-forming metastases observed in the clinic. This approach allows “cross species analysis” to integrate the host component, recapitulating the contributions of the tumor microenvironment, with the tumor of human origin.

Mice with tumor-bearing and contralateral non-tumor-bearing bones were treated with vehicle or Ra-223. Bone parameters were assessed by TRAP, Von Kossa and H&E staining and bone histomorphometry. Magnetic Resonance Imaging (MRI) was used to monitor tumor volume. Micro-Computed-Tomography (microCT) was applied to evaluate bone integrity and structural changes. Differential gene expression was analyzed by RNA sequencing of treated and control groups.

Results

Histomorphometric analysis showed a significant decrease in osteoclast activity in Ra-223-treated non-tumor-bearing bones compared to controls. Tumor-bearing bones also exhibited decreased osteoclast parameters, to a lesser degree. Analysis of MRI scans revealed significant reduced tumor burden of Ra-223-treated tumors. microCT illustrated altered bone architecture. Transcriptome analysis highlighted significant differential expression in genes associated with bone metabolism and immune response in Ra-223-treated tumors compared to controls.

Conclusions

The comprehensive transcriptomic and pathway analyses provide a deeper understanding of the dual role of Ra-223 in modulating both the tumor and bone microenvironment, by decreasing tumor volume while promoting bone remodeling. Ra-223 treatment significantly alters osteoclast activity, bone structure, and gene expression in both tumor-bearing and non-tumor-bearing bones. These findings provide deeper insights into Ra-223 targeting metastatic lesions and modulating the bone microenvironment, accounting for its therapeutic efficacy in CRPC patients with bone metastases. Further studies are needed to explore the long-term effect and clinical implications of these alterations.

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Behavior of basal prostate stem cells in resolving and chronic prostate inflammation models

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Abstract

Inflammation is present in the majority of human benign prostatic hyperplasia (BPH) samples, and the severity of BPH symptoms correlates with the extent of inflammation. However, how inflammation contributes to BPH etiology remains unclear. We have previously demonstrated, using an inducible prostatitis model (POET3, Prostate Ovalbumin Expressing Transgenic 3), that inflammation promotes the luminal differentiation of basal prostate stem cells (bPSC) via the activation of androgen receptor (AR) signaling. In the current study, we examined the behavior of bPSC in a chronic inflammation model, the *Aire* (Autoimmune Regulator) knockout model (*Aire*$^{-/-}$ in the C57BL/6 background). Unlike POET3, in which prostate inflammation peaks at 1 week and resolves within one month after the induction of inflammation via the adoptive transfer of pre-activated T cells, inflammation in the *Aire*$^{-/-}$ prostates persisted even when mice were 1.5 years old. The composition of inflammatory cells was similar between the two models, with CD3+ T cells being the most abundant population. To study bPSC differentiation *in vivo*, lineage tracing models were established with either the POET3 (POET3^{het};mTmG^{het};*Krt5*-creERT2^{het}) or *Aire*$^{-/-}$ background (*Aire*$^{-/-}$;mTmG^{het};*Krt5*-creERT2^{het}). Luminal differentiation was more extensive in *Aire*$^{-/-}$ prostates compared to POET3 prostates one month after the tracing started, and majority of the luminal cells in the *Aire*$^{-/-}$ prostates were replaced by cells derived from the basal epithelium after two months. Interestingly, unlike in the POET3 model, where differentiation was similar between the anterior and dorsal lobes, it was significantly more pronounced in the anterior lobes in the *Aire*$^{-/-}$ model. Consistently, the number of bPSC from the *Aire*$^{-/-}$ anterior lobes was significantly higher than that from the dorsal lobes. Organoid cultures also corroborated the findings from the lineage tracing studies, with bPSC from anterior lobes giving rise to significantly more organoids with more differentiated structures than bPSC from dorsal lobes. The differentiation of *Aire*$^{-/-}$ bPSC relies on AR signaling, as enzalutamide treatment led to fewer organoids with less differentiated structures. Taken together, chronic inflammation exerts more potent effects on bPSC differentiation, and these chronic effects appear to be lobe-specific. Investigation into the differentially expressed factors in different prostate lobes during resolving and chronic inflammation is currently ongoing and may provide critical insight into the mechanisms underlying prostate hyperplasia.

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miR-140-3p Promotes Prostatic Fibrosis as Part of a ceRNA Regulatory Network

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Abstract

Title: miR-140-3p Promotes Prostatic Fibrosis as Part of ceRNA Regulatory Network

Background: IL-4/IL-13 have been shown to increase fibrosis-related extracellular matrix (ECM) proteins in prostate fibroblasts in conjunction with Lower Urinary Tract Dysfunction (LUTD) development. Moreover, translation of these ECM proteins can be highly affected by different types of regulatory RNAs (rRNAs). miRNAs can bind to and destabilize linear mRNAs, while circular RNAs (circRNAs) are able to “sponge” and inhibit miRNAs. These binding interactions can be elucidated and analyzed to develop a competitive endogenous RNA (ceRNA) network, which can function to modulate and increase ECM protein production during fibrotic development.

Methods: RNA was extracted from N1 prostate stromal fibroblasts treated with IL-4 or IL-13. circRNA levels were analyzed via microarray (ArrayStar) while miRNA and mRNA levels were analyzed via bulk next-gen sequencing. All three datasets were then analyzed through miRNAda and CircR to establish likely binding interactions between ceRNA species (I.e., upregulated miRNA / downregulated mRNA / downregulated circRNA) and networks were generated and visualized using Cytoscape. miRNAs/circRNAs that show potential binding with known profibrotic mRNAs were experimentally knocked down with siRNA transfection to identify alteration in ECM protein levels via western blot.

Results: Several miRNAs, mRNAs, and circRNAs were significantly differentially regulated in response to IL-4or IL-13. Of note, miR-140-3p was significantly downregulated in both IL-4 and IL-13 treated cells and was shown to have a potential binding site on the 3’ UTR of Cell Migration Inducing Hyaluronidase 1 (CEMIP) mRNA , which encodes a known pro-fibrotic ECM protein. Further knockdown of miR-140-3p in IL-4 and IL-13 treated cells resulted in a significant increase in CEMIP protein levels, suggesting that loss of miR-140-3p results in CEMIP protein upregulation in IL-4/IL-13 treated cells. Additionally, miR-141-3p was also significantly downregulated in IL-13 treated cells and further knockdown of miR-141-3p in IL-13 treated cells may result in an increase of Leukemia Inhibitory Factor Receptor (LIFR), another profibrotic protein.

Conclusions: Further validation of this network will include miRNA overexpression as well as circRNA overexpression and inhibition. Testing and analysis of this ceRNA network may identify interacting regulatory RNAs that may dysfunctionally cause up-regulation of pro-fibrotic proteins contributing to fibrotic pathobiologies in the lower urinary tract.

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The mechanosensory transient receptor potential vanilloid 4 mediates its effects through prostanoids in the urinary bladder

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Abstract

Background: One of the key regulators of normal urinary bladder function is mechanotransduction, where ion channels detect bladder filling. When a certain level of wall tension is reached, signaling pathways are activated, leading to the sensation of needing to urinate. The transient receptor potential vanilloid type 4 (TRPV4) is a major representative of these ion channels. Several functional urological conditions are known, such as overactive bladder syndrome, which are characterized by frequent, urgent urination. It is widely believed that altered mechanotransduction function underlies the development of these symptoms. However, the detailed mechanisms of these signaling pathways are not yet fully understood. Our objective is to study bladder activation signaling mediated by the key mechanosensory TRPV4 ion channel, aiming to identify potential new intervention points.

Methods: We conducted experiments on adult male and female wild-type mice, as well as cyclooxygenase 1 knock-out (COX1-KO) mice. We evaluated bladder function in live subjects using transurethral cystometry and under isolated conditions through myographic experiments. The levels of various mediators were measured using ELISA kits.

Results: Activation of the TRPV4 ion channel significantly enhanced bladder function, an effect that was almost entirely abolished by the non-selective COX inhibitor indomethacin, both in vivo and ex vivo. In COX1-KO animals, the effect was partially diminished, while it was completely eliminated when combined with a COX2 inhibitor. The levels of Prostaglandin E2 and Thromboxane A2, products of the COX enzyme, increased significantly 5 minutes after TRPV4 activation and even more so after 10 minutes.

Conclusions: The activation of the TRPV4 ion channel, which is abundantly expressed in the bladder wall, leads to bladder activation through inflammatory mediators, a process that occurs largely locally. The production of these mediators is linked to both cyclooxygenase subtypes. We have identified a signaling pathway where intervention at several points can inhibit the increased excitability of the bladder. We hope that by describing this signaling pathway, we can contribute to the identification of more specific therapeutic targets that could be utilized in the treatment of various functional urological conditions associated with bladder hyperactivity.

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ADIPOSE TRIGLYCERIDE LIPASE (ATGL) REGULATES A LIPID-MICROTUBULE ORGANIZING CENTER (MTOC) AXIS TO SUPPRESS PROSTATE TUMOR GROWTH

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Abstract

Background: Prostate tumor and stromal cells are known to undergo metabolic reprogramming by increasing the rate of fatty acid synthesis to provide energy and sustain proliferation. This high rate of fatty acid turnover or lipid flux in the tumor microenvironment (TME) is maintained, in part, by increasing the key enzyme in triacylglycerol lipogenesis, diacylglycerol transferase 1 (DGAT1), and decreasing the rate-limiting enzyme in lipolysis, adipose triglyceride lipase (ATGL). This net gain in lipid can promote amplification of centrosomes, the most common microtubule organizing center (MTOC), leading to clustering which can be used by cells as a mechanism for forming pseudo-bipolar spindles to avert cell death. Since proliferating cells are the target of anti-mitotic chemotherapeutic agents, these drugs bypass a significant number of tumor and stromal cells that are in interphase. Emerging data suggest that agents directed at de-clustering centrosomes can target both proliferating and interphase cells. We postulated that ATGL regulates prostate growth, and it may be a new centrosomal de-clustering anti-tumor agent capable of normalizing both lipid metabolism and centrosomal amplification in the prostate TME.

Methods: Prostate cancer (PCa) specimens from low and high grade tumors and PCa cell lines, PC-3 and LNCaP, were immunostained for ATGL, perilipin 2 and DGAT1. Normal fibroblasts and primary cancer-associated fibroblasts (CAFs) were cultured, and Western blots performed. Immunofluorescent studies included ATGL, Bodipy (lipid droplets, LDs) and MTOC markers pericentrin, centrin, and γ-TuRC. ATGL heterozygous male mice were aged for up to 18 months and prostates evaluated for histopathology and mitoses.

Results: High grade PCa had the lowest expression of ATGL while the DGAT1 protein was consistently elevated in high grade PCa. In contrast to ATGL-expressing normal fibroblasts, CAFs had increased LDs and low ATGL levels. $ATGL^{low}$ stromal and epithelial cells demonstrated amplified centrosomal and non-centrosomal MTOCs. ATGL co-localized with centrin-positive centrosomes and microtubule nucleator, γ-TuRC. ATGL deficient mice exhibited atypical prostate hyperplasia with age and multifocal PCa developed by 9 months of age. Asymmetric multi-polar mitoses and excessive MTOCs with clustering were evident in lipid-enriched prostate epithelial cells.

Conclusions: Our data suggest that ATGL is a previously unrecognized regulator of centrosomes through a lipid-MTOC axis. Further, restoration of ATGL may exert anti-tumor activity by normalizing lipid metabolism and de-clustering centrosomes in the TME.

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FOXA1 control of MYC-driven metabolic adaptations in aggressive prostate cancer

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Abstract

Background: Lifestyles-related factors such as obesity and excessive saturated fat intake (SFI) increase the risk of developing lethal prostate cancer (PCa). We showed that high SFI-induced obesity boosts the oncogenic c-MYC (MYC) transcriptional program and gives rise to tumour glycolytic features that create an immunosuppressive and protumourigenic microenvironment. Upon MYC overexpression or under SFI-induced obesity conditions, the enhancement of the MYC transcriptional program creates new epigenetic and metabolic liabilities. However, the mechanisms underpinning these liabilities thus far has been uncharacterized.

Methods: We leveraged murine models of MYC-driven PCa that recapitulates several molecular features of human prostate adenocarcinoma. We performed CRISPR/Cas9 knock-out (KO) screens *in vitro* using custom libraries targeting epigenetic-related genes in murine PCa cellular models driven by MYC overexpression, loss of *Pten* alone or combined with loss of *Rb1*. We generated stable KO cells together with doxycycline-inducible re-expression and performed *in vitro* (*e.g.*, proliferation, Seahorse) and *in vivo* characterization under conditions of either a control diet (CTD; 10% kCal fat) or SFI-induced obesity (60% kCal fat) using next-generation sequencing-based experiments and analyses (*e.g.*, RNA-seq, ATAC-seq, ChIP-seq).

Results: Our *in vitro* CRISPR/Cas9 KO screens identified the pioneer transcription factor forkhead box A1 (FOXA1) as a top candidate unique in promoting MYC-driven PCa cellular proliferation. This was

confirmed by independent genetic KO. Gene set enrichment analysis identified FOXA1 as a positive regulator of the glycolysis transcriptional signature. Supporting this finding, FOXA1 KO clones demonstrates reduced compensatory glycolysis following mitochondrial inhibition as well as reduced ATP production. Importantly, SFI enhances chromatin accessibility to binding sites of the FOX transcription factor family in MYC-overexpressing prostates in comparison to CTD-fed animals. Critically, SFI was not able to sustain the same growth rate in FOXA1 KO allografts or induce glycolysis in the absence of FOXA1. Along this line, we found that FOXA1 controls the expression of hexokinase 2 (HK2), the rate-limiting enzyme that catalyzes the irreversible conversion of glucose to glucose-6-phosphate.

Conclusions: Our studies revealed FOXA1 as a mechanistic link underlying metabolic adaptation in MYC-driven PCa and provides the basis to counteract SFI-associated and FOXA1-mediated PCa progression by interfering with glycolysis.

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Acute versus chronic CDK12 loss in prostate cancer drives different phenotypes and therapeutic vulnerabilities

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Abstract

BACKGROUND. DNA repair pathway mutations occur in ~20% of metastatic castration resistant prostate cancer (mCRPC), leading to opportunities for targeted therapies. PARP inhibitors (PARPi) have demonstrated clinical benefit for patients with mutations in core homologous recombination (HR) genes (e.g. *BRCA2)*, whose tumors show an HR-deficiency (HRd) phenotype. Cyclin dependent kinase 12 (*CDK12*) is lost by biallelic (BAL) mutation in 5-7% of mCRPC. Loss of *CDK12* was reported to lead to HRd via impaired phosphorylation of RNA Polymerase II causing aberrant splicing and polyadenylation of long HR genes. Unfortunately, *CDK12* ^{BAL} patients have shown limited responses to PARPi. We sought to test the hypothesis that the HRd phenotype ascribed to *CDK12* loss is predominantly limited to an acute context, while *CDK12* ^{BAL} tumors restore HR activity and necessitate alternate targeting strategies.

METHODS. We analyzed whole genome and RNA sequence data from four human mCRPC datasets. Cell lines were treated with CDK12/13 inhibitor to model acute *CDK12* loss, while CRISPR-mediated KO lines and LuCaP189.4 (a natural *CDK12*^{BAL} line) were used to model adapted loss. HR activity was assayed by immunostaining for RAD51 foci following ionizing radiation. Cells were treated with compounds *in vitro* to generate dose response curves and LuCaP xenografts were treated *in vivo* with SR4835 (CDK12/13 inhibitor).

RESULTS. Our analysis found that *CDK12*^{BAL} tumors are not enriched for genomic scar signatures associated with HRd and show limited splicing and polyadenylation defects in core HR genes. Acute CDK12 inhibition in cell lines recapitulated aberrant polyadenylation and downregulation of long HR genes, but such effects were greatly diminished in *CDK12*^{BAL} lines. *CDK12*^{BAL} cells were competent at inducing RAD51 foci and showed limited sensitivity to PARPi but were highly sensitive to targeting of *CDK13* by sgRNA or CDK12/13 inhibitor. Increased sensitivity was also observed with compounds that antagonize transcription (5-fluorouracil) or target RNA Polymerase II (α-amanitin).

CONCLUSIONS. Our data show that HR gene downregulation is primarily a consequence of acute *CDK12* loss, which is largely compensated for in stable *CDK12*^{BAL} cells. These results provide an explanation for why PARPi monotherapy has not consistently benefited *CDK12*^{BAL} patients as it has those with core HR

mutations. Alternate (or combination) therapies that target CDK13 or transcription are candidates for future research.

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Enhancing the effect of PARP inhibitors by targeting DGAT1-induced lipogenesis

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Abstract

Background: Despite advancements in monotherapy and combination therapy, treatment response of metastatic castration-resistant prostate cancer (mCRPC) is still challenging. Recent studies revealed the therapy resistant mechanism in prostate cancer (PCa) to Olaparib, a poly (ADP-ribose) polymerase (PARP) inhibitor, is due to the induction of autophagy. Autophagy increases free fatty acids (FFA) levels. Cancer cells can adapt to excess FFA via lipid droplet (LD) biogenesis regulated by diacylglycerol O-acyltransferase 1 (DGAT1) to prevent lipotoxicity. We hypothesize that DGAT1 inhibition induces reactive oxygen species (ROS) mediated prostate cancer cell death and enhances the anti-cancer efficacy of Olaparib.

Methods: Prostate cancer cell lines were exposed to Olaparib, DGAT1 inhibitor (DGAT1i) alone or in combination to evaluate the effects on cell proliferation by Incucyte. To assess the impact on lipid metabolism and oxidative stress, we performed free fatty acid assay, along with fluorescence staining of LD (NileRed), reactive oxygen species (Cell ROX Green), and mitochondria integrity (Mitoview). To evaluate the extent of apoptosis induced by the treatment, we performed Annexin V staining followed by PI staining and flow cytometry analysis to evaluate cell cycle (PI). Expression analysis of proteins involved in autophagy, lipogenesis, and apoptosis was performed by western blot to provide insights into the underlying molecular mechanisms.

Results: DGAT1i in combination with Olaparib significantly inhibited the growth of prostate cancer cells. While treatment with Olaparib alone led to an increase in lipid droplet formation, the combination therapy reduced the number of lipid droplets, indicating a shift in lipid storage. Additionally, Olaparib alone elevated FFA levels, and this effect was further amplified by the combination treatment. The levels of ROS and mitochondrial damage were also notably higher with the combination therapy, suggesting that DGAT1i enhances ROS-driven cell death mechanisms. These effects were further supported by flow cytometry and western blot analysis, which confirmed increased apoptosis in the combination treatment group.

Conclusion: Our study proposes a novel therapeutic strategy to enhance Olaparib efficacy by targeting LD biogenesis through DGAT1 inhibition. The synergistic mechanisms of Olaparib and DGAT1 inhibitor suggest a potential role on ROS-driven apoptosis. Future in vivo studies will elucidate the translational utility of PARP inhibitors in combination with LD lipogenesis blockers to provide a foundational basis for clinical trials in individuals with mCRPC.

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ARHGEF2 regulates lineage plasticity promoting neuroendocrine differentiation and treatment resistance in advanced prostate Cancer

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Abstract

Background Neuroendocrine prostate cancer (NEPC) is an incurable, highly aggressive subtype of prostate cancer; approximately 20% of metastatic castration-resistant prostate cancer (CRPC) have progressed toward treatment-emergent NEPC. Tumor cells, upon AR-targeted treatment, acquire stem-like features and transform toward neuroendocrine differentiation. The GEF-H1 encoded by ARHGEF2 is aberrantly overexpressed in treatment-resistant CRPC and NEPC patients, however, the role in neuroendocrine differentiation (NED) remains unknown.

Methods Transcriptomic analysis for neural lineage gene sets was performed in enzalutamide-resistant MDVR, 42D, and small-cell NEPC H660 cell lines. ARHGEF2 expression was evaluated in patient samples by IHC staining. Cell proliferation and NE markers were examined after knocking down ARHGEF2. The correlation of Rac1 with ARHGEF2 in NEPC patients was examined, and GTPase activation assay (GLISA) was employed to verify the activation of Rac1. Anti-tumor effects of GEF-Rac1 inhibitor Ehop-016 were investigated in ex vivo NEPC patient-derived LuCaP49 organoids and in vivo LuCaP49 xenograft and H660 cell xenograft models. The targeting effects of Ehop-016 were interrogated by performing RNAseq analysis to see whether blocking ARHGEF2-Rac1 diminishes trans-differentiation toward neuroendocrine.

Results The bioinformatic analysis demonstrates that ARHGEF2 is predominantly upregulated in NEPC cohorts, and enzalutamide treatment significantly increased ARHGEF2 expression, further confirmed by IHC staining in patient samples. In MDVR and H660 cells, ARHGEF2 knocking down significantly inhibited cell viability and the expression of NE markers. The GTPase Rac1 expresses a higher level in NEPC and positively correlates with ARHGEF2. Rac1 inhibitor EHop-016 showed anti-tumor effects in H660 and MDVR cells. Ehop-016 effectively reduced the LuCaP49 PDX organoid formation and viability ex vivo. Inhibition of the ARHGEF2-Rac1 activity by Ehop-016 treatment exhibited anti-tumor effects in vitro and ex vivo in inhibiting NEPC tumor growth.

Conclusions ARHGEF2 is aberrantly overexpressed in enzalutamide-resistant and NEPC patients. Our findings show that targeting ARHGEF2-activated Rac1 by EHop-016 suppresses NEPC tumor viability in vitro and in vivo, providing a novel therapeutic strategy for lethal prostate cancer patients.

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Evaluation of RK33:AA pharmaceutical cocrystals as a candidate therapeutic in AR low/- CRPC

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Abstract

Background: With the development of second-generation anti-androgens (AAs), the prevalence of AR low/negative subtypes of castration resistant prostate cancer (CRPC) has increased. Hormonal therapies are rendered ineffective by the absence of AR protein expression in these CRPC subtypes. Previously, disruption of DDX3:*AR* mRNA complexes with the small molecule DDX3 inhibitor RK33 was demonstrated to alleviate the translational repression of *AR* mRNA in models of AR low/- CRPC. After the subsequent rise in AR protein levels, cells were determined to be re-sensitized to AAs. However, RK33 is a poor drug candidate due to its aqueous solubility and bioavailability profile. An emerging technique for improving biopharmaceutical aspects of candidate drugs involves the formulation of pharmaceutical cocrystals. This study aims to evaluate the utility of RK33 and AA cocrystals as a mechanism for improving the biopharmaceutical potential of RK33 while simultaneously offering a combination therapeutic approach for AR low/- CRPC.

Methods: *In vitro* profiling of RK33:Flutamide (RK33:Flut) cocrystals was completed using AR low/- cell lines. MTT assays were completed to determine the IC50 of RK33:Flut, RK33, flutamide, and RK33 + Flutamide combination treatment. Using this same dosing scheme, the impacts of RK33:Flut treatment on DDX3 inhibition and AR expression were profiled by western blot. Alterations to activation of PSA and profiling of AR mRNA in response to treatments were completed via qPCR.

Results: IC50 data demonstrated that RK33:Flut treatment does not significantly alter the viability of cells compared to traditional combination dosing. WB analysis demonstrated retention of efficacy in DDX3 inhibition and induction of AR expression across formulations of RK33 *in vitro*. Furthermore, AR pathway activation was observed by increased PSA levels after RK33 treatments regardless of the presence of flutamide.

Conclusions: RK33:AA pharmaceutical cocrystals may be a viable formulation method for improving the aqueous solubility of RK33 given that the functional capacities of RK33:Flut in inhibiting DDX3 and inducing AR expression are retained through this method of synthesis. Notably, we suspect that biopharmaceutical advantages are likely to be more apparent after moving into *in vivo* studies. Prior to this, we propose that improvements to the cocrystals including formulation with more potent AAs and increased ratios of RK33:AA will improve the relevance of this approach in AR low/- CRPC.

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High-Density Lipoprotein-Like Nanoparticle Targets Scavenger Receptor Class B Type 1 and Sensitizes Prostate Cancer Cells to Radiation Therapy

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Abstract

Background: Ionizing radiation therapy (XRT) treatment is common for all stages of PCa. The dose of XRT administered to patients to treat PCa is balanced against the likelihood of the patient developing side effects. There are strategies to deliver XRT more specifically to PCa and reduce side effects, such as using more precise imaging and XRT delivery. Another strategy is to employ a radiosensitizer. Ideally, the radiosensitizer would localize only to cancer cells and make them more vulnerable to XRT. We have developed a nanoparticle radiosensitizer that is targeted to a novel protein biomarker present on the surface of PCa cells, scavenger receptor class B type 1 (SR-B1). The expression of SR-B1 increases in PCa with correlation to grade and stage. Radiosensitizer binding SR-B1 engages multiple physical and molecular mechanisms to enhance cancer killing by XRT. This study was designed to validate the mechanisms of the radiosensitizer agent, called high-density lipoprotein-like nanoparticle (HDL NP), in PCa and to study its ability to sensitize PCa cells to XRT.

Methods: We conducted in vitro clonogenic assays to assess synergy of HDL NPs with XRT to promote cell death in androgen-dependent and castration-resistant PCa cell lines. Sensitizer enhancement ratios (SERs) were calculated to determine radiation dose enhancement. Additionally, we performed molecular studies to measure HDL NP-mediated control over redox [e.g., glutathione peroxidase 4 (GPx4)] and androgen pathways. Oxidized cell membrane lipids were quantified using the C11-BODIPY dye and flow cytometry to measure lipid peroxidation. Specific studies were conducted to quantify oxidative damage to nuclear DNA. Finally, in vivo studies are currently underway to quantify efficacy of HDL NPs to modulate XRT sensitivity in an immunocompetent mouse model of PCa.

Results: Data from in vitro clonogenic assays demonstrate that HDL NPs effectively sensitize androgen-dependent (LNCaP, Myc-CaP) and castration-resistant (CWR-R1, 22rv1) PCa cells to XRT. Combination treatment with HDL NPs further enhances lipid peroxidation in PCa cells caused by XRT through C11-BODIPY analysis. Additionally, data show that HDL NP treatment promotes DNA damage upon XRT through increased levels of ɣ-H2AX foci formation, which localize at double-strand breaks.

Conclusions: Synthetic HDL NP target SR-B1 in PCa and sensitize them to XRT through multiple mechanisms. Accordingly, this project will set the groundwork for a new strategy to treat PCa more effectively while reducing the devastating side effects common to PCa treatment.

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Racial genetic alterations in the EphB2 receptor in prostate epithelial cells increases “reverse” ephrin signaling promoting carcinoma associated fibroblasts activation

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Abstract

Background: EphB2 mutations in the extracellular domain or intracellular truncated mutations lacking the conserved tyrosine kinase domain and C-terminal tail can disrupt the “reverse signaling” with profound effects in Ephrin ligand expressing cells. The consequences of common nonsense (K1019X) EphB2 mutations associated with prostate cancer (PCa) in AA (African American) on Ephrin “reverse signaling” in non-cancerous stromal cells are unknown. Here we determined the impact of racially associated EphB2 point mutations on Ephrin reverse signaling in fibroblast activation toward a carcinoma associated fibroblasts (CAF) phenotype.

Methods: Human EphB2 guide RNA primers and Cas9 protein were transfected using electroporation into benign prostate epithelial cell lines. In addition, prostate cancer cells expressing EphB2 wild-type, K1019X and truncated extracellular domain were generated. Engineered cells lines were fully characterized for their in vitro proliferation and motility as well as their transcriptomes by RNASeq. Cells were co-cultured in vitro and xenograft in vivo with fibroblasts expressing cognate Ephrin B ligands (B1, B2 and B3). Expression of CAF markers both in vitro and in vivo were assessed as well as the consequences of EphB2 mutations on stromal and epithelial cytoskeleton (Grb4, SH3, RhoA, Rac and Cdc42). The effects on tumor growth, invasion and stromal remodeling were evaluated.

Results: Expression of the mutant as well as truncated forms of EphB2 altered the proliferative capacity of benign and prostate cancer cell lines. Loss of EphB2 function in prostate epithelial cells induce an increased ephrin reverse signaling in stromal cells leading to expression of CAF markers as well as enhanced motility in fibroblasts. Compared to control groups, expression of EphB2 mutations commonly observed in AA led to larger tumors with a distinct pattern of stromal changes promoting collagen deposition, fibroblast activation and neoangiogenesis in the tumor microenvironment (TME). Transcriptome analysis revealed differential expression of genes in cells expressing mutant and truncated forms of EphB2 compared to control cells. Further gene ontology data identified critical molecular functions and biological processes regulated by EphB2.

Conclusion: Loss of EphB2 function (due to nonsense K1019X) in epithelial cells induce a strong activation of CAF in the TME. In addition, we identified a set of genes potentially regulated by EphB2 in prostate epithelial cells. Further studies are still needed to characterize the intrinsic molecular effectors of EphB2 tumor suppressor effects in AA men with PCa.

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LX1 Targets Androgen Receptor Variants and AKR1C3 to overcome Therapy Resistance in Advanced Prostate Cancer

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Abstract

Background: The development of resistance to current standard-of-care treatments, such as androgen receptor (AR) targeting therapies, remains a major challenge in the management of advanced prostate cancer. There is an urgent need for new therapeutic strategies targeting key resistant drivers such as AR variants like AR-V7 and steroidogenic enzymes such as AKR1C3 to overcome drug resistance and improve outcomes for patients with advanced prostate cancer. We have designed, synthesized, and characterized a novel class of LX compounds targeting both the AR/AR-Variants and AKR1C3 pathways. **Methods:** A library of the LX compounds was designed and synthesized according to structure-based computer modeling. The effects of the lead LXs on the expression and activity of AR/AR-variants and AKR1C3 were evaluated. RNA-seq was performed on the lead LXs. Resistant cell sublines generated from C4-2B cells resistant to enzalutamide (MDVR), apalutamide (ApalR), darolutamide (DaroR), or abiraterone (AbiR) were treated with LX1 or their respective antiandrogen. Mice bearing VCaP xenograft tumors and LuCaP35CR PDX tumors were treated with LX-1 and effects on tumor growth were assessed. **Results:** Molecular docking studies and in vitro experiments demonstrated that LX compounds effectively bind to the AKR1C3 active site and inhibit AKR1C3 enzymatic activity. LX1 was also shown to reduce AR/AR-V7 expression and downregulate their target genes, inducing G0/G1 arrest in anti-androgen-resistant cell lines. LX1 treatment reduced tumor volumes and decreased intratumoral testosterone in both xenograft tumor and PDX models. LX1 effectively inhibited the conversion of androstenedione into testosterone in tumor-based ex vivo enzyme assays. Moreover, LX1 synergized with enzalutamide and abiraterone as well as docetaxel, suggesting its potential to enhance the anti-tumor activity of these standard therapies in resistant prostate cancer. Furthermore, LX1 improved enzalutamide treatment in resistant prostate cancer tumor models. **Conclusions**: Our study unveils the dual effect of LX compounds, especially LX1 in reducing intratumoral testosterone and AR and AKR1C3 signaling, along with its synergy with standard therapies in resistant models, underscores its potential as a valuable treatment option for advanced prostate cancer.

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Truncal Drivers as Key Predictors of Treatment Outcomes in High TMB and HRD Co-Phenotype Tumors

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Abstract

Background

PARP inhibitors (PARPi) are a promising treatment for prostate cancer with homologous DNA repair deficiencies (HRd), particularly in BRCA1/2 loss patients. However, 35-40% of these patients do not respond to PARPi. Our small case study identified three BRCA mutated HRd patients with high tumor mutation burden(TMBh) (TMB >10 mutations/mb or mut/mb); all responded well to immunotherapy but no response to PARPi, highlighting the need for a deeper genomic evaluation of metastatic prostate cancer (mPC) to understand the variations in clinical outcomes. We hypothesize that there may be two subsets of TMBh-HRd: i) HRd mutations as truncal drivers. ii) Mismatch Repair Deficiency (MMRd)/Microsatellite Instability (MSI) as causal genomic aberrations and truncal drivers.

Methods

We analyzed high-quality exomes from 632 metastatic prostate cancer (mPC) patients, focusing on germline and somatic mutations across 43 DNA repair pathway genes. Our evaluation included TMB, MSI scores, and various HRd signature scores. We distinguished between truncal and subclonal mutations using advanced tumor evolution analysis tools & supported our findings with inferred mutation signature scores.

Results & discussion

Previously, we found that 4% of mPC cases have a high tumor mutational burden (TMB >10 mut/mb) with HR pathway gene mutations. In our extended analysis, 9% of mPC cases were TMBh (≥10 mut/mb,

n=62), with 3% being MSI-high. Notably, 21 of 23 TMBh tumors were also MSI-high. Among the 62 TMBh cases, 16 (~26%) had HRD scar signatures, and 2 showed BRCA2 biallelic loss due to loss of function mutations.

We observed a significant decrease in HRD-scar positivity ($p < 0.05$) when the TMBh threshold was raised to ≥20 mut/mb. Approximately 45% of tumors with TMB between 10 & 20 mut/mb were HRd sig positive, while only 9% with TMB ≥20 mut/mb were HRD sig positive. Notably, ~61% of tumors with TMB ≥20 mut/mb were also MSI-high. These findings suggest that combining HRd signature analysis with TMB assessment can differentiate between pure TMB and TMB+HRd phenotypes, potentially guiding PARPi or immunotherapy selection. We further derived temporally spaced mutations & copy number events from tumor exomes and genomes using GRITIC & MesKit tools and performed COSMIC signature analysis to determine if HRd is a truncal event. Our findings are validated in six clinical genomes with treatment outcomes, suggesting that tumors with TMB ≥20 mut/mb are predominantly driven by MMRd mechanisms, with HRd as a potential passenger event, making them less suitable for PARPi therapy. Conversely, TMBh tumors with HRd signatures are more likely to respond favorably to PARPi therapy.

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Development of an NEPC cell line from androgen sensitive Myc-Cap

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Abstract

Development of a NEPC cell line from androgen sensitive Myc-Cap

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Abstract:

Prostate adenocarcinoma responds to treatment with androgen deprivation therapy (ADT), evolving over time into castration-resistant prostate cancer (CRPC), and eventually progress to neuroendocrine prostate cancer (NEPC). We aimed to establish an NEPC mouse cell line to enable study in an immunocompetent mouse. MyC-CaP cells were cultured in androgen-free media and injected into syngeneic pre-castrated FVB mice. Enzalutamide therapy was administered when the tumor volume was 100-200mm^3. Harvested tumors were processed for cell line production, and re-implanted into castrated mice. The re-implanted tumors were processed similarly and yielded a targeted cell line for NEPC. An in vivo experiment, involving a castrated group of mice carrying the NEPC cell line and a group of intact mice carrying parental MyC-CaP, was conducted for confirmation and characterization. H&E staining exhibited neuroendocrine tumors' characteristic "Salt and Pepper" granular chromatin appearance. Androgen receptor (AR) expression in NEPC tumors had a characteristic AR cytoplasmic localization, while MyC-CaP tumors displayed intense AR nuclear localization. Neuroendocrine marker chromogranin A was significantly over-expressed in NEPC tumors. Additionally, bulk RNA sequencing data analysis revealed that NEPC cell line had overexpressed neuroendocrine markers like NCAM1 and had upregulated AR expression, while androgen-regulated genes were down-regulated. Transcription factors known to be related to neuroendocrine differentiation like *Myc* and *Ascl1* were upregulated in the developed cell line. NEPC development is associated with loss of *Trp53, Rb1*, and *Pten*, the NEPC cell line has low expression of *Trp53* and deactivated pathways of *Rb1* and *Pten*. In conclusion, the NEPC cell line has demonstrated histological features of NEPC upon growth in an androgen-deprived environment. The development of the NEPC mouse cell line will provide a model for studying disease biology, resistance mechanisms, and the development of new therapeutic strategies.

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Targeting Synaptotagmin 4 to Overcome Enzalutamide and Abiraterone Resistance in Castration Resistance Prostate Cancer

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Abstract

Background: Castration-resistant prostate cancers (CRPCs) are predominantly driven by androgen receptor (AR) signaling. Although next-generation anti-androgen therapies (NGATs), such as enzalutamide (Enza) and abiraterone (Abi), have been shown to extend patient survival, resistance to these therapies often develops. The underlying mechanisms driving resistance to these treatments remains unclear. Synaptogamin 4 (SYT4), a membrane protein involved in vesicle trafficking, is highly expressed in our established Enza and Abi resistant CRPC cells. Here, we explore the contribution of SYT4 in disease progression and NGATs resistance in CRPC.

Methods: Differences in SYT4 expression were determined in Enza-resistant C4-2B MDVR (MDVR) and Abi-resistant C4-2B AbiR (AbiR) cells versus parental C4-2B cells by RNA-sequencing, real time PCR, western blot and immunofluorescence microscopy. SYT4 expression was analyzed in GEO and cBioPortal databases from patients with prostate cancer. Specific siRNAs were used to inhibit SYT4 expression. The effect of SYT4 knock down on global gene expression, cell growth and sensitivity to Enza and Abi was assessed in our resistant cell lines.

Results: SYT4 gene expression was found to be markedly elevated in MDVR and AbiR cells compared to their parental C4-2B counterparts. Analysis of clinical data further revealed that SYT4 expression is significantly upregulated in patients with advanced prostate cancer; with high SYT4 levels being associated with poorer overall survival outcomes. Transcriptomic profiling identified enrichment of genes involved in calcium-mediated cell adhesion in MDVR cells, which is suppressed upon transfection with SYT4-specific siRNA. Additionally, silencing SYT4 expression via siRNA not only inhibited prostate cancer cell growth but also restored sensitivity of MDVR and AbiR cells to their respective treatments, underscoring SYT4 as a promising therapeutic target for treatment-resistant prostate cancer.

Conclusions: Our findings indicate that elevated expression of SYT4 promotes resistance to Enza and Abi; correlating with poorer outcomes in prostate cancer patients. Targeting SYT4 reduces the expression of genes involved in calcium-dependent cell adhesion and reduces cellular viability, thereby restoring sensitivity to NGATs like Enza and Abi, offering potential new strategies for overcoming treatment-resistant prostate cancer.

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MEIS1 and HOXB13 Mediated Proteoglycan Regulation Suppresses Prostate Cancer

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Abstract

Background: Prostate cancer (PrCa) continues to pose a burden to adult men in the United States, having the highest incidence and second highest mortality rates. A prostate cancer diagnosis in an immediate family member increases risk 2-3 fold. Germline mutations in the developmental transcription factor HOXB13 is associated with early development and potentially more rapid progression of prostate cancer. We recently reported that both MEIS1 and HOXB13 act as tumor suppressors through mediation of extracellular matrix proteoglycans Deocrin (DCN) and Lumican (LUM) (VanOpstall et al., eLife 2021). Further, loss of MEIS expression is associated with a more rapid time to biochemical recurrence and metastasis (Bhanvadia et al., Clin Can Res 2018). These data support a hypothesis whereby HOXB13 mutations impact MEIS-HOXB13 transcriptional activity, leading to decreased expression of tumor-suppressive proteoglycans.

Methods: CWR22Rv1 and LAPC4 cells were modified to express ectopic MEIS1 (LV-MEIS1); HOXB13 was deleted using CRISPR-Cas9 targeting. MEIS1 ChIP-Seq and RNA-Seq were conducted and bioinformatics analyses were done as previously described (VanOpstall et al., eLife 2021). CWR22Rv1 cells harboring heterozygous G84E HOXB13 mutants were generously shared from Dr. Sandor Spisak, Ph.D. and Matthew Freedman, M.D.. *in vivo* study utilizing SCID mice were used to xenograph cell types mentioned. Prominent HOXB13 mutants were evaluated for binding and downstream targets.

Results: Analyses of paired RNAseq and ChIP-seq of MEIS1 in the presence or absence of HOXB13 prioritized proteoglycan signaling, and in particular direct regulation of DCN and LUM by MEIS and HOXB13. DCN and LUM expression was validated using western blotting and qPCR. Induction of DCN and LUM expression in cells harboring HOXB13(G84E) mutations was decreased compared to wild-type HOXB13. G84E tumors grew less quickly than WT as well as had a lowered overall DCN and LUM load.

Conclusions: These data support our hypothesis that HOXB13 mutations decrease extracellular proteoglycan expression and thus provide a potential mechanism to understand how HOXB13 mutations promote tumorigenesis and progression. Further studies are needed to 1) test whether other HOXB13 mutants likewise impact proteoglycan expression; 2) determine the role of Androgen Receptor (AR) in mediating MEIS and HOXB13 transcriptional function; and 3) delineate the role of HOXB13 mutations in castration-resistance and metastatic progression using in vivo models.

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The miR-888 Cluster Accelerates Prostate Neuroendocrine Transdifferentiation in LNCaP Cells

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Abstract

Background:

Prostate cancer (PCa) is the second leading cause of male cancer deaths. Prolonged androgen deprivation therapy (ADT), a first-line treatment for advanced PCa, leads to the development of aggressive castration-resistant prostate cancer (CRPC). Clinicians have noticed an association between ADT and treatment-emergent neuroendocrine PCa (T-NEPC), a subset of CRPC characterized by tumor acquisition of neuroendocrine (NE) characteristics and down-regulation of androgen receptor (AR). Since CRPC is a lethal disease with mean survival of 2-3 years, new therapeutic targets are needed. Following initial research that showed this microRNA cluster to be upregulated in prostatic fluids from high-grade PCa patients, we hypothesize that the noncoding RNA miR-888 cluster promotes PCa progression and NE transdifferentiation.

Methods:

LNCaP cells were modulated for miR-888 and miR-891a expression using lentiviral miRNA mimic overexpression (OX) vectors and CRISPR miRNA knockout (KO) cell lines. Cells cultured in in phenol-free RPMI media with 10% charcoal-stripped fetal bovine serum to simulate androgen deprivation. KO cells were harvested at 0 day and weekly for 8 weeks; and OX cells were harvested at 0 day and every 48 hours for 3 weeks. Cells were stained with neurite marker beta-3-tubulin and neurite length was measured using NeuronJ. Total RNA and protein lysate from these timepoints were analyzed for stem cell markers (H19, SOX-2, NANOG, BRN2) and NE markers (SCGN, ENO2) by qRT-PCR and western blot.

Results:

LNCaP miR-888 and miR-891a OX cells displayed significantly accelerated neurite outgrowth compared to controls, while miR-888 and miR-891a KO cells demonstrated delayed neurite outgrowth. Western blot analysis demonstrated accelerated beta-3-tubulin and ENO2 expression in OX cell lines, and delayed expression of these markers in KO cell lines. Quantitative RT-PCR showed upregulation of H19, SOX-2, NANOG, BRN2, and SCGN in cells overexpressing miR-888 and miR-891a compared to controls.

Conclusions:

The miR-888 cluster accelerates NE transdifferentiation in LNCaP cells. These findings support the development of the miR-888 cluster as a potential therapeutic target or prognostic marker.

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ADC Co-targeting of Metastatic Castration-Resistant Prostate Cancer

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Abstract

Background: Antibody-Drug Conjugates (ADCs) are monoclonal antibodies targeted against an antigen on the cancer cell surface conjugated to a cytotoxic drug (payload) via a chemical linker. To date no ADCs are approved for metastatic castration-resistant prostate cancer (mCRPC) therapy due to a lack of efficacy and/or systemic toxicity. The rational design of ADCs and the development of combinatorial ADC-based approaches may enhance the therapeutic window for effective mCRPC treatment.

Methods: Distribution of mCRPC surface targets (B7-H3, PSMA, and STEAP1) was evaluated in mCRPCs using multiplex immunofluorescent staining to identify antigen pairs suitable for ADC co-targeting. Monoclonal antibodies binding target antigens were expressed in 293-F cells, purified, and characterized for ADC production. To select the payload pairs with synergistic interaction, a combinatorial payload screen was completed, and drug synergy was validated in a panel of mCRPC cell lines. Molecular mechanisms of drug interaction were studied in loss-of-function experiments *in vitro*. Synergistic ADCs were produced and tested in cellular and xenograft mCRPC models.

Results:

(1) The majority of mCRPC patients present with B7-H3 & STEAP1 double-positive metastasis.

(2) Genotoxic payloads (duocarmycin, doxorubicin, camptothecin analogs, pyrrolobenzodiazepines, calicheamcycin), as well as ADCs bearing genotoxic payloads, synergize with the Bcl-xl inhibitor A-1331852.

(3) P53 activity is predictive of mCRPC response to the combination of DNA damaging ADCs with systemic A-1331852.

Conclusions: This work addresses challenges associated with ADC monotherapy in mCRPC and explores the potential of rational ADC combinations. We nominate B7-H3 and STEAP1 as targets for dual ADC therapy, and we propose to use DNA-damaging agents and Bcl-xl inhibitor A-1331852 as payloads for B7-H3 and STEAP1-targeting ADCs. Synergistic interactions between genotoxic ADCs and ADCs bearing A-1331852 have the potential to elicit effective tumor killing in mCRPC. Because the prototypes of such ADCs have already demonstrated safety in early phase human studies, the data generated in this work enables near-term clinical development of new agents for mCRPC therapy.

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Staphylococcus epidermidis-derived lipoteichoic acid promotes PD-1 inhibitory signaling and reduced endoplasmic reticulum stress in sensory neurons to mediate anti-nociception.

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Abstract

***Staphylococcus epidermidis*-derived lipoteichoic acid promotes PD-1 inhibitory signaling and reduced endoplasmic reticulum stress in sensory neurons to mediate anti-nociception.**

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Introduction — Chronic pelvic pain syndrome (CPPS), the most prevalent form of prostatitis, is a complex and multifaceted syndrome characterized by multiple symptoms, an unknown etiology, and limited treatment options. We identified a novel lipoteichoic acid derived from a commensal strain of *Staphylococcus epidermidis* (SELTA) that has shown promise in reducing tactile allodynia in Experimental Autoimmune Prostatitis (EAP), a murine model of CPPS. Our previous studies indicated that the modulation of immune homeostasis within the prostate plays a significant role in alleviating allodynia in this model. In the current study, we established a sensory neuron-specific PD-1 conditional knockout mouse model to test our hypothesis that the analgesic effect of SELTA is mediated through neuronal PD-1. We focused on the impact of SELTA on neuronal PD-1 expression and activation, cytosolic Ca^{2+} homeostasis, and endoplasmic reticulum (ER) stress in peripheral nociceptive neurons at lumbar-sacral levels.

Methods — Experimental Autoimmune Prostatitis (EAP) was induced in mice via subcutaneous injection of rat prostate antigen combined with adjuvant, as previously described (1). Pain responses were measured using von Frey filament testing for tactile allodynia every seven days. On day 28 post-injection, animals were sacrificed, and their prostates were collected for further analysis. For ex vivo experiments, dorsal root ganglia (DRGs) were harvested from B6 mice at the L4-S2 levels and co-cultured with SELTA for 24 hours. Intracellular calcium levels in adult DRG neurons were assessed using live calcium imaging with the fura-2 ratiometric dye. To study endoplasmic reticulum (ER)-related stress, SERCA-mediated Ca^{2+} uptake and the unfolded protein response were evaluated using calcium imaging in a minimal calcium environment and qPCR, respectively. Confocal microscopy was used to capture images, which were subsequently analyzed with NIH ImageJ software. Statistical analyses were conducted using Microsoft Excel and GraphPad Prism software.

Results — Following SELTA instillation, PD-1 immunoreactivity in EAP-induced prostate tissue was significantly increased on day 28, with localization primarily to βIII-tubulin-labeled nerve fibers within

the stroma and nerve endings surrounding epithelial cells. Similarly, elevated PD-1 immunoreactivity was observed in L4-S2 DRGs treated with SELTA ex vivo. A dye-labeled PD-1 RNA probe confirmed that SELTA induced an increase in PD-1 mRNA expression in both the prostate and DRG. Functionally, SELTA inhibited ATP-induced Ca^{2+} influx in cultured DRG neurons. This inhibitory effect of SELTA on neuronal Ca^{2+} influx was reversed by the application of PD-1 neutralizing antibodies and in sensory neuron-specific PD-1 conditional knockout (PD-1 CKO) mice. Additionally, SELTA attenuated calcium signaling induced by thapsigargin, an ER stressor, in a PD-1 dependent manner. Importantly, reduction of ER stress by SELTA was associated with downregulation of the unfolded protein response including spliced XBP1, ATF4, ATF6, and GRP78. *In* vivo, the abrogation of PD-1 in PD-1 CKO mice eliminated SELTA's ability to mediate pain relief in the EAP model of chronic pelvic pain.

Conclusions — Our data demonstrate that SELTA is a novel exogenous inducer of neuronal inhibitory PD-1 signaling that contributes to its anti-nociceptive effects. SELTA appears to modulate neuronal function through PD-1 mediated regulation of endoplasmic reticulum (ER) stress to achieve homeostasis. These findings identify novel mechanisms for SELTA that may underpin its activity as an anti-nociceptive therapeutic.

1. Murphy SF, Schaeffer AJ, Done JD, Quick ML, Acar U, Thumbikat P. Commensal bacterial modulation of the host immune response to ameliorate pain in a murine model of chronic prostatitis. Pain. 2017;158(8):1517-27.

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Twist-1 levels are increased during PCa progression, and it is a major driver of cellular plasticity.

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Abstract

Twist-1 levels are increased during PCa progression, and it is a major driver of cellular plasticity.

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Running Title: Twist-1 and PDEF in PCa Plasticity

Background: Metastatic Castration Resistant Prostate cancer (mCRPC) results in over 35, 000 deaths annually in the USA. mCRPC patients initially respond to AR-targeted therapies (Enzalutamide etc.) but once therapeutic resistance to these drugs emerges, there is no curative treatment for these patients to date. Twist-1 is a master regulator of Epithelial-Mesenchymal transition and SAM pointed domain-containing Ets transcription factor (SPDEF) is a DNA-binding transcription factor. Our previous studies have shown that SPDEF plays an anti-metastatic role in prostate cancer. We also observed an inverse relationship between Twist-1 and SPDEF, but an interplay between Twist-1 and SPDEF in response to Enzalutamide resistance has not been investigated.

Methods: We analyzed publicly available data sets from TCGA (GDC TCGA Prostate Cancer, and TCGA Prostate Cancer (PRAD) for expression of Twist-1 and SPDEF. LNCaP and PC3 cells were purchased from ATCC and maintained according to ATCC guidelines. PDEF was cloned with an amino-terminal FLAG tag, and inserted into retroviral vectors pBABE, while Twist-1 gene was obtained from: Origene NM_00047 and cloned into plvx-neo. Xenograft experiments with LNCaP cells and LNCaP-Twist-1 cells were performed in nu/nu mice (Jackson). Protein levels were measured by Western blot analysis and gene expression was measured by RT-PCR.

Results: Our results show that Twist1 mRNA levels are increased in PCa tissues as compared to normal tissue. Moreover, there is a gradual increase in Twist-1 expression with tumor progression (increasing Gleason score). We observed that treatment of PCa cells with Enzalutamide increased Twist-1 and that enzalutamide-resistant PCa cells had increased levels of Twist-1. Furthermore, expression of Twist-1 in LNCaP cells increased clonogenic activity, cell migration and invasion *in vitro*, and tumor growth *in vivo*. Mechanistically Twist-1 expression was associated with decreased expression of luminal markers and increased expression of mesenchymal and neuroendocrine markers. These results show that Twist-1

expression is sufficient to promote cellular plasticity in prostate cancer cells. In previous studies, we have shown that ectopic expression of PDEF in PC3 cells resulted in decreased Twist-1 levels. Here we show that ectopic expression of Twist-1 failed to decrease levels of PDEF in these cells, suggesting that Twist-1 expression impacted PDEF expression by regulating transcriptional output via regulation of PDEF promoter activity. Moreover, ectopic expression of Twist-1 in PC3-PDEF cells failed to impact clonogenic activity, cell migration, or cell invasion.

Conclusions: Taken together, these results reveal that Twist-1 may play a role in enzalutamide-induced cellular plasticity in PCa. Our results also suggest that PDEF blocks the cellular plasticity of PCa.

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Mitochondrial Heteroplasmy Burden is Associated with Lower Urinary Tract Symptom Severity in Older Men

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Abstract

Mitochondrial Heteroplasmy Burden is Associated with Lower Urinary Tract Symptom Severity in Older Men

Background: Lower urinary tract symptoms (LUTS) affect millions of aging men worldwide. Men with LUTS may have severe symptoms that reduce quality of life, increase morbidity, and may require prostate surgery. The etiology of LUTS is multifaceted and poorly understood. Recently, mitochondrial dysfunction, a hallmark of aging, has been proposed as a contributor to LUTS. Mitochondrial heteroplasmy, defined as the presence of potentially pathogenic subpopulations of mitochondrial DNA (mtDNA) mutations, is one marker of mitochondrial dysfunction associated with aging. This study aims to investigate the association between leukocyte heteroplasmy burden and LUTS severity in community-dwelling older men.

Methods: Participants were men enrolled in the Health, Aging, and Body Composition Study. Blood samples were collected at the baseline visit and participants were administered the American Urological Association Symptom Index (AUASI) to evaluate LUTS severity. DNA was extracted from the buffy coat (leukocytes) of blood samples, 371 of which underwent mtDNA sequencing and were analyzed for heteroplasmy burden for 43 known pathogenic mutations. Multivariable linear regression models adjusted for age and clinical site were used to evaluate the association between heteroplasmy burden and AUASI severity. A Bonferroni correction was used to account for multiple comparisons ($p<0.0012$).

Results: Four (of 43) pathogenic mtDNA mutations (m.12315G>A, m.7471_7472insC, m.8344A>G, m.3302A>G) were associated with AUASI score, although only one (m.12315G>A) met the threshold for statistical significance after multiple comparisons correction. All four loci encode for mitochondrial tRNA elements and are associated with mitochondrial diseases. Older men with higher heteroplasmy at three mutation sites (m.12315G>A, m.7471_7472insC, m.8344A>G) had higher AUASI scores. Conversely, higher m.3302A>G heteroplasmy was associated with lower AUASI scores, although this may be due to higher rates of prior prostate surgery.

Conclusions: Better understanding the cellular processes driving the development of LUTS in older men is critical for identifying new biomarkers and treatment targets. This study demonstrates that mitochondrial heteroplasmy burden for specific mutations, measured from blood samples, is associated

with LUTS severity in older men. The findings further support the role of mitochondrial dysfunction in age-related LUTS. Additional studies are necessary to further validate these findings in a cohort with additional prostate measures and to investigate the potential role of mtDNA mutations in LUTS progression.

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DISSECTING THE MOLECULAR INTERPLAY BETWEEN AR AND AP-1 IN PROSTATE TUMORIGENESIS

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Abstract

BACKGROUND

Activator protein 1 (AP-1) is a complex heterodimeric transcription factor constituent of bZIP superfamily of proteins including Jun, Fos, Maf, and ATF proteins, each of whose abundance and activity is determined by external stimuli and effectively regulate cell function in a tissue and context dependent manner. The prostate – an organ responsible for seminal fluid production is primarily regulated by the dihydrotestosterone (DHT)-activated androgen receptor (AR). Although AP-1 has been studied in instructing key cellular events including cell cycle, survival, proliferation, and apoptosis through studies in multiple cell types, its distinct genomic and transcriptional regulation of prostate epithelial cell (PrEC) identity and function in an androgen signaling-dependent context remains elusive. Our preliminary studies have revealed that prior to androgen stimulation, genetically normal murine PrECs exhibit significant chromatin accessibility of AP-1 (FOSL1/2) bound sites. Upon androgen stimulation, these AP-1 sites are intriguingly depleted with the concurrent opening of androgen-response elements (AREs) across the chromatin. **We thus hypothesize that the AP-1 occupancy is antagonized by AR upon androgen stimulation specifying terminal differentiation towards a PrEC type.**

METHODS

We have engineered a temporally-controlled DNA-binding-incompetent state of AP-1 in the backgrounds of two AR-dependent models: genetically normal mouse prostate organoids and human prostate cancer cells (LNCaP) to simultaneously toggle the activity of AR and AP-1.

RESULTS

Inhibiting AP-1 DNA-binding markedly increases proliferation, S-phase entry in cell cycle activity, AR target gene expression and luminal cell fate specification in AR-dependent prostate cells. Furthermore, the AR transcriptome upregulates proliferative MYC and E2F-associated cell-cycle programs and downregulates apoptosis. Lastly, we have found that the androgen receptor cistrome is redistributed towards oncogenic co-factor recruitment upon AP-1 inhibition. To nominate a mechanism, we will employ integrated analyses of chromatin accessibility(ATAC-seq), AR ChIP-seq and bulk transcriptome

data in addition to targeted genetic deletion of AP-1 subunits to identify the responsible factor driving opposition with the androgen receptor.

CONCLUSIONS

AP-1 activity may govern a stem-cell transcriptional architecture in a normal prostate epithelial cell. Upon androgen exposure, the cells differentiate towards terminal luminal prostatic lineage.

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Targeting Cadherin-X with CAR-T Therapy: A Novel Approach for the Treatment of Prostate Cancer

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Abstract

Background: Prostate cancer (PCa) is a hard-to-treat malignancy that often exhibits resistance and unresponsiveness to current therapies. Cadherin-X (CDHx) is a tumor-associated antigen upregulated in basal-like PCa, making it a potential target for chimeric antigen receptor T-cell (CAR-T) immunotherapy. We hypothesize that CDHx-positive (CDHx+) PCa can be eradicated by anti-CDHx CAR-T cells both *in vitro* and *in vivo*.

Methods: We assessed the efficacy of anti-CDHx CAR-T cells against prostate cancer cell lines, specifically DU145 and DU145-CDHx$^{-/-}$, in both *in vitro* and *in vivo* models. *In vitro* studies focused on evaluating the target-selective cytotoxicity of anti-CDHx CAR-T cells, while *in vivo* studies involved engrafting NOD SCID mice with PCa cell lines and treating them with CAR-T therapy, with or without the combination therapy of immune checkpoint inhibitors.

Results: Anti-CDHx CAR-T cells demonstrated significant target-selective cytotoxicity against CDHx+ DU145 cell lines compared to CDHx$^{-/-}$ counterparts *in vitro*. Preliminary *in vivo* data suggest that CDHx-high tumor-bearing mice exhibited extended survival following anti-CDHx CAR-T treatment, especially when combined with PD-1 blockade.

Conclusions: Our study highlights the potent cytotoxicity of anti-CDHx CAR-T cells against CDHx-positive prostate cancer cells *in vitro* and suggests promising therapeutic potential *in vivo*. These findings may pave the way for the development of a novel immunotherapy approach for advanced prostate cancer, potentially enhancing current treatment regimens. Future research will focus on further validating these results and optimizing combination therapies for better clinical outcomes.

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SPDEF modulates transcriptional output to regulate multiple pathways: Immune and Inflammatory pathways as potential hubs targeted by SPDEF in RC77/T cells, a line of prostate cancer cells of African American origin.

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Abstract

SPDEF modulates transcriptional output to regulate multiple pathways: Immune and Inflammatory pathways as potential hubs targeted by SPDEF in RC77/T cells, a line of prostate cancer cells of African American origin.

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Background: Metastatic Castration Resistant Prostate cancer (mCRPC) results in over 30, 000 deaths annually in the USA. There are no curative therapies for patients suffering from mCRPC. Moreover, African American men are twice as likely to die from PCa as compared to Caucasian American men, but the underlying mechanisms for such disparity are not fully understood. SAM pointed domain-containing Ets transcription factor (SPDEF) is a DNA-binding transcription factor known to play roles in the prostate gland by targeting sets of genes via binding to 5'-GGAT-3' DNA sequences. Our previous studies revealed that SPDEF plays an anti-metastatic role in Prostate cancer, but the role of SPDEF in African American PCa cells has not been investigated. In the present study, we evaluated the effects of PDEF in RC77/T cells, a line of prostate cancer cells of African American origin.

Methods: RC77/T cells were stably transfected with an amino-terminal FLAG-tagged PDEF using a pBABE vector. Total RNA samples in triplicate from both SPDEF- and Vector control-RC77/T cells after confirming the overexpression by immunoblotting. The RNA quality control, cDNA library preparation, and sequencing were performed at the Louisiana Cancer Research Center (LCRC) using an in-house NGS device. RNA seq was performed to evaluate the effects of SPDEF on gene expression. And gene expression was confirmed by RT-PCR. The data were received as fold-changes, and p value of less than 0.05 was considered significant. To have an insightful interpretation of this complex omics data and to find the significantly altered molecules and pathways changed by SPDEF overexpression, we used the QIAGEN Ingenuity Pathway Analysis (IPA) web-based software. Clonogenic activity, cell migration, and invasion assays were performed to measure the effects of PDEF on cell functions.

Results: Our results reveal that PDEF expression in RC77/T cells resulted in a significant decrease in cell migration and suppression of genes associated with EMT. Analysis of RNA seq data revealed increases in

multiple pathways including hypercytokinemia/hyperchemokinemia (21 molecules, 25% match), interferon signaling (13 molecules, 35% match), and pathogen recognition receptor in infectious disease pathogenicity (17 molecules, 15% match). The IPA analysis of disease- and functional-related changes suggests significant upregulation of genes associated with inflammatory responses, hematological system development of function, immune cell signaling and trafficking, and cellular movement. Our network prediction analysis also highlights the central role of the inflammatory cytokine IL-6 and inhibitor of NFKB (NFKBIA). Interestingly, when overlaid with the publicly available prostate signaling pathway, our data showed a unidirectional increase in NFKBIA and HSP90 which control the cell survival and AR signaling, respectively. Collectively, these data suggest the immune and inflammatory pathways as potential hubs that SPDEF is targeting at least in the transcriptional levels which opens a new door to look at the SPDEF role in prostate cancer pathogenesis with special emphasis in African American Men. Further studies are under progress to functionally test these pathways and their correlation with the anti-metastatic role of SPDEF in PCa.

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Elucidating global metabolic alterations associated with phosphoserine phosphatase (PSPH) suppression in prostate cancer cell lines

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Abstract

Background

Metabolic reprogramming is a crucial feature of tumor and its microenvironment. L-3-phosphoserine phosphatase (PSPH) is one of the five rate-limiting enzymes in the biosynthesis of serine. We have found that mRNA levels of PSPH are significantly higher in African American (AA) prostate cancer (PCa) patients compared to European American (EA) patients and predict poor survival. Suppression of PSPH expression in prostate cancer cells results in inhibiting cell proliferation and tumor growth. Here we aim to identify what metabolic changes are globally associated with PSPH suppression and intent to dissect potential underlying mechanisms of action.

Methods

PSPH expression in PCa cell lines MDAPCa2b (derived from an AA PCa patient) and LNCaP cells (from an EA PCa patient) was stably knocked down by short-hairpin RNAs (shRNAs). mRNA and metabolites from the knockdown and control cells were extracted and processed with bulk RNA sequencing and untargeted metabolomics analysis.

Results

mRNA expression profiling demonstrates that suppression of PSPH expression by shRNAs in LNCaP and MDAPCa2b cells is associated with altered expression of genes related to metabolism and immunity. We also find lower L-serine levels in both LNCaP and MDAPCa2b PSPH knockdown cells compared to ShRNA control knockdown cells. Moreover, KEGG enrichment analysis of metabolites from untargeted metabolomics approach reveals that nucleotide metabolism and oxidative phosphorylation pathways in LNCaP PSPH knockdown cells, and sphingolipid signaling pathway in MDAPCa2b PSPH knockdown cells are significantly altered. The expression trend of all identified metabolites in central carbon metabolism is up regulated in control cells of MDAPCa2b compared to LNCaP control cells. Metabolites that participate in glycolysis like 3-Phosphoglyceric acid, DL-Glyceraldehyde 3-Phosphate and D-Glucose 6-Phosphate are markedly increased in LNCaP PSPH knockdown cells. α-Ketoglutaric Acid (α-KG), citric acid, and malic acid involved in TCA cycle as well as folic acid of one carbon metabolism present significant lower levels in MDAPCa2b PSPH knockdown cells.

Conclusions

Our results indicate that knockdown of PSPH significantly inhibited PCa cell metabolism and growth. Differently altered metabolic pathways upon PSPH knockdown between MDAPCa2b and LNCaP cells

provide clues for further understanding prostate cancer disparity and developing therapeutic strategy targeting cancer cell metabolism.

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IGFBP3 Signaling Promotes Resistance to Next-Generation Antiandrogens Therapeutics in Advanced Prostate Cancer

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Abstract

Background: Next-generation antiandrogen therapeutics (NGATs) have irrefutably improved the survival of prostate cancer patients. However, treatment resistance to NGATs remains a clinical challenge for men with castration-resistant prostate cancer (CRPC). The molecular mechanisms underlying resistance to NGAT remain confounding. Insulin growth factor binding protein 3 (IGFBP3) is associated with prostate cancer tumorigenesis and progression. It is known that IGFBP3 induces sphingosine-1-kinase (SphK1) activation which subsequently stimulates sphingosine-1-phosphate (S1P) production. Previously, we found that IGFBP3 is necessary for PARP inhibitor resistance in acquired resistant models (LN-OlapR and 2B-OlapR cells). We hypothesize that IGFBP3 is required for NGAT resistance.

Methods: Enzalutamide-resistant (MDVR), Abiraterone-resistant (AbiR), Apalutamide-resistant (ApalR), and Darolutamide-resistant (DaroR) C4-2B subline cells and Enzalutamide-resistant models, 42D/42F and 22Rv1, were utilized. Transcriptomic analysis was performed on Enzalutamide-treated patient cohorts and NGAT resistant cells. RNAseq, real time qPCR, western blot, and ELISA were used to determine expression and secretion levels. Cell growth and survival was determined in NGAT resistant cells and spheroids treated with siRNA-IGFBP3 and -SphK1 or SphK1 inhibitor, SKI-II.

Results: This study found that IGFBP3 was upregulated in NGAT resistant models. We found that NGAT resistant models highly express IGFBP3 and SphK1 using RNA-seq, qPCR, and western blot. SphK1 is a lipid kinase that phosphorylates sphingosine to form S1P metabolite which promotes tumor growth and therapeutic resistance. We discovered high levels of IGFBP3 and S1P secretion in the resistant models. We found that inhibition of IGFBP3/SphK1 decreased both SphK1 expression and S1P secretion. Mechanistically, IGFBP3 activated the Sphingolipids pathway to promote cell survival and subsequently enforced the growth factor signaling to enhance cell proliferation. Ablating the expression of IGFBP3 promotes the formation of ceramide which induces the apoptotic cascade.

Conclusion: We demonstrated that increased IGFBP3 expression by NGAT treatment leads to increased sphingolipid metabolism by simultaneously demoting ceramide-induced apoptosis and fostering S1P production through SphK1 activation. Thus, IGFBP3 induces the activation of SphK1 to hijack the sphingosine metabolism to fuel NGAT resistance. Our findings demonstrate that targeting the IGFBP3/SphK1 signaling axis enhances our ability to resensitize resistant CRPC to NGAT therapeutics.

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PDEF restricts cellular plasticity and resistance to AR pathway inhibitor Enzalutamide in prostate cancer

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Abstract

PDEF restricts cellular plasticity and resistance to AR pathway inhibitor Enzalutamide in prostate cancer

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Background: Prostate cancer (PCa) is the second leading cause of cancer death among men in the United States. In 2024, an estimated 0.3 million new cases of PCa are expected to be diagnosed, with approximately 35,000 deaths, most notably due to the therapy resistance. Enzalutamide (ENZ) is a second-generation androgen receptor antagonist that has demonstrated its ability to enhance overall patient survival. However, the response to ENZ is heterogeneous with almost half of CRPC patients either not responding at all or developing resistance within 8 to 18 months, due to the dynamic nature of lineage plasticity and phenotype switching. Therefore, it is urgent to investigate novel approaches to overcome enzalutamide resistance. Our previous studies have demonstrated that PDEF promotes luminal differentiation in androgen-independent PCa cells (PC3 and DU145) cells and knockdown of PDEF in Androgen responsive (LNCaP cells) promoted EMT. In this study, we evaluated the role of PDEF in enzalutamide-induced cellular plasticity and phenotype switching.

Methods: LNCaP and C4-2 cell lines were cultured in an androgen-deprivation medium and treated with 10nM DHT and/or 10µM enzalutamide for 3 days. Following this treatment period, the media was changed to assess the comparative effects of DHT versus enzalutamide over an additional 3 days. Enzalutamide-resistant (LNCaP and C4-2) cells were developed by maintaining them in a medium containing 5µM enzalutamide for a minimum of six months. Proliferation assay was assessed over 4 days using Incucyte live cell imaging software with 8 replicates. Colony formation assay was conducted over 10 days by crystal violet staining. Cell migration was assessed by in vitro scratch wound healing assays. Protein and mRNA levels were determined by western blot and RT-PCR techniques. Cellular morphology was captured under phase contrast and fluorescence microscopy.

Results: Our results show that treating LNCaP and C-4 cells with Enzalutamide and androgen deprivation decreased PDEF expression and induced EMT and NEPC-associated genes. Ectopic expression of PDEF, in these cells reduces EMT and NEPC-associated hallmark genes under androgen deprivation conditions. Furthermore, enzalutamide-resistant (LNCaP-ENZR) cells show a robust increase in EMT, stemness, and NEPC-related markers and a concomitant decrease in luminal markers viz. PDEF, PSA, and KRT18,

suggest cellular plasticity. In line with the above results, we also observed a gradual transformation from epithelial morphology to neuroendocrine phenotype, characterized by spindle-shaped and elongated structures in LNCaP-ENZ-R cells. We also observed that enzalutamide significantly inhibited the proliferation and clonogenic ability of enzalutamide-sensitive LNCaP cells but had no effect on LNCaP-ENZ-R cells. Moreover, ectopic expression of PDEF in LNCaP-ENZ-R cells reversed many of the changes in gene expression associated with EMT and NEPC Intriguingly, the above results support the plausible role of PDEF in treatment-induced cellular plasticity and reprogramming in PCa.

Conclusion: Overall, our findings suggest that PDEF promotes the androgen-sensitive luminal phenotype, limiting therapy-induced cellular plasticity, and increasing prostate cancer cells' sensitivity to existing therapies.

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Regulation of YAP1/TAZ transcriptional targets in Prostate Cancer by PDEF

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Abstract

Regulation of YAP1/TAZ transcriptional targets in Prostate Cancer by PDEF

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Objective: Most prostate cancer-related deaths are attributed to Androgen indifferent prostate cancer (AIPC). Three often overlapping variants of AIPC, neuroendocrine prostate cancer (NEPC), double-negative prostate cancer (DNPC), and aggressive variant prostate cancer (AVPC) are commonly observed in men with castrate-resistant prostate cancer (CRPC) following treatment with Androgen Receptor signaling inhibitors (ARSi) and may represent therapy induced Prostate cancer plasticity. The Hippo TEAD-transcriptional regulators YAP1 and TAZ play essential roles in cancer cell growth and metastasis. However, the function of YAP1/TAZ-TEAD in prostate cancer has not been well characterized until recently. Our previous studies revealed that the expression of PDEF is decreased during prostate cancer progression and re-expression of PDEF limits prostate cancer metastasis in part by promoting luminal epithelial phenotype, but the mechanisms of PDEF action are not completely understood. In the present study, we evaluated the effects of the SPDEF on YAP1/TAZ levels and expression of YAP1/TAZ regulated genes in prostate cancer (PCa) cells. We also evaluated the expression of SPDEF, YAP1, TAZ, and YAP/TAZ-TEAD regulated genes in clinical cohorts of prostate cancer patients.

Material & Method: Prostate cancer (PC3 and DU145) cells were transfected with PDEF or respective vector control. shRNA was used to knock down SPDEF in AR-positive and androgen-responsive LNCaP cells. Enzalutamide resistant LNCaP cells were generated by continual exposure of LNCaP cells to ENZ (5mM) for at least 6 months. Protein levels were analyzed by western blotting. Gene expression analysis was done by microarray/RNA seq and confirmed by RT-PCR assays. PDEF expression was analyzed from publicly available gene expression data in several prostate cancer cohorts.

Results: We observed that androgen-dependent PCa (LNCaP) cells express high levels of PDEF and YAP1, and lower levels of TAZ, while androgen-independent PCa (PC3 and DU145) cells express reduced levels of PDEF and YAP1, and higher levels of TAZ. We also observed that expression of PDEF in PC3 and DU145 cells resulted in increased phospho-YAP1(Ser127) and phospho-TAZ(Ser89) protein levels, as compared to the respective vector control. Moreover, knockdown of PDEF in LNCaP cells resulted in

overexpression of YAP/TAZ target genes, while stable expression of PDEF in PC3 and DU145 cells resulted in inhibition of YAP1/TAZ target genes, as compared to the respective vector controls. These results demonstrate that PDEF plays a critical role in modulating YAP1/TAZ-TEAD transcriptional activity, and by extension in the regulation of the Hippo pathway. Analysis of data from metastatic prostate cancer revealed a negative correlation between TAZ expression and survival from hormone therapy and an appositive correlation between PDEF expression and survival from hormone therapy, suggesting that these pathways could also play a role in therapy resistance in PCa.

Conclusions: Taken together these data suggest that PDEF limits YAP1/TAZ-TEAD driven transcriptional output in prostate cancer cells, and cellular plasticity associated with loss of PDEF in subsets of PCa patients could result from aberrant activation of YAP1/TAZ-TEAD pathway.

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Modulation of FOXM1 isoform expression in cisplatin resistant bladder cancer

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Abstract

Background: The first line of treatment for muscle invasive bladder cancer (MIBC) is platinum-based chemotherapy prior to and/or following radical cystectomy. However, only ~40% of patients respond to systemic platinum. Consequently, there is an unmet need to understand the molecular drivers contributing to cisplatin resistance in MIBC to aid in the development of novel treatment modalities. FOXM1, a member of the FOX family of transcription factors, has been identified as a key driver of cisplatin resistance in other solid tumors. The *FOXM1* gene contains 10 exons, composing four major splice variants, *FOXM1A-D*. FOXM1A is transcriptionally inactive, whereas FOXM1B and FOXM1C are transcriptional activators with oncogenic traits, whereas FOXM1D enhances EMT and metastasis via protein-protein interactions. However, the individual roles of the various FOXM1 isoforms in aggressive and chemoresistant disease in bladder cancer (BlCa) have not been elucidated. Herein, we demonstrate that individual FOXM1 isoform activities, and subsequent EMT and cisplatin resistance, is regulated by the novel RNA-binding protein ANGEL2.

Methods: Multi-orthogonal approaches, including 2D and 3D cell culture, RNA-seq, LC-MS/MS and 10x Genomics Visium Spatial Gene Expression system analyses were utilized for this project to elucidate how loss of *ANGEL2* in MIBC contributes to isoform specific FOXM1-mediated EMT and cisplatin resistance in MIBC.

Results: Exploration of the TCGA Pan-Cancer dataset revealed a significant negative correlation between *ANGEL2* and *FOXM1* expression ((r) = -0.302, p= <0.0001). Clinically, *ANGEL2*^{Low}/*FOXM1*^{High} expression was correlated with worse BlCa patient overall survival. 10x Genomics Visium Spatial Gene Expression analyses on two BlCa tumor samples, coupled with corresponding PDX drug response analyses demonstrated that *ANGEL2* and *FOXM1* expression are inversely correlated and that a *ANGEL2*^{Low}/*FOXM1*^{High} tumor (BL0293) was not sensitive to cisplatin, whereas a *ANGEL2*^{High}/*FOXM1*^{Low} tumor (BL0440) was sensitive to cisplatin treatment. Finally, *ANGEL2*-null cells treated with/without cisplatin showed significant upregulation of the oncogenic FOXM1 isoforms *FOXM1B*, *FOXM1C*, and *FOXM1D,* upregulated EMT markers *SLUG*, *TWIST1*, and *OCT4*, and *conferred* cisplatin resistance.

Conclusion: We demonstrate that ANGEL2 expression modulates oncogenic FOXM1 isoform expression contributing to EMT and cisplatin resistance. We hypothesize that ANGEL2 expression may function as a prognostic biomarker for PBC sensitivity for MIBC patients.

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Hematuria protects Urothelium from Cell Death

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Abstract

Background: Hematuria is a common and frightening complication of many urinary diseases. Hematuria is induced by urinary tract infections (UTIs) and may be pathogenic because heme carries lipophilic Fe^{2+} which may damage cells. In addition, iron is limiting for bacterial growth, and urinary bacteria express heme transporters (Chu and Hma) as well as ferric siderophore receptors. Hence, hematuria may accelerate bacterial growth and tissue damage.

Methods: We iron loaded the urinary system with ferric iron or with heme by induced hemolysis using phenylhydrazine (PHZ). UTI was induced by titration of UTI-89 inoculation in male and female mice (20uL of 107 CFU), and monitoring tissue damage by urothelial shedding from the luminal surface of the bladder (CK20+cells) compared to preserved basal cells (CK5+cells). Urothelial RNA was captured by (1) physical isolation of urothelial cells from bladder mucosa (2) and by 4-thiouracil pulse labeling of nascent RNA in vivo in Upk2Cre;Uprtf/f urothelial cells followed by capture of nascent RNA with thio-biotin-avidin beads. Steady state and nascent RNA as used for RT-PCR. RNAscope probed anatomical expression of induced genes.

Results: UTI induced detachment and shedding of superficial cells (between 6-12hrs post infection). Ferric iron loading worsened, and ferric iron deficiency suppressed bacterial growth and urothelial shedding. In contrast, hematuria neither induced bacterial growth nor enhanced cell shedding. In fact, hematuria prevented cell shedding (n=75 mice). Retention of urothelial superficial cells and suppression of erythema was grossly visibile even when invasive bacterial intracellular colonies were present.

Urothelial RNA from dissected cells and from Upk2 directed RNA pulldowns suggested that pyroptosis genes may mediate cell death in UTI. Indeed, we discovered that GsdmD KO mice suppressed urothelial shedding and accordingly hematuria suppressed GsdmD RNA expression. Upstream regulators of GsdmD, such as Nlrp3 and cleaved casp1 and 11 proteins were depressed by hematuria. In situ demonstrated that while Gsdmd and Nlrp3 RNA were enhanced by UTI, both were suppressed by hematuria. Pyroptosis associated cytokines, chemokines, neutrophil chemoattractants, interleukins and inflammatory cell death regulators were systematically and quantitatively downregulated by hematuria. In accordance, neutrophil invasion of the infected bladder was suppressed.

We found that urothelial cells express a heme-machine. Heme is captured in urothelial cell layers by HRG1, which in turn transports heme into the cytoplasm where upregulated BMAL, NPAS2, and HMOX1 actively metabolizes heme to Biliverdin and Carbon Monoxide the emission of which was detectable with our Palladium-based fluorescence quenched probes. Iron release was detected by Perls and Turnbull's staining in superficial cells and intermediate cells. However, both HRG1 KO and HMOX KO still demonstrated a complete suppressive effect of UTI induced cell shedding in the presence of hematuria.

Conclusions: Hematuria is a powerful anti-inflammatory agent suppressing GSDMD mediated cell death, cytokine expression, and neutrophil invasion. While HMOX plays a critical role in urothelial survival, the import and metabolism of heme cannot explain heme rescue. Rather, we have accumulated evidence that a members of the TLR superfamily are cell surface heme receptors mediating heme's anti-inflammatory activity by MAPK suppression. In sum, hematuria, a frightening sign of urological disease, is cell protective.

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Enzalutamide resistance may be mediated by amino acid transport in prostate cancer cells

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Abstract

Background: Castration-resistant prostate cancer (CRPC) cells develop resistance to enzalutamide within a relatively short span of time. Several mechanisms including androgen receptor variants, alternative signaling mechanisms, metabolic pathways, and androgen receptor by-pass mechanisms have been proposed to contribute to the development of enzalutamide resistance. We showed earlier that metabolic alterations during the development of resistance to enzalutamide may offer unique opportunities to target metabolic vulnerabilities in prostate cancer (PCa) cells. Exploration of the mechanisms using metabolomics indicated that amino acid metabolism and amino acid transporters may play an important role in the resistance to enzalutamide. Hence, we investigated the role of amino acid transporters in enzalutamide resistance in PCa cells.

Methods: We analyzed the expression levels of amino acid transporters in PCa tissues using public datasets. We treated enzalutamide-sensitive and -resistant PCa cells with varying concentrations of amino acid transport inhibitors JPH203 or V9302 either singly or in combination with enzalutamide and assessed cell survival, cell viability, proliferation, and survival in 3-D models. We analyzed synergism between the combinations using SynergyFinder.

Results: Expression levels of several amino acid transporters were higher in PCa tissues compared with benign tissues. Expression of amino acid transporters was significantly upregulated in enzalutamide-resistant PCa cells. Treatment with amino acid transport inhibitors JPH203 and V9302 inhibited enzalutamide-resistant PCa cell survival preferentially, while their combinations with enzalutamide achieved greater suppression of enzalutamide-resistant PCa cell survival.

Conclusions: We found that increasing amino acid import may be one of the metabolic mechanisms employed by PCa cells during the development of enzalutamide resistance. These results imply that inhibiting elevated amino acid import into cancer cells may be an attractive strategy to overcome enzalutamide resistance.

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Kidney in a bag: a low-cost strategy for establishing high-performance normothermic ex vivo organ perfusion

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Abstract

Background: Normothermic *ex vivo* organ perfusion technology has great therapeutic and disease-modeling potential and is a growing frontier for biomedical discovery. There are considerable barriers to entry for new investigators interested in this area of research, however, such as the restricted availability of commercial *ex vivo* perfusion devices, the high cost of organs and reagents, and a steep technical learning curve.

Methods: To overcome these barriers and originate a normothermic *ex vivo* kidney perfusion (NEVKP) program at our institution, a *de novo* NEVKP circuit was constructed using recycled, repurposed, and low-cost components. Freshly discarded porcine kidneys and autologous blood sourced primarily from a local abattoir were then used to iteratively optimize the circuit design, and discarded human donor kidneys were perfused with expired blood products to demonstrate proof of concept. Key design innovations included a parallel flow resistor to facilitate low-flow perfusion in small or high-resistance organs and a containment bag to provide external compression and recycling of venous hemorrhage.

Results: An NEVKP system capable of maintaining viability and urine production for up to 36 hours was thus developed over a series of 20 porcine kidneys. Three human donor kidneys were then perfused for 24 hours using this system. Both porcine and human kidneys exhibited the ability to maintain potassium homeostasis via excretion, and increased urine output was observed in response to furosemide administration. Histologic assessment demonstrated preserved tubular and glomerular architecture. The total cost of circuit components, organs, and reagents was less than $5,000.

Conclusions: Proficiency in NEVKP can be achieved using low-cost and readily available materials. While much remains to be understood and improved in *ex vivo* organ perfusion, our experience provides a road map to aid investigators seeking entry into this field.

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DEVELOPMENT AND CHARACTERIZATION OF A SELECTIVE ARGININE VASOPRESSIN RECEPTOR 1A (AVPR1A) ANTAGONIST FOR TREATING CASTRATION RESISTANT PROSTATE CANCER

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Abstract

Castration resistant prostate cancer (CRPC) is a lethal and incurable stage of PC. We previously showed that the GPCR arginine vasopressin receptor 1A (AVPR1A) is expressed in CRPC and is crucial for cell growth (Zhao et al. *Sci Transl Med* 2019). In preclinical xenograft models, relcovaptan, a selective AVPR1A antagonist, decreases growth of newly emergent, established and end-stage bone CRPC (Zhao et al. *Sci Transl Med* 2019, Heidman et al. *Mol Cancer Res* 2022). Unfortunately, there are currently no FDA-approved AVPR1A-selective antagonists. Although the potent and selective antagonists relcovaptan and the more recently developed balovaptan were proven safe in human clinical trials for non-cancer disorders, these compounds are no longer available for clinical studies. To advance our findings toward clinical application, we report here the development and characterization of novel AVPR1A antagonists and their effects in preclinical models of CRPC. Seven novel compounds (UMFs) were evaluated for efficacy and selectivity using the BRET-based TRUPATH (Olsen et al. *Nat Chem Biol* 2020), which detects GPCR activity by measuring dissociation of the heterotrimeric G protein complex. HEK cells expressing AVPR1A, Gαq-Rluc8, Gβ3 and Gγ9-GFP2 were incubated with UMFs plus the agonist arginine vasopressin (AVP) and activity determined by the ratio between acceptor and donor emission intensities. UMFs antagonized AVPR1A with an IC50 in the nanomolar range. Using TRUPATH we demonstrated that UMFs were selective for AVPR1A and did not antagonize the closely related AVPR1B, AVPR2 or oxytocin receptor. UMFs similarly decreased AVP-induced intracellular calcium release, ERK phosphorylation and cell proliferation in CRPC cell lines. No effect on the proliferation of AVPR1A non-expressing cells was observed. Based on superior potency and selectivity, UMF-504 was selected as our lead compound. UMF-504 had excellent *in vitro* and, following oral administration, *in vivo* DMPK. Orally administered UMF-504 decreased CRPC tumor establishment and growth in C4-2B xenograft models. Neither animal weights nor behavior were affected by UMF-504, indicative of a lack of adverse effects of this compound like other AVPR1A antagonists tested in humans. These results show that UMF-504 is an orally available, potent and selective antagonist for AVPR1A with potential benefits for the treatment of CRPC.

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Exploiting BUB1B as an untapped vulnerability in castration resistant prostate cancer.

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Abstract

Men with high-risk or advanced prostate cancer (PC) are treated with androgen deprivation therapy but unfortunately incurable castration-resistant (CR) tumors often emerge. Ligand-independent androgen receptor (AR) signaling including expression of constitutively active AR variants (AR-Vs) is one mechanism underlying progression to CRPC. We previously identified a clinically relevant seven gene set that is driven by ligand-independent AR signaling and contains potential therapeutic targets including BUB1B. BUB1B, a mitotic serine/threonine kinase, is an essential component of the spindle assembly checkpoint and serves as a master regulator of the seven gene set in CRPC cells. BUB1B is overexpressed in PC compared to benign prostate tissue and is higher in metastatic CRPC compared to primary disease. Depletion of BUB1B blocked the proliferation of all tested CRPC cell lines, including AR-null cells, suggesting that BUB1B may function through both AR-dependent and -independent pathways in CRPC. Ectopic expression of BUB1B in androgen-dependent PC cells conferred castration resistant growth, which was not blocked by the clinically used AR antagonist enzalutamide. This finding suggests that BUB1B drives castration resistance and enzalutamide resistance, a significant clinical problem. To evaluate the role of BUB1B kinase activity in CRPC growth, we depleted BUB1B in CRPC cells stably expressing shRNA-resistant BUB1B wild type (wt) or three previously characterized kinase-dead BUB1B mutants. Only expression of BUB1B wt rescued growth inhibition after BUB1B depletion, suggesting that BUB1B kinase activity is required for CRPC cell proliferation. Therefore, targeting the catalytic function of BUB1B represents a potential therapeutic approach for CRPC. BUB1B has no tractable small molecule inhibitors and no experimental structure of its kinase domain. To develop a chemical probe, we employed a scalable computational screening pipeline integrating deep neural network predictors and molecular simulations. We generated several high quality BUB1B 3D homology models based on homologous kinases and applied a computational screening pipeline to prioritize putative BUB1B inhibitors/ binders. Based on docking scores, we identified a predicted low molecular weight potent binder. In addition, we mined recent proteomics data from Kinome-wide PROteolysis Targeting Chimera (PROTAC) profiling and identified promiscuous compounds predicted to bind to the BUB1B kinase domain. Our modeling data will be used to develop specific inhibitors of BUB1B kinase. These results support BUB1B as an exploitable and untapped therapeutic vulnerability for enzalutamide resistant CRPC.

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Vitamin D deficiency leads to a proinflammatory microenvironment in the prostate that supports carcinogenesis

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Abstract

Vitamin D deficiency leads to a proinflammatory microenvironment in the prostate that supports carcinogenesis

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Abstract:

Background:

Prostate cancer (PCa) is the second leading cause of cancer-related deaths in men. However, its impact is pronounced among African American (AA) men, who face double the mortality rate compared to their European American (EA) counterparts. Melanin-related vitamin D (vitD) deficiency in AA men may contribute to the heightened risk of lethal and aggressive PCa. VitD is a hormone with a pivotal role in skeletal maintenance and bone health, however, it also has anti-tumor, immuno-modulatory, and pro-differentiation effects. The prostate microenvironment, comprising a complex network of cells and signaling molecules, plays a crucial role in regulating various physiological processes within the prostate. Prostate immune infiltrates play a pivotal role in maintaining homeostasis by secreting cytokines crucial for effective immune surveillance and defense against abnormal cell growth. Therefore, vitD deficiency may shift immune cell subpopulations in the prostate, fostering an inflamed environment. The prostate stroma actively influences the epithelial cells by secreting growth factors, chemokines, and cytokines. Here, we examined how vitD deficiency in male mice alters molecular profiles of stromal and immune cells within the prostate.

Hypothesis/Aims:

We hypothesize that vitD deficiency alters the prostate microenvironment to support carcinogenesis.

Methods:

Male C57BL/6 mice were fed either a vitD deficient or control diet for 6 months. Dorsolateral prostate lobe and cells were isolated for single-cell RNA sequencing. Gene expression differences and cell population analyses were conducted in “R”.

Results:

Multiple types of immune cells and stroma were identified in the scRNAseq analyses. Numerous differentially expressed genes were identified within the stroma, with known roles in inflammation, paracrine signaling to the epithelium, and tumor microenvironment remodeling. Additionally, our analysis revealed a profound shift in immune cell infiltrates in the prostate. We observed increased expression of activated T cell markers in mice with vitD deficiency, which may contribute to an immunosuppressive environment. In contrast, mice on a sufficient diet expressed markers of central memory T cells, poised to respond against cancerous cells, suggesting differing cancer surveillance capabilities based on vitD status.

Conclusions:

These data suggest that maintaining adequate vitD levels may be essential for regulating the prostate microenvironment and preventing the molecular changes that may contribute to earlier and more aggressive PCa development. VitD deficiency status may lead to heightened inflammation and T cell activation. As vitD is easily supplemented, our studies offer a rationale for preventing vitD deficiency to support prostate health and function, potentially reducing the risk of aggressive PCa. Given the vulnerability of AA men to vitD deficiency and lethal PCa, this research aims to mitigate the disparity by addressing the underlying factors. As our findings highlight shifts in immune infiltrates within the prostate due to vitD deficiency, interventions to maintain optimal vitD levels could potentially improve treatment outcomes for AA patients facing PCa.

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Explore biological vectors to deliver CRISPR machinery against androgen receptor in prostate cancer cells

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Abstract

Background: Prostate cancer (PCa) is the most diagnosed malignancy among men in the United States in 2024. Approximately 10-20% of cases progress to castration-resistant stage, which poses significant treatment challenges. Androgen receptor (AR) signaling remains active in most castration-resistant prostate cancer and is a crucial driver of the disease progression. CRISPR/Cas9 system offers a promising approach to silence the AR gene at the genomic DNA level. However, efficient and safe delivery of CRISPR machinery remains a significant obstacle.

Experimental Methods: 1) Bioengineering of Cas9/sgRNA against AR or other genes and fusion of myristoylation consensus sequence at the N-terminus of Cas9 gene were carried out by molecular cloning. **2)** mCas9/sgRNA was transfected by transient transfection or lentiviral infection. **3)** Gene editing efficiency in 293T-AR or C4-2B PCa cells was analyzed by T7E1 assay, sanger sequencing, and/or next-generation sequencing. **4)** Cell growth rate was determined by MTT assay. **5)** EVs were isolated from the conditional medium by differential ultracentrifugation with/without the assistance of Exodus. **6)** Expression levels of proteins in the isolated EVs were analyzed by immunoblotting.

Results: Fusion of an octapeptide to Cas9 induced N-myristoylation and facilitated the encapsulation of the mCas9/sgRNA (against luciferase or AR) into EVs. Additionally, the designed sgRNAs successfully knocked out luciferase or both ectopic and endogenous AR genes. Next, the chromatid accessibility regulates gene editing efficiency. The sgRNA against the LBD or NTD of AR exhibited different gene editing efficiency between C4-2B and 22Rv1 cells by lentivirus-based delivery. The sgRNA against the NTD of AR had significantly higher gene editing efficiency in C4-2B cells than that in 22Rv1 cells. The different accessibility of the sgRNA to the target sites was further confirmed by the ATAC-seq data analysis. Furthermore, gene editing on AR in C4-2B cells resulted in 50% growth inhibition and significantly decreased expression levels of AR-downstream genes. Finally, Cas9/sgRNA ribonucleoprotein complex was also encapsulated into EVs for delivery. However, EVs containing the RNP had much lower gene editing efficiency than lentivirus-based delivery in 293T-AR or C4-2B PCa cells.

Conclusions: 1) N-myristoylation promoted the enrichment of CRISPR-Cas9 into EVs. Myristoylated Cas9 is functional in gene editing. **2)** Lentivirus-based CRISPR delivery resulted in different gene editing efficiencies in PCa cells, due to chromatin accessibility pattern of sgRNA. 3) AR gene editing led to approximately 50% inhibition of C4-2B cell proliferation and a significant decrease of AR downstream gene expression. **3)** EVs-based Cas9/sgRNA RNPs delivery was not as effective as lentivirus-based delivery in gene editing. Further study should focus on improving EVs-mediated gene editing efficiency to target PCa cells.

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The association between obesity, diabetes, and prostate cancer aggressiveness in Louisiana.

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Abstract

The association between obesity, diabetes, and prostate cancer aggressiveness in Louisiana.

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Introduction

In the US, Black men are more likely to be diagnosed with prostate cancer (PCa), especially aggressive types, compared to other races and ethnicities. Black men are more than twice as likely to die from the disease. There is conflicting literature on the association between obesity, diabetes and PCa development. We aim to assess if there is an association between diabetes and PCa aggressiveness at diagnosis, and if this association is modified by race or obesity status.

Methods

Data on primary invasive locoregional prostate cancer cases diagnosed in 2011-2020 were obtained from the Louisiana Tumor Registry. PCa aggressiveness was defined in accordance with the TNM stage and Gleason score. Obesity was defined as BMI of 30 or higher, and type 2 diabetes was identified using ICD-10 codes. Linear trends were assessed with Cochran-Armitage Trend tests and highly aggressive disease at diagnosis was modeled using logistic regression. Analyses were performed overall and stratified by race.

Results

The study included 22,554 cases. The majority were White (62%), while 35% were Black, and 2% were Hispanic. The overall distribution of PCa aggressiveness was low (23%), intermediate (47%), and high (30%). PCa aggressiveness was associated with older age, Black race, obesity, and diabetes ($p<.001$). Stratified by race, the relationship between diabetes and aggressiveness was significant among whites ($p<.001$) and Hispanics ($p=0.037$) but not Blacks ($p=0.398$). In multivariable models controlling for age and obesity, diabetes was significantly associated with high PCa aggressiveness among lean White

(OR=1.40 (1.18,1.66)) and Hispanic (OR = 2.68 (1.13,6.36)) men, but not among lean (OR=1.08 (0.90,1.29)) or obese (OR= 0.94 (0.78,1.12)) Black men.

Conclusion

We found that obesity is associated with an increased risk of aggressive PCa in both Caucasian and Black men, with obesity and diabetes interacting as risk factors in PCa patients. In Louisiana, diabetes was associated with more aggressive disease in lean White and Hispanic men but not in Black men. Future research should explore social and metabolic risk factors across racial and ethnic cohorts, as the risk and the underlying mechanisms may differ.

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Delineating the role of miR-21 in the prostate tumor immune microenvironment

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Abstract

Background: Prostate Cancer (PCa) develops an immune-suppressed tumor microenvironment (TME) that ultimately limits the efficacy of cancer immunotherapy. The molecular underpinnings of this immune suppression, and its relationship with therapeutic resistance, remain incompletely understood. Our laboratory previously reported that an oncogenic microRNA (miRNA), miR-21, is highly expressed in both the stromal and epithelial compartments of human PCa. Here we assessed the role of miR-21 in the tumor microenvironment (TME) using syngeneic murine cancer models and examine how miR-21 influences tumor development, gene expression, and immune cell infiltration.

Methods: The expression of miR-21 in murine prostate tumor tissues was measured by RT-qPCR and in situ hybridization. To study effects of miR-21 in TME, we developed syngeneic tumor models, where miR21$^{+/+}$ MycCaP or NT2.5 cancer cell lines were implanted subcutaneously in Wild Type (WT) or *Mir21a* (the mouse homolog of miR-21) knockout (KO) FVB mice. In both models, we studied tumor growth patterns, and performed bulk RNA sequencing to identify differentially expressed genes. Gene Set Enrichment Analysis (GSEA) was performed to identify differentially enriched pathways. Immunohistochemistry was performed on formalin fixed and paraffin embedded tumor sections to evaluate cell proliferation and death. Immune cell populations were studied in MycCaP tumors using flow cytometry.

Results: The expression of miR-21 was elevated in Hi-Myc prostate tumors, with the highest levels detected in the tumor-associated stroma. Loss of miR-21 from the TME significantly delayed tumor take and attenuated tumor growth in both MycCap and NT2.5 models. Most remarkably, miR-21 KO tumors presented with altered tumor immune microenvironments and cytokine and inflammatory gene expression, as well as significant increase in anti-tumor immune cell populations and activated immune checkpoints.

Conclusions: In light of these discoveries, we demonstrate that elevated miR-21 expression in the prostate TME contributes to a pro-tumorigenic cytokine signaling environment, altered immune cell infiltration and checkpoint activation, and enhanced tumor development and growth. These studies support the potential for miR-21 inhibition as a therapeutic approach for improving PCa response to cancer immune therapy.

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Targeted delivery of AR-V7 siRNA with bivalent PSMA aptamers effectively suppresses the growth of enzalutamide-resistant prostate cancer

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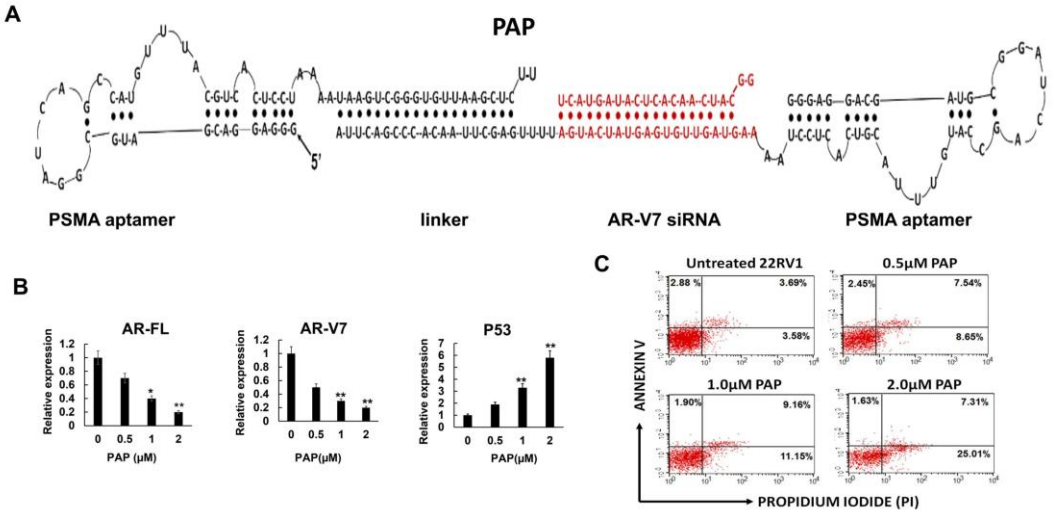
Abstract

Background: Androgen deprivation therapy has been the primary treatment strategy for advanced prostate cancer (PCa). But most patients develop castration resistance over time. For FDA approved next-generation androgen receptor (AR) signaling inhibitors including enzalutamide (ENZ) and abiraterone (AA), patients who initially respond to them eventually develop resistance. The key mechanism for the resistance to ENZ/AA involves AR splicing variants (AR-Vs) and specifically AR-V7. Current AR inhibitors cannot target AR-V7 due to its lack of C-terminal ligand binding domain (LBD) but keeping AR N-terminal domain (NTD) which still can active androgen responsive genes. Therefore, targeting the AR NTD and AR-V7 is critically important to overcome ENZ resistance. Unfortunately, AR NTD has been considered an "undruggable" target due to difficulty in defining its 3-D structure. In this context, siRNA is highly suitable to address this "undruggable" target. However, siRNA cannot freely diffuse into cells and a carrier is needed. In this regard, nucleic acid-based aptamers are highly suitable for cell-type specific delivery of siRNA *in vivo.*

Methods: In this study, we have developed a PSMA aptamer-ARV7 siRNA-PSMA aptamer (PAP) chimera . To increase the overall chimera size, the monovalent PSMA aptamer-ARV7 siRNA chimera was linked to another chimera composed of a PSMA aptamer and a linker (random sequence) of the same structure as a siRNA through hybridization. This structure with one AR-V7 siRNA sandwiched between two PSMA aptamers is termed PAP. The selected AR-V7 siRNA targets the N-terminal of AR. This AR-V7 siRNA has potential to silence both AR-FL, AR-V7, and other NTD containing splicing variants.

Results: The results show PAP can knock down both AR-full length and AR-V7 in PSMA expressing castration resistant cells. It can re-sensitize ENZ in cell lines and PCa xenografts. ENZ combined with PAP can significantly inhibit 22Rv1 xenograft growth in mice without experiencing castration



Conclusion: Owing to the low toxicity, PAP will be a novel RNA-based biologic against AR-V7. PAP has potential to offer a new treatment for current PCa patients who have developed resistance to the next-generation AR signaling inhibitors.

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PRDM16 transcriptionally regulates an intrinsic prostate cancer dormancy program

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Abstract

Background: Prostate cancer (PCa) cells can disseminate to bone and remain dormant for extended periods after primary tumor treatment. Upon awakening, these cells give rise to incurable metastases that greatly contribute to patient mortality. Thus, understanding the molecular mechanisms that govern dormancy could lead to therapeutic interventions that would prevent future metastatic relapse thereby, potentially reducing mortality rates.

Methods: We developed a model of PCa dormancy in multiple PCa cell lines (RM1, 22Rv1, LAPC4). Bulk RNA-Seq analysis was performed on active, dormant, and reawakened PCa cells. We used genetic approaches to validate our findings *in vitro* & *in vivo*.

Results: *In vitro,* experimentally induced dormant PCa cells could remain in this state for long periods (>21 days) and be reactivated when exposed to normal culture conditions. Dormancy was validated via cell cycle analysis, membrane dye retention and ERK/p38 ratio. RNASeq identified 14 common genes upregulated across dormant PCa cell lines. Of these, the transcription factor PRDM16 was the most highly induced. We prioritized PRDM16 given its role in hematopoietic stem cell quiescence and negative correlation with PCa disease specific recurrence in patients. Silencing PRDM16 led to impaired dormancy and increased cell death while forced expression significantly enhanced the ability of PCa cells to enter into a dormant state. *In vivo,* forced expression of PRDM16 resulted in a significant reduction of bone metastasis outbreaks upon intrailiac artery injection and extended survival. Histology revealed significantly more ki67 null PCa solitary cells/clusters (<20 cells) in the PRDM16 over expressing group vs. controls. In defining the program through which PRDM16 controls dormancy entry, bioinformatic analysis of putative transcription factor binding sites revealed several targets including RB1, a known cell cycle inhibitor. We found that PRDM16 expression significantly enhanced RB1 at the transcriptional and protein level. Using ChIP-qPCR, we are validating the specific PRDM16 binding sites on RB1.

Conclusions: Our studies reveal PRDM16 as a novel regulator of PCa dormancy. Mechanistically, we posit that PRDM16 controls dormancy via the induction of genes that inhibit the cell cycle such as RB1. Translationally, inhibiting PRDM16 function could block PCa entry into dormancy thereby rendering the cells susceptible to apoptosis/systemic therapy and ultimately, preventing metastatic relapse.

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Identification of a distinct subgroup exhibiting luminal signatures via single-cell RNA sequencing of prostate stem cell populations

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Abstract

Benign prostate hyperplasia (BPH) is a prevalent and costly condition affecting elderly men. The mechanisms underlying continued prostate growth as men age are not known. Transit-amplifying cells with high proliferative potential are crucial for adult stem cell regeneration. Given their role in tissue expansion, we considered their involvement in BPH conditions. Inflammation commonly associated with BPH, is linked to cellular proliferation, leading us to hypothesize that inflammation within the prostate could induce a transitional state in prostate epithelial cells. This transitional state is characterized by both basal and luminal markers, which may initiate basal-to-luminal differentiation, contributing to continued prostate growth, possibly involving transit-amplifying cells. To address this hypothesis, our laboratory has established an induced prostatitis model, POET3 (Prostate Ovalbumin Expressing Transgenic 3) to study the effects of infiltrating inflammatory cells on the prostate. Pre-activated T cells recognizing ovalbumin peptide were injected into POET3 to induce prostate inflammation. To explore the transcriptomic profiles of stem cell populations associated with inflammation, we performed single-cell RNA sequencing (scRNA-seq) analysis on 26,314 preprocessed prostate stem cells pooled from naïve and inflamed prostates across three independent batches. Our analysis identified 10 distinct clusters, with clusters-2, 3, 4, 6 and 9 expanding in size in the inflamed prostates. Notably, cluster 9 (C9) exhibited a distinct expression pattern of basal (*Krt5*, *Krt14*, *Trp63*) and luminal markers (*Krt8*, *Krt18*, *Nkx3-1*, *Pbsn*). Further examination of differentially expressed genes (DEGs) across these clusters revealed that C9 had high expression of genes related to carcinoma, immune inhibition, cytokine-chemokine signaling, the major histocompatibility complex (MHC class I and II), and luminal progenitor cells. The involvement of C9 in the differentiation process was validated using approaches such as RNA velocity, cellRank and Monocle, confirming that C9 is a subgroup most committed to luminal differentiation. Hence, our findings suggest that C9 could represent a distinct cluster of transit-amplifying epithelial cells present in the inflamed prostates. This analysis provides insight into the impact of inflammatory cells on prostate epithelial differentiation, and this transitional cluster could serve as a novel transitional cell population that contributes to continued prostate growth.

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Exploring Biomarkers in a Phase II Trial of Sacituzumab Govitecan in Advanced Prostate Cancer

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Abstract

Background: Prostate cancer is the second leading cause of cancer related death in men. Next generation antiandrogens have improved survival time for patients with advanced prostate cancer, however many cases inevitably progress to lethal castration resistant prostate cancer (CRPC). Identifying new treatment strategies for CRPC is critical for improving outcomes for patients currently resistant to available therapies. One promising therapeutic target for CRPC is the trophoblastic cell surface antigen (Trop-2). Found to be highly expressed in many solid tumors, Trop-2 has oncogenic activities implicated in CRPC progression and could provide a mechanism to therapeutically target treatment resistant prostate cancer. Sacituzumab Govitecan (IMMU-132) is an anti-Trop-2 antibody drug conjugate (ADC) which provides targeted delivery of the topoisomerase I inhibitor SN-38. Methods: As part of a phase II clinical trial testing the efficacy of Sacituzumab Govitecan in CRPC, we performed integrative whole exome DNA-seq and RNA-seq on metastatic biopsies obtained from 29 patients. Results: We successfully sequenced DNA from 28/29 patients (15 matched screening and on-treatment) and RNA from 26/29 patients (12 matched screening and on-treatment). We characterized the gene expression profiles and mutational landscapes at baseline and on-treatment to evaluate the prognostic value of differentially expressed genes and acquired genetic alterations. Within the on-treatment biopsies, we identified differential gene expression profiles belonging to pathways of cell proliferation, metabolism and DNA repair. Conclusions: These studies provide a framework for interpreting response to Sacituzumab Govitecan that can inform the future development of laboratory models to enhance mechanistic knowledge and pre-clinical studies, including combination treatment regimens.

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GAPDH overactivation promotes treatment-induced neuroendocrinal progression in prostate cancer

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Abstract

Background: AR antagonist-induced neuroendocrine progression in prostate cancers (t-NEPC) [1] is a lethal and aggressive subtype of advanced prostate cancer without means to cure. With the wide use of potent AR antagonists in CRPC patients, t-NEPC has accounted for more than 25-30% mortality of prostate cancers. Recent studies with patient-derived xenografts (PDX) revealed that t-NEPC model LTL-331R exerted a highly elevated glycolytic activity [2], one of the malignant hallmarks featured in metabolic reprogramming [3]. Targeting the altered glycolysis pathway in cancer cells has emerged as a potent cancer therapy [2, 4], including glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a critical glycolytic enzyme [5]. In this report, we sought to determine how AR antagonist promotes glycolysis via GAPDH overactivation in CRPC cells and to test the effect of glycolysis (GAPDH) inhibition on NEPC tumor growth.

Methods: We utilized human NEPC PDX models and prostate cancer cell lines with NE and CRPC features. The effect of AR antagonist Enzalutamide treatment on GAPDH expression at the mRNA and protein levels was determined in castration-resistant prostate cancer cell lines, C4-2B and PC-3. We analyzed the glucose uptake rate in CRPC cell lines after Enzalutamide treatment and a cellular thermal shift assay (CETSA) in C4-2B cells to examine Enzalutamide interaction with GAPDH. We performed a metabolomics analysis on C4-2B cells after Enzalutamide treatment. GAPDH expression was knocked out using the CRISPR/Cas9 plus HDR system. The orthotopic xenograft tumors model was established using the parental and GAPDH-KO C4-2B cell lines in castration male nude mice. PDX LTL331R models were established in male NOD/SCID mice.

Results: Our results indicated that Enzalutamide treatment drastically enhanced GAPDH expression at the mRNA and protein levels in a time-dependent manner (Fig 1). Enzalutamide treatment significantly enhanced glucose uptake rate and consumption rate in all prostate cancer cell lines tested in a time- and concentration-dependent manner (Fig 2). Enzalutamide treatment did not largely alter GAPDH thermal stability in C4-2B cells, similar to the Actin curve, indicating that Enzalutamide is not interacting with GAPDH directly (Fig 3). In contrast, Alternol treatment in PC-3 (panel C) and C4-2B (panel D) cells significantly altered the GAPDH thermal shifting curve, as reported [6]. Metabolomics analysis on C4-2B cells after Enzalutamide treatment showed that Enzalutamide treatment increased the levels of glycolysis intermediates like L-lactic acid and pyruvate (Fig 4). GAPDH Knockout in C4-2B cells severely reduced glucose uptake (Fig 5), resulting in a significant reduction of glucose consumption and L-lactate production. Reinstallation of GAPDH expression in the KO subline cells produced an excellent recovery of glycolytic activity. GAPDH inhibitor Korningic Acid (KA, 1.0 mg/kg, ip.) dramatically inhibited LTL331R PDX tumor growth without affecting animal body weight (Fig 6A-6B). KA treatment also substantially inhibited C4-2B xenograft tumor growth (Fig 6C). Consistently, xenograft tumors derived from C4-

2B/GAPDH-KO cells exhibited a significant growth retardation compared to the parental wild-type cells (Fig 6C).

Conclusion: GAPDH is overactivated by AR antagonist and GAPDH-specific inhibitor Koningic Acid provided an excellent therapeutic effect on NEPC tumor growth.

1. Puca L, Vlachostergios PJ, Beltran H: **Neuroendocrine Differentiation in Prostate Cancer: Emerging Biology, Models, and Therapies**. *Cold Spring Harb Perspect Med* 2019, **9**(2).

2. Choi SYC, Ettinger SL, Lin D, Xue H, Ci X, Nabavi N, Bell RH, Mo F, Gout PW, Fleshner NE *et al*: **Targeting MCT4 to reduce lactic acid secretion and glycolysis for treatment of neuroendocrine prostate cancer**. *Cancer Med* 2018.

3. Fouad YA, Aanei C: **Revisiting the hallmarks of cancer**. *Am J Cancer Res* 2017, **7**(5):1016-1036.

4. Gill KS, Fernandes P, O'Donovan TR, McKenna SL, Doddakula KK, Power DG, Soden DM, Forde PF: **Glycolysis inhibition as a cancer treatment and its role in an anti-tumour immune response**. *Biochim Biophys Acta* 2016, **1866**(1):87-105.

5. Liberti MV, Dai Z, Wardell SE, Baccile JA, Liu X, Gao X, Baldi R, Mehrmohamadi M, Johnson MO, Madhukar NS *et al*: **A Predictive Model for Selective Targeting of the Warburg Effect through GAPDH Inhibition with a Natural Product**. *Cell Metab* 2017, **26**(4):648-659 e648.

6. Li C, He C, Xu Y, Xu H, Tang Y, Chavan H, Duan S, Artigues A, Forrest ML, Krishnamurthy P *et al*: **Alternol eliminates excessive ATP production by disturbing Krebs cycle in prostate cancer**. *Prostate* 2019, **79**(6):628-639.

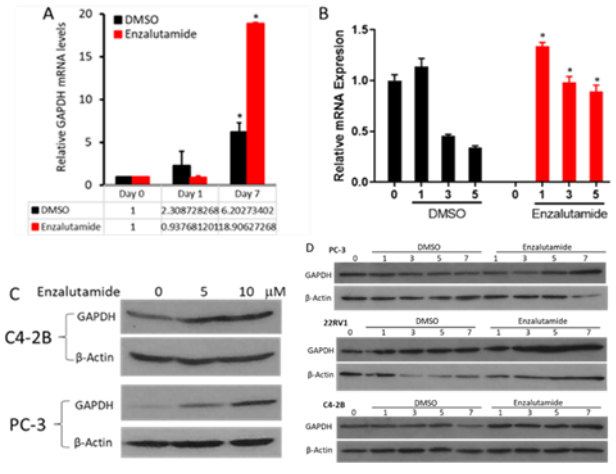

Fig 1. Enzalutamide treatment enhances GAPDH expression. Prostate cancer C4-2B and PC-3 cells were seeded with media containing 10% FBS overnight and then changed to 2% charcoal-stripped FBS with DMSO or Enzalutamide (10 μM). After the period as indicated, cells were harvested for total RNA extraction and qPCR assays were conducted to analyze GAPDH gene expression (panel A & B). Expression levels of the 18S gene were used as the internal control for normalization. Data represent the mean from three independent repeats and the error bar indicates the SEM. The asterisk indicates a significant difference comparing to the DMSO control. For panel C&D, cells were harvested for total protein extraction and western blot assays were conducted to determine GAPDH levels. Actin blots served as a protein loading control.

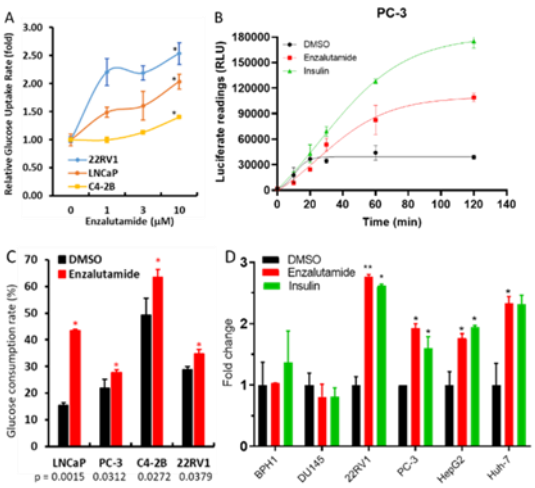

Fig 2. Enzalutamide enhances glucose uptake and consumption. A. Prostate cancer cells, LNCaP, C4-2B, 22RV1, were seeded in 6-well plates overnight and then treated with DMSO or Enzalutamide at indicated concentrations for 4 h in 2% cFBS condition. B. PC-3 cells were treated with DMSO, Enzalutamide (10 μM), or Insulin (10 ng/ml) for the indicated period in 2% cFBS condition. Glucose uptake assay was conducted using the Promega assay kit. C&D. Cells were treated as indicated with DMSO, Enzalutamide (10 μM), or Insulin (10 ng/ml) for 4 h in 2% cFBS condition. Glucose consumption [(before - after)/before x 100%] was determined by comparing glucose levels before vs after treatment with a pre-assembled kit from Signa (catalog #GAGO20). (* $p < 0.05$; ** $p < 0.01$, Student t-test compared to DMSO control). HepG2 and Huh7 cells were used as insulin action control.

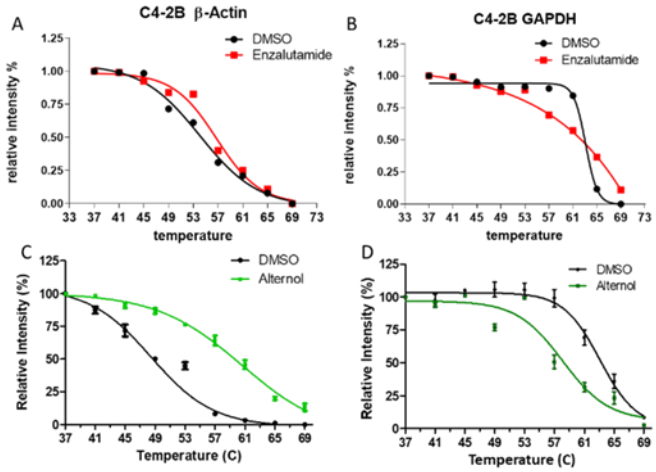

Fig 3. CETSA assay. Cells were treated with DMSO, Enzalutamide (10 μM), or Alternol (10 μM), as indicated and then harvested for CETSA assay. The CETSA assay can determine the interaction of a given compound with its binding protein target in intact cells and tissue, without modifications to the target protein but comparing the measured cellular thermal stability of the protein in the presence and absence of the test compound. Compound engagement with its intended target will affect the thermal stability of its associated proteins in the cell.

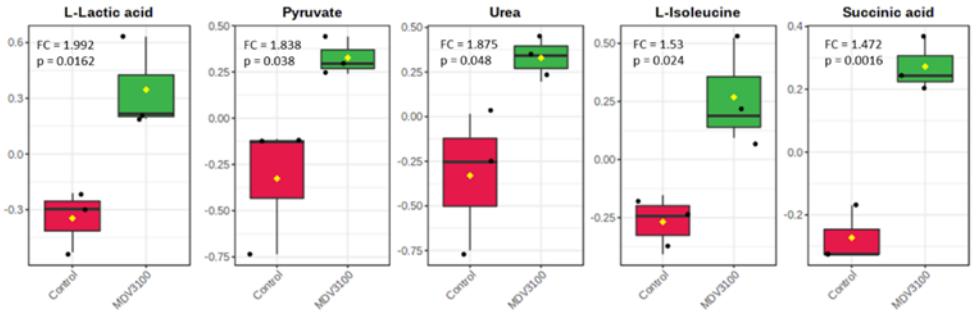

Fig 4. Enzalutamide treatment enhances glycolysis. C4-2B cells were treated with DMSO or Enzalutamide (10 μM) for 3 days and then harvested for metabolomic analysis using a gas chromatography-mass spectrometry (GC-MS) at the University of Utah Metabolomics core facility. Data statistical analysis was performed using Metaboanalsyt 3.0.

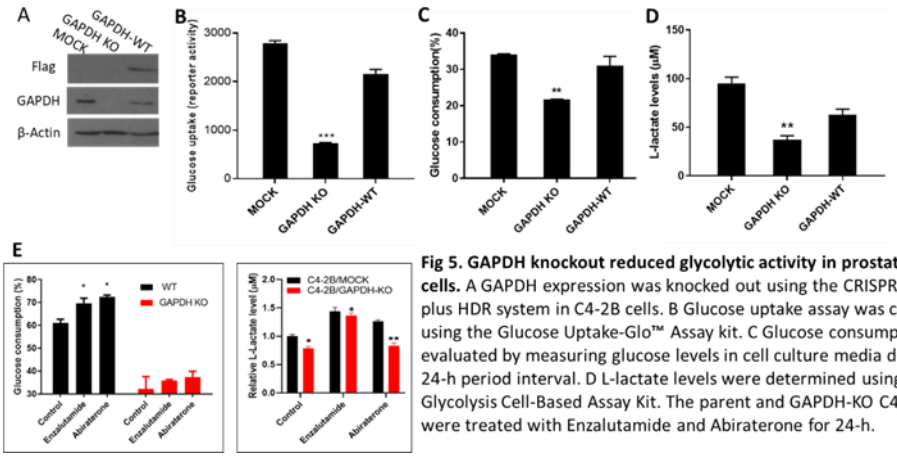

Fig 5. GAPDH knockout reduced glycolytic activity in prostate cancer cells. A GAPDH expression was knocked out using the CRISPR/Cas9 plus HDR system in C4-2B cells. B Glucose uptake assay was conducted using the Glucose Uptake-Glo™ Assay kit. C Glucose consumption was evaluated by measuring glucose levels in cell culture media during a 24-h period interval. D L-lactate levels were determined using the Glycolysis Cell-Based Assay Kit. The parent and GAPDH-KO C4-2B cells were treated with Enzalutamide and Abiraterone for 24-h.

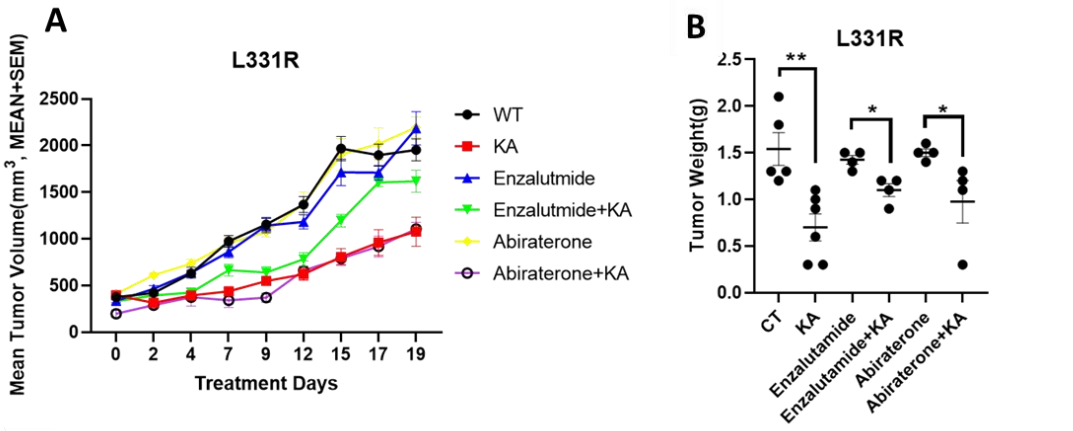

Fig 6. GAPDH inhibition reduces NEPC xenograft tumor growth in vivo. A&B NEPC PDX L331R tumors were treated with the drugs as listed for 3 weeks. C. GAPDH-KO C4-2B cells were used for establishing orthotopic xenograft tumors in nude mice. C4-2B/GAPDH-KO models received no treatment.

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The Role of GATA2 and TWIST1 in Prostate Cancer Disrupted Differentiation

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Abstract

Background: Prostate cancer is hypothesized to arise from a defect in the differentiation process. A healthy prostate gland consists of a bilayer of epithelial luminal and basal cells. These cell types express unique cell-specific markers that allow us to identify them. In prostate development, we observe basal cells able to differentiate to luminal cells which is why a bilayer of epithelial cells is observed in prostate glands. However, in a prostate tumor the cells lose the bilayer and form a monolayer of tumor cells that express both luminal and basal markers. This suggests a defect in the differentiation process. Previous research in our laboratory demonstrated that the transcription factor, CREB1, is aberrantly activated in prostate cancer cells and blocks differentiation. RNA sequencing results identified GATA2 and TWIST1 as possible CREB1 target genes in prostate cancer. Both genes have been shown to be elevated in human prostate cancers, but their role in preventing differentiation or driving prostate cancer oncogenesis is unclear.

Approach: We used ChIP assays to measure CREB1 binding to GATA2 and TWIST1 promoters. We constructed doxycycline inducible cell lines or used shRNA adenovirus to knockdown GATA2 and TWIST1 to see if this rescues differentiation and reverses tumorigenicity. We used IF, migration, and invasion assays to measure tumorigenic and differentiation states.

Results: ChIP assay results demonstrate that CREB1 can possibly regulate GATA2 and TWIST1 by binding to the CREB1 binding site, CRE, within their promoters. We were able to knock-down GATA2 and TWIST1 as validated by immunoblotting. We are testing differentiation, migration and invasion in the knock-down cells relative to control cells.

Conclusions: Our results from the proposed study will provide insight into potential drivers that disrupt differentiation and promote tumorigenesis in prostate cancer.

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Regulation of WNT Signaling in Prostate Stromal Cells: Impact of Androgen on Gene Expression and Implications for Prostate Development and Disease

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Abstract

Background: WNT signaling regulates prostate tissue homeostasis, influencing cell proliferation, differentiation, and function. Dysregulation of WNT signaling has been implicated in the development of prostate cancer and benign prostatic hyperplasia. Despite its importance, the specific role of stromal WNT signaling in prostate development and disease remains poorly understood. Investigating how WNT signaling in the prostate stroma affects epithelial cells and interacts with other signaling pathways is essential for a comprehensive understanding of its role in prostate pathology and for identifying potential therapeutic targets.

Approach: To explore the role of WNT signaling in the prostate stroma, we examined the expression of various WNT ligands and inhibitors in cultured prostate stromal cells treated with R1881, a synthetic androgen analog. Gene expression levels were quantified using quantitative PCR (qPCR), and protein expression was assessed through Western blot analysis. We performed statistical analyses to evaluate relative expression changes and determine the significance of our findings.

Results: Our analysis revealed distinct patterns of WNT expression in fibroblasts and smooth muscle cells within the prostate stroma. Notably, WNT7B expression was significantly upregulated in smooth muscle cells following treatment with R1881. These results suggest that androgen signaling can modulate WNT expression in the prostate stroma, potentially influencing stromal-epithelial interactions.

Conclusions: The observed changes in WNT expression in response to androgen treatment suggest a dynamic role for WNT pathways in regulating prostate tissue development and differentiation. These findings provide a foundation for further research into how WNT signaling contributes to prostate disease and its potential as a target for therapeutic intervention.

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Mechanical Regulation of Prostate Differentiation and Tumorigenesis

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Abstract

Background: Recent research has implicated matrix stiffness in tumor aggressiveness; however, the role of matrix stiffness in prostate oncogenesis is unclear. Notch signaling has been implicated in prostate cancer progression; however, the mechanism of Notch mediated tumor progression is poorly understood. Notch is a family of four mechanical responsive proteins which function in regulating proliferation, differentiation, survival, apoptosis, features that are critical to cancer cell survival. Previously, we have reported that NOTCH3 is required for prostate cell differentiation to form a suprabasal layer and to differentiate into luminal epithelial cells. Given the mechanical properties of Notch proteins, we explored the role of matrix stiffness in PrEC cell differentiation and it's dysregulation in prostate oncogenesis.

Methods: We cultured normal and transformed PrECs (EMP: ERG, Myc, shPTEN) on **soft** (0.5kPa) and **stiff** (35kPa) matrix poly-acrylamide hydrogels and induced differentiation for 7 and 14 days. Concurrently, PrECs and EMP cells were subjected to NOTCH3 knockdown or overexpression of NOTCH3 intercellular domain (NICD3) and cultured on **so**/**st** matrix. Cells were either collected for RNA/qPCR or fixed in 4% PFA and immunostained for differentiation markers (CK5/14, 8/18, TP63, TMPRSS2) and for adhesion markers (Paxillin and Actin).

Results: Our data show that in normal PrECs, NOTCH3 expression increased 3.6-fold leading to a 6.5-fold increase in differentiation markers on soft relative to stiff matrix. This differentiation phenotype was diminished or not present in the tumorigenic EMP cells. Normal PrECs exhibit sensitivity to mechanical stimuli with increased protrusions and spreading on stiff relative to soft matrices. However, in the tumorigenic EMP cells this phenotype is absent. EMP cells exhibit increased mechanical responses as indicated by protrusion and spreading on soft matrix relative to normal PrECs. PrECs with NOTCH3 knockdown showed decreased differentiation on soft matrix relative to normal, whereas cells overexpressing NICD3 showed increased differentiation status, even on stiff matrix.

Conclusion: Our results highlight the dysregulation in mechanical responses in prostate tumor cells. This model can be used to determine how oncogene events lead to this dysregulation of mechanobiology in prostate cancer initiation and progression.

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Bone Stromal Cell Matrix Enhances Osteoblast Differentiation

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Abstract

Background: Prostate cancer, the second most common cancer among men, has an increased 5-year mortality rate dropping from 90% in locally advanced cancers to 30% once it has metastasized. Overwhelmingly, prostate cancer metastasis to bone, but the reasons and mechanisms behind this are poorly understood. To combat this issue, the development of a Prostate-Bone metastasis model is needed to examine the signaling that occurs between the two organs to promote bone metastasis. Previously, our lab has developed a Prostate-on-a-Chip (PoC) model which we aim to attach to a Bone-on-a-Chip (BoC) model, allowing for the examination of signaling, cell migration and the interactions between the bone and prostate that drive prostate cancer metastasis. Bone itself is a living, metabolically active organ whose 3-dimensional structure is primarily determined by the interactions of osteoblasts, bone building cells, and osteoclasts, bone resorbing cells. Our first goal is to test the feasibility of establishing long term cultures of osteoblasts by differentiating human mesenchymal stem cells (hMSC) on a human bone-specific matrix.

Methods: Human bone-derived stromal cells, HS-5 secrete an extracellular matrix rich in collagens specifically seen in bone. HS-5 cells were plated onto plastic plates and allowed to reach confluency and secrete their matrix onto the plate. HS-5 cells were then removed, leaving their matrix behind. hMSC's were then plated on top of this remaining matrix or placed on plastic. The hMSC's were treated with dexamethasone and Vitamin C so they would differentiate into osteoblasts. qPCR, Alizarin Red, and Alkaline Phosphatase staining was performed to analyze differentiation at different times post induction of differentiation.

Results: Plating hMSCs on bone stromal cell matrix resulted in more extensive osteoblast differentiation compared to cells on plastic based on qPCR and cell staining. A greater induction of classical osteoblast gene markers including ALP, BSP, OCN, OPN and OSX was observed at earlier times on matrix than on plastic. Alizarin Red Staining illustrated extensive calcium ion deposition within the matrix that was secreted by the osteoblasts. Plating on plastic leads to reduced staining and eventual loss of adhesion of the osteoblasts from the plate.

Conclusion: These data demonstrate the importance of including a bone matrix to support Osteoblast differentiation for long term culture. These studies have identified ideal conditions for generating a bone-on-chip model for future prostate cancer bone metastasis modeling.

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CHD1 Loss Fuel SPOP-deficient Prostate Cancer via Reprogramming Lipid Metabolism

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Abstract

Background: Chromatin remodeling proteins contribute to DNA replication, transcription, repair, and recombination. The chromodomain helicase DNA-binding (CHD) family of remodelers plays crucial roles in embryonic development, neurogenesis, and carcinogenesis. As the founding member, CHD1 is capable of assembling nucleosomes, remodeling chromatin structure, and regulating gene transcription. Dysregulation of CHD1 at genetic, epigenetic, and post-translational levels is common in prostate cancer and other malignancies. Through interacting with different genetic alterations, CHD1 possesses the capabilities to exert oncogenic or tumor-suppressive functions in context-dependent manners. Cancer genome studies have uncovered a unique subtype of prostate cancer (PCa) characterized by speckle-type BTB/POZ protein (SPOP) mutations, which stabilize AR and its co-activators and have been associated with prolonged responses to androgen deprivation therapies and favorable prognoses. SPOP mutations frequently co-occur with CHD1 loss, however, how concurrent CHD1 loss impacts SPOP-mutated PCa remains unclear.

Methods: We generated a new genetically engineered mouse (GEM) model containing CHD1 deletion and SPOP mutation to recapitulate this emerging molecular subtype of PCa. Then, we tested the impact of these genetic alterations on prostate tumorigenesis and response to castration using MRI imaging. For mechanistic studies, we performed single-cell transcriptomic, lipidomic, epigenetic, and proteomic profiling in the newly developed GEM models.

Results: We provided genetic evidence supporting that CHD1 loss accelerates SPOP-mutated PCa progression and confers resistance to castration in this context. We uncover a non-canonical epigenetic function of CHD1 and demonstrate that CHD1 reprograms lipid metabolism by governing SREBPs. CHD1 loss–induced lipid reprogramming supplies intratumoral androgen biosynthesis and retains AR transcriptional activity in SPOP-mutated prostate tumors upon castration. Furthermore, the mechanistic studies facilitate the development of a novel biomarker-driven combinatorial therapy for CRPC harboring CHD1 loss and SPOP mutations.

Conclusions: These findings advance our understanding of an emerging PCa subtype and mechanism of action and provide novel therapeutic strategies for men with CRPC.

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Interferon-gamma signaling represses FOXA1 and drives a basal transcriptional program in muscle-invasive bladder cancer (MIBC)

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Abstract

Background: Luminal muscle-invasive bladder cancer (MIBC) can change into the more aggressive, basal-squamous (Ba/Sq) molecular subtype during disease progression. Previous studies revealed that the reduced expression of forkhead box A1 (*FOXA1*) is a hallmark and driver of the Ba/Sq transcriptional program and squamous differentiation (SqD). Ba/Sq MIBCs are highly inflamed. However, the specific inflammatory pathways contributing to the Ba/Sq program remain unclear.

Methods and Results: In this study, we performed transcriptomic analyses of The Cancer Genome Atlas MIBC cohort to determine whether immune response gene signatures are associated with MIBC molecular states. The results revealed that the interferon-gamma (IFNγ)-dominant signature is observed in Ba/Sq MIBCs, which increased IFNγ/JAK/STAT pathway activity and reduced FOXA1 regulon activity. Next, immunohistochemistry analysis of human specimens demonstrated that JAK1 expression was significantly increased in tumor areas with SqD.

IFNγ treatment of luminal MIBC cell lines (SW780, RT112, and UMUC1) significantly downregulated expression of luminal transcriptional drivers, including *FOXA1*, while considerably increasing expression of Ba/Sq markers. RNA sequencing analyses of these samples identified IFNγ as a driver of the Ba/Sq program. The ability of IFNγ to repress *FOXA1* in luminal MIBC cells was blocked by Ruxolitinib (JAK1/2 inhibitor). Additionally, pharmacological inhibition or siRNA targeting for JAK1 restored *FOXA1* expression in the Ba/Sq SCaBER cell line.

Conclusions: Taken together, these findings are the first to identify IFNγ as a critical extrinsic factor to repress *FOXA1* and drive the Ba/Sq state in MIBC.

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Aneuploidy-associated SQLE gain promotes prostate cancer aggressiveness by altering lipid metabolism

Ms. Chloe Springer BS[1,2], Mr. Thomas Janas BS[1,2], Ms. Elise DeArment MS[1,2], Dr. Konrad Stopsack MD[3,4], Dr. Daniel Schmidt MD[5,6,7], Dr Duanduan Ma PhD[5,8], Dr Zhe Li PhD[7,9], Dr. Matthew Vander Heiden MD/PhD[5,10,11], Dr Kathryn Penny PhD[3,7,9], Dr Paul Scheet PhD[12], Dr Tamara Lotan PhD[13], Dr Ayesha Shafi PhD[1,2], Dr Angelika Amon PhD[5,11], Dr Lorelei Mucci PhD[5], Dr Xiaofeng Su PhD[1,2,5,11,14]

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Abstract

Background:

Prostate cancer (PCa) remains the second leading cause of cancer-related mortality among men in the United States. Aneuploidy, characterized by imbalanced chromosome numbers, correlates with lethal PCa progression. Chromosome 8q (chr8q) gain is one of the most frequent aneuploidy events, occurring in 23% of PCa cases. Recently, we ranked odds ratio (OR) for each chr8q gene to assess the long-term risk of metastases and death from PCa (lethal disease) within 403 patients in the HPFS and PHS cohorts. We observed that the squalene monooxygenase (SQLE), is one of the top ranked genes on chr8q, with an OR of 2.2. SQLE, encoding the key enzyme in cholesterol biosynthesis pathways, has been shown to be associated with poor prognosis in PCa, suggesting it as a potential target to treat aggressive PCa.

Methods:

Datasets such as The Cancer Genome Atlas (TCGA) and the Prostate Cancer Atlas were used to correlate the copy number and mRNA expression levels and the progression of PCa. We focused on mouse prostate organoids (both normal and cancer-like) and human metastatic prostate cell line, VCaP cell, expressing TMPRSS2-ERG gene, which approximately 50% of PCa cases harbors. We used lentiviral transduction to introduce either SQLE overexpression in prostate organoids (which harbor euploid SQLE) or SQLE shRNA in VCaP cells (which harbor increased SQLE). Molecular and cellular approaches were employed to study the influences of altered SQLE levels on target prostate cells. Ultra performance liquid chromatography (UPLC) with mass spectrometry (MS) was used to analyze the changed levels of squalene and lipid profiles when SQLE levels were altered.

Results:

Overexpression of SQLE in cancer-like mouse prostate organoid models led to increased invasive structures and proliferation. RNA sequencing data suggested that alteration in SQLE levels changes gene signatures related to lipid metabolisms. Interestingly, SQLE overexpression decreased Tmprss2-ERG protein levels in the organoids. In contrast, SQLE knockdown in VCaP cells resulted in significantly increased TMPRSS2-ERG protein levels. ERG is associated with fatty acid metabolism in PCa cohorts. Using UPLC-MS, we found that SQLE knockdown in VCaP cells significantly decreased squalene levels and changed lipid composition and heterogeneity.

Conclusion:

Our findings suggest that aneuploidy-associated overexpression of SQLE drives PCa aggressiveness by modulating lipid metabolism, particularly through its impact on cholesterol biosynthesis and TMPRSS2-ERG regulation. Targeting SQLE could present a novel therapeutic approach to treat aggressive PCa..

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Elucidating a Role for Bacterial in Calcium Oxalate Stone Formation through High-Resolution Imaging

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Abstract

BACKGROUND: Understanding the microbiological factors contributing to calcium kidney stone formation is vital for prevention. While it is well known that urease-producing bacteria contribute to struvite stone growth, it is less clear how bacteria may contribute to the development of other types of stones. Bacteria such as E. coli and P. aeruginosa have been detected in patient-derived calcium oxalate (CaOx) stones even in the absence of infection. Prior studies investigating the effect of bacteria on CaOx crystal growth have utilized pre-crystallized chunks, which are less likely to resemble physiological stone growth. Our study aims to elucidate the impact of bacteria on CaOx stone formation, through high-resolution imaging of patient-derived kidney stones and de novo CaOx crystals.

METHODS: Patient-derived kidney stones were obtained and evaluated for intrastructural bacteria using Scanning Electron Microscopy (SEM) and two-photon confocal microscopy. In separate experiments, CaOx crystals were grown in vitro, using a novel method of de novo crystallization in a nonstatic in vitro environment that mimics the flow of urine. CaOx crystals were grown in the presence of *E. Coli* and *P. aeruginosa* with growth monitored for 3 and 10 days, respectively as compared to controls. RAMAN spectroscopy confirmed the chemical composition of crystals. Confocal and SEM assessed crystal (1) size, (2) morphology, and (3) formation kinetics. SEM with energy dispersive spectroscopy was used to analyze crystal morphology and aggregation. Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) provided high resolution 3D crystal structure visualization.

RESULTS: Bacteria and biofilm were identified and confirmed within CaOx kidney stones. SEM showed the morphology of bacteria-influenced crystals significantly differs from controls and revealed rapid clustered aggregation of *E. Coli* -mediated crystals. Evaluation of formation kinetics revealed *P. aeruginosa* mediated up to 280% increase in CaOx crystal size over 10 days, with 250% growth rate versus controls. Further analysis with FIB-SEM revealed larger crystals with hollow internal structure versus solid controls.

CONCLUSIONS: Biofilm-forming bacteria were confirmed present within kidney stone layers, suggesting a role for biomineralization in kidney stone development. Complementary analytical methods demonstrate that biofilm-forming bacteria alter the morphology of CaOx crystals in an in vitro model. These morphological changes may promote crystal aggregation leading to accelerated stone growth. Collectively, these findings give more explicit insight into the mechanistic link between bacterial biofilm formation and CaOx nephrolithiasis.

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Identifying and targeting novel kinase regulators for cancer metastasis

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Abstract

Background: Metastasis is responsible for >90% of cancer death. However, metastasis is still poorly understood and the current approaches to prevent or treat human metastatic cancers are largely unsuccessful.

Methods and Results: Through RNAi and cDNA functional screening, genomics analysis, and functional validations, we have identified several critical but previously unknown/understudied kinase regulators for metastasis. As an example, shRNA screening revealed that GPCR-kinase 3 (GRK3) is essential preferentially for highly metastatic cancer cells as compared to lowly metastatic cancer cells. GRK3 is significantly overexpressed in metastatic prostate tumors from patients, especially in the aggressive variant of prostate cancers, so called treatment-related neuroendocrine prostate cancer (NEPC). We further found that GRK3 is a key missing link between two prominent phenotypes of NEPC, i.e. angiogenesis and neuroendocrine feature. Mechanistically, GRK3 phosphorylates histone deacetylase 2 (HDAC2) at S394 and enhances HDAC2's epigenetic activity. Through compound library screening, we have identified several compounds that block the kinase activity of GRK3 much more potently than that of GRK2, the closest-related kinase to GRK3. Of note, our GRK3 inhibitors could significantly inhibit NEPC cell growth in culture and in mouse xenografts.

Another group of potential metastasis regulators we identified are new EMT-regulating kinases from our kinome cDNA screening for epithelial-mesenchymal transition (EMT). For example, mixed lineage kinase ZAK positively regulates EMT phenotypes and migration of cancer cells in culture, as well as metastasis in mice. ZAK overexpression correlates with poor prognosis in cancer patients. We are carrying out in silicon docking analysis and chemical genomics to identify potent and specific inhibitors for ZAK as research tools and drug candidates for metastatic cancers.

Conclusions: Our robust and unbiased functional screening has identified several novel kinase regulators and potential drug targets for cancer metastasis. For example, GRK3 has emerged as a critical kinase for metastatic prostate cancer cells and a key regulator of aggressive neuroendocrine prostate cancer phenotypes, while ZAK has been identified as a potent new regulator of epithelial-to-mesenchymal transition (EMT).

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Iron in Tumor microenvironment Regulates Prostate Cancer Proliferation

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Abstract

Background:

Tumor-associated macrophages (TAMs) are well-known as anti-inflammatory immune cells that contribute to various facets of prostate cancer development. An emerging aspect of TAMs is their "iron-releasing" phenotype which provides iron to promote cancer progression. However, the key role of TAMs in regulating iron balance within the prostate tumor microenvironment (TME) remains unexplored.

Methods:

We induced prostate tumorigenesis in the ***NP*** mice using Tamoxifen containing diet. The NP mice (*Nkx3.1*$^{CreERT2/+}$; *Pten*$^{flox/flox}$; *Rosa26-CAG-LSL-EYFP/+* mice) develop prostate intraepithelial neoplasia and localized prostate adenocarcinoma by 12 months of age. We then stained prostate tissues using different immune cell markers and Perls's method which stains "non-heme" iron . Additionally, live imaging was employed to assess the impact of iron on the proliferation of various prostate cancer cell lines.

Results:

Prostate tissues from two distinct transgenic prostate cancer models, spanning different developmental stages (12 and 18 months) exhibited high level of non-heme iron in stromal regions compared with non-tumor bearing controls. The areas with high iron abundance exhibited high expression of F4/80 and the iron exporter Ferroportin-1**,** also known as solute carrier family 40 member 1 (SLC40A1). We then investigated the effects of the anticipated released iron on proliferation of a range of cancer cell lines. We then found that the ability of iron to regulate prostate cancer cell proliferation is concentration-dependent.

Summary:

The availability of non-heme iron correlates with the abundance of TAMs in prostate tumor microenvironment. Regulating iron level is crucial to support rapid proliferation and survival of prostate cancer. Understanding how TAMs are regulating iron availability in the dynamic tumor environment is key for developing targeted therapies.