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Meeting report of the 2023 Society for Basic Urologic Research (SBUR) annual meeting: advances in basic urological research-cellular diversity and crosstalk

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The Society for Basic Urologic Research (SBUR) 2023 Annual Meeting convened on November 9-12, 2023, at the Sheraton Gunter Hotel at San Antonio, Texas. The meeting was officially kicked off with the keynote Leland W.K. Chung lecture delivered by Dr. Owen Witte from the University of California at Los Angeles followed by six plenary sessions focused on: 1) New models and technologies for urologic research; 2) Cellular heterogeneity and interaction in urological tissues; 3) Genetic and epigenetic alterations of epithelia in urologic pathologies; 4) Stromal microenvironment of urologic pathologies; 5) Immunoregulation of urological diseases; 6) Confounding factors in urologic pathologies. Dr. Aria Olumi from the Beth Israel Deaconess Medical Center delivered this year's American Urological Association (AUA) lecture. There were 225 registrants from 4 countries that participated in the meeting.

The keynote Leland W.K. Chung lecturer Dr. Owen Witte was introduced by Dr. Li Xin from the University of Washington at Seattle. Dr. Witte described the studies in his laboratory trying to understand how neuroendocrine prostate cancer evolves. Investigators in Dr. Witte's laboratory isolated human prostate basal epithelial cells using flow cytometry and infected the cells using lentiviruses that mediate five different oncogenic signaling: overexpression of AKT1, MYC, and BCL2 and suppression of TP53 and RB1. The infected basal cells were able to form tumorous tissues containing cells that display a neuroendocrine phenotype. The lung epithelial cells transduced with the same set of the oncogenic events also underwent neuroendocrine differentiation, demonstrating a generalized cellular mechanism of lineage plasticity. By RNA-seq and proteomics analyses, the Witte lab and collaborators identified proteins that are highly expressed in the neuroendocrine tumors that are crucial drivers of neuroendocrine differentiation, potential diagnostic markers, and therapeutic targets. Single cell analyses of the tumors formed from the transduced basal cells at different stages revealed a bifurcating developmental trajectory of the neuroendocrine tumors that express ASCL1 and ASCL2, respectively. Metabolic analysis of the neuroendocrine tumors show that mitochondrial activity of the tumors was significantly elevated, which is consistent with upregulation of PGC-1α, a master regulator of energy metabolism.

Dr. Witte also described highly collaborative studies to identify T cell receptors (TCRs) with reactivity for the antigen prostatic acid phosphatase (PAP) which is expressed highly in prostate cancer with minimal normal tissue expression except in the prostate. A secreted major histocompatibility complex (MHC) reagent combined with mass spectrometry was employed to identify epitopes of PAP presented on HLA-A*02. This information was then applied to a series of novel technologies including hydrogel nanovials (decorated with peptide-MHC I complexes and reporters of T cell activation signals) to isolate reactive T cells from healthy donor peripheral blood mononuclear cells, which were isolated and sequenced to determine their TCR sequences. A subset of TCR clones were found to induce antigen-specific T cell activation in functional assays. However, cytotoxic killing of PAP-expressing target cells was limited. To overcome this, the complementarity determining regions of a leading TCR were mutated to enhance "catch bonds" which substantially increased cytotoxic killing. Additional studies are ongoing to identify a candidate for clinical translation. Dr. Witte also described collaborative work leveraging high-throughput sequencing including long-read sequencing to identify alternatively spliced neoantigens that are specifically enriched in aggressive neuroendocrine carcinomas of the prostate and lung. The TCR discovery platform (used for PAP) has also been applied here with the preliminary identification of TCR clones reactive to these neoantigens that could potentially expand the reach of TCR T cell therapy to these deadly tumors.

The first plenary session discussed new models and technologies for urological research. Drs. Travis Jerde from Indiana University and Rosalyn Adams from Harvard University were the discussion leaders for this plenary session. Dr. Kenny Roman from University of Pittsburgh presented data that established a link between activation of the mTOR pathway and changes to neurobiological and neuroinflammatory systems in relevant cortices during the transition from acute to chronic pelvic pain in a novel experimental model of autoimmune prostatitis (EAP) and chronic pelvic pain syndrome (CPPS). Dr. Nicholas Reder from the Alpenglow Inc. at Seattle discussed the application of a novel open-top light-sheet (OTLS) microscope. All optical components in the system are positioned below the glass-plate sample holder, which allows the microscope to accommodate specimens of arbitrary size and thickness without physically interfering with the illumination and collection optics. Next, Dr. Reder presented successful applications of this novel system in visualizing rare cell populations in mouse prostate and complicated neuronal network in mouse brain etc. Dr. Qianben Wang from Duke University described a chemically engineered lipid nanoparticle (LNP) system encapsulating CRISPR-Cas13d that is developed to specifically target lung protease cathepsin L (Ctsl) messenger RNA to block SARS-CoV-2 infection in mice. The nanosystem decreases lung Ctsl expression in normal mice efficiently and safely and extends survival of mice lethally infected with SARS-CoV-2. By developing a novel LNP enabling potent delivery of CRISPR-Cas13d mRNA, and further modifying its surface with an E3 aptamer that selectively recognizes prostate cancer cells, Dr. Wang effectively, specifically, and safely knocks down the expression of HOXB13 and MYCN genes in prostate cancer cells that metastasize to the liver and/or lungs in mouse models. This gene therapy approach helps suppress prostate cancer metastasis and extends the survival of the mice. Dr. Meghan Behringer from Vanderbilt University described a coculture system of E. Coli and Lactobacillus to screen for genetic mutations that suppress the biofilm formation of E. Coli, which may inspire novel strategies for treating urinary tract infections (UTIs). In the end, Dr. Antonina Mitrofanova from Rutgers University described a novel mechanism-centric network-based algorithm by combining transcriptional regulatory modeling and pathway-based modeling. Using the algorithms to analyze the SU2C East coast patient cohort, Dr. Mitrofanova discovered that both Nucleoside diphosphate kinase B (NME2) and MYC are upregulated in prostate adenocarcinoma cells in patients at risk of enzalutamide resistance and identified NME2 as a new upstream regulator of MYC and a valuable candidate for future clinical trials. Immediately following this session, Dr. Christine Riordan, Director of Research at the AUA, provided an update on the education, diversity, advocacy, and funding programs at the AUA.

The second plenary session focused on cellular heterogeneity and interaction in urological tissues. Drs. Cindy Miranti from Arizona State University and Donald Vander Griend from

the University of Illinois were the discussion leaders for this plenary session. This session was kicked off by Dr. Douglas Strand from the University of Texas Southwestern Medical Center, who described the effort of the lab and collaborators in characterizing the cell lineages in normal urological organs including the prostate, urethra, and bladder as well those in BPH patients using different platforms from 10x and Nanostring. One of the surprising observations is that gene expression profiles of stromal cell components in normal transition zone tissue, glandular nodules, and stromal nodules are not distinctively different from each other. With the markers for individual lineages defined from the single cell analyses, Dr. Chad Vezina in the University of Wisconsin at Madison is generating Split-Cre systems that only activate in cells that simultaneously express two antigens, which will target cell lineages in urological organs more specifically. Next, Dr. Cathy Mendelsohn from Columbia University discussed her research showing that Pparg promotes urothelial/luminal differentiation and suppresses basal/squamous differentiation. Dr. Mendelsohn also discussed the efficacy of a 2-drug treatment consisting of rosiglitazone, a synthetic Pparg agonist, and trametinib, a MEK/ERK inhibitor. Either drug alone can restrict tumor growth in a mouse model of basal/squamous muscle invasive bladder cancer. In contrast the 2-drug combination promotes luminal differentiation and induces tumor cell death within 7 days of treatment. Pparg regulates urothelial differentiation partly by up-regulating retinoid signaling. This is partly through Kdm6a independent of its demethylase activity. Next, Dr. Zijie Sun from City of Hope described his research in understanding how prostatic stromal androgen signaling affects prostate development and cancer by knocking out the androgen receptor in prostate stromal cells using a Shh-Cre model. Loss of AR in prostatic stromal cells resulted in smaller prostates in the wild type of background and suppressed tumor growth in the Hi-Myc model of prostate cancer. The suppressive effect is mediated by an increased expression of insulin-like growth factor binding protein 3 (IGFBP3), which impairs IGF1-induced Wnt signaling activation in prostate basal epithelial cells. In the end, Dr. Lori O'Brien from the University of North Carolina described the neuronal network in developing kidneys using light-sheet microscopy. Developing kidney is innervated by both sympathetic and sensory neurons that follow the vasculature to reach nephrons. Genetic ablation of the NGF receptor TrkA causes kidney innervation, which affects both nephron numbers and morphology. Further studies are necessary to dissect the functions of different types of neurons and whether the neuron affects nephrons directly or indirectly by blood flow.

The third plenary session focused on genetic and epigenetic alterations of epithelia in urological pathologies. Drs. Tanya Stoyanova from the University of California at Los Angeles and Xiaoqi Liu from University of Kentucky were the discussion leaders for this plenary session. This session was kicked off by Dr. Chris Barbieri from Weill Cornell Medicine. Dr. Barbieri emphasized the notion that the androgen responding element (ARE) in normal prostate cells are tumor suppressive regulatory elements because AR occupancy at ARE is decreased in primary tumors. Dr. Barbieri described the novel Modifiers of ARE containing Chromatin (MACC). MACCs are fusion proteins of the AR DNA binding domain and VP64 or KRAB that can be driven to nuclear by tamoxifen to activate or suppress ARE regulated genes. Because of the absence of the AR N-terminal domain (NTD), binding of MACC to ARE is free of regulation by the interaction between NTD and other transcriptional modulators. Inactivation of ARE enriched enhancers by the repressive MACC disrupted normal prostate cells in the in vitro organoid assay. In contrast, AREs are tumor suppressive regulatory elements. Reactivating of "normal-like" program via ARE activation suppressed tumor growth in vitro and in vivo. These results imply that AREs are a tumor suppressive regulatory element that is dispensable for tumorigenesis. Next, Dr. Jun Luo from Johns Hopkins University discussed germline genetic risk factors that modulate AR signaling. Specifically, HOXB13 (X285K) is significantly enriched in self-reported black patients, and X285K carriers detected in clinics have aggressive prostate cancer features like the BRAC2 carriers. Functionally, the X285K mutant gained the capability to upregulate E2F/Myc signaling. Other HOXB13 variants may have similar gain of function features. Dr. Luo also discussed an ongoing international collaboration with Jamaica to collect information and specimens from patients with other germline mutations. Dr. Michael Freeman from Cedar Sinai Medical

Center discussed the function of ONECUT2 in prostate cancer progression. Published studies from the Freeman group demonstrated that ONECUT2 is a master transcription factor that drives AR-indifferent prostate cancer and serves as a critical node in a circuit that controls multiple prostate cancer phenotypes. A novel family of small molecules has been developed by the Freeman group to target ONECUT2. An interesting feature of ONECUT2 is its extremely long 3'UTR that functions as a miRNA sponge. Surprisingly, the ONECUT2 3'UTR has a cooperative function independent of the protein coding region to regulate gene expression. Next, Dr. Himisha Beltran from Dana Farber Cancer Institute discussed the application of HPN-328 for the treatment of neuroendocrine prostate cancer. DLL3 is often upregulated in NEPC. HPN-328 is designed to simultaneously engage DLL3 on tumor cells and CD3 on a T cell resulting in T cell activation, proliferation, and the eventual lysis of the target cancer cells. Preliminary response data for patients is encouraging, with a 54% overall response rate across all neuroendocrine (NE) tumor types. Dr. Beltran also discussed the role of Notch signaling in neuroendocrine diseases. Knocking down the Notch 2 intracellular domain (NICD2) is insufficient to drive NE differentiation but overexpressing NICD2 attenuates NE phenotype.

The fourth plenary session discussed the stromal microenvironment of urological pathologies. Drs. Christina Jamieson from the University of California at San Diego and Ping Mu from University of Texas Southwestern Medical Center were discussion leaders for this plenary session. Dr. Xiaohong Li from University of Toledo discussed the impact of the bone microenvironment on prostate cancer dormancy, metastasis, and therapeutic resistance. Dr. Li showed that enzalutamide decreased TGFBR2 protein expression in osteoblasts through PTH1R-mediated endocytosis. PTH1R blockade rescued enzalutamide-mediated decrease in TGFBR2 levels and enzalutamide responses in C4-2B cells that were co-cultured with osteoblasts. This result demonstrates the contribution of the bone microenvironment to enzalutamide resistance and identifies PTH1R as a feasible target to overcome the resistance in PCa bone metastases. Dr. Li also described unpublished data in which rare dormant prostate cancer bone metastasis formed after primary C4-2b subcutaneous xenografts were removed in mice. Next, Dr. Subhamoy Dasgupta from Roswell Park Comprehensive Cancer Center showed that the androgen receptor and its coregulator steroid receptor coactivator-2 (SRC-2) recruit histone deacetylase 2 to the promoter of mitochondrial deacetylase sirtuin 3 (SIRT3), transcriptionally repress SIRT3, limit acetylation of mitochondrial aconitase (ACO2) at lysine258 by SIRT3, and reduce the enzymatic activity of ACO2, thereby suppressing mitochondrial citrate synthesis and de novo lipogenesis. Prostate tumors with increased SRC-2 expression with concomitant reduced SIRT3 were found to enrich in bone metastatic lesions. Dr. Dasgupta also discussed some unpublished work regarding the role of nuclear ACO2 in production of acetyl-CoA which may play a critical role in epigenetic plasticity of prostate tumors. Dr. Ming Lam from the University of Washington introduced the bladder cancer rapid autopsy program at the University of Washington and the established CocaB patient-derived bladder cancer models. Dr. Lam also discussed the effort of the team to profile genetic mutations and spatial transcriptomic of the models, particularly in the context of liver metastasis using various technologies. Dr. Will Ricke from the University of Wisconsin at Madison discussed some mouse models for studying BPH/LUTS, including an aging model and a model induced by treatment with testosterone and estradiol ($T+E_2$). Both models display some features characteristic of human BPH/LUTS such as bladder obstruction and fibrosis. Dr. Ricke further discussed the role of fibrosis in BPH/LUTS. Expression of a gain-of-function of collagen-I promotes LUTD in mouse models. Use of anti-fibrotic in mouse models improves urinary frequency, supporting a future clinical trial in men with BPH/LUTS.

The fifth plenary session discussed Immunoregulation in urological diseases. Drs. Leigh Ellis from the Center for Prostate Disease Research and Yan Dong from Tulane University were discussion leaders for this plenary session. Dr. Flaminia Talos from State University of New York discussed how immune mechanisms shape the clonal landscape during early progression of prostate cancer using mouse models for prostate cancer in conjunction with multicolor lineage tracing and single-cell transcriptomics. Dr. Talos showed that dominant and minor clones display distinct capacity for immu-

noediting. Minor clones are marked by an increased expression of IFNγ-response genes and the T cell-activating chemokines Cxcl9 and Cxcl11. Next, Dr. Simon Hayward from North Shore Research Institute discussed the role of inflammation as a driving force in BPH pathogenesis. Dr. Hayward showed that as prostate size increases, the T cell lineages decrease while the myeloid lineages increase. The macrophages are an important source of TNFα. Suppressing TNFα signaling reduces inflammation and prostate growth. Dr. Hayward also pointed out that Type 2 cytokine driven inflammatory signaling are alternative drivers of prostate growth and formation of fibrosis and may be potentially therapeutically exploited. Next, Dr. Nikki Delk from the University of Dallas discussed her research that acute IL-1 exposure represses prostate cancer androgen receptor (AR) expression via RELA, which is concomitant with the upregulation of pro-survival proteins, causing de novo accumulation of castration-resistant prostate cancer cells. Dr. Sushant Kachhap from Johns Hopkins University discussed molecular mechanisms underlying the bipolar androgen therapy (BAT) during which serum testosterone is cycled monthly from supraphysiologic down to near-castrate levels. Dr. Kachhap reported that a supraphysiologic level of testosterone induces two parallel autophagy-mediated processes (ferritinophagy and nucleophagy). Autophagosomal DNA activated the STING-TBK1-NFκB signaling axis to upregulates Cxcl10, which recruits cytotoxic immune cells and inhibits prostate cancer growth. Next, Dr. William Kim from the University of North Carolina discussed how oncogene activation affects immune response in bladder cancer. Data presented by Dr. Kim showed that expression of a S249C FGFR3 mutant in a mouse model induces a luminal bladder cancer phenotype and an intermediate T-cell-inflamed tumor. RNA-seq analysis revealed that FGFR3 suppresses basal tumor expression patterns by the activation of Pparg signaling. FGFR inhibitor abrogates anti-PD-1 induced T_{reg} proliferation and promotes highly T-cell-inflamed tumors.

Right before the sixth plenary session was the AUA lecture delivered by Dr. Aria Olumi from the Beth Israel Deaconess Medical Center. Dr. Olumi first inspired the audience by sharing his own journey to an independent career and his perseverance that leads to the success for extramural funding. Then Dr. Olumi described his long-term interest in the role of Steroid 5α-reductase 2 (SRD5A2) in BPH and summarized early work from his laboratory showing that inflammation, age, and BMI are associated with SRD5A2 promoter methylation. In human prostate specimens, methylation at the SRD5A2 promoter is concomitant with the phosphorylation of estrogen receptor-α (ER-α) in prostatic stroma and a significant upregulation of estrogen response genes. Tumor necrosis factor (TNF)-α activates NF-kB and promotes expression of DNA methyltransferase 1 (DNMT1), which methylates the promoter of SRD5A2 and suppresses its expression. Meanwhile, TNF-α promotes the phosphorylation of ER-α, and subsequently the expression of aromatase, the enzyme responsible for conversion of testosterone to estradiol. This leads to an androgenic to estrogenic switch. Finally, Dr. Olumi also described the unpublished study on a *Srd5a2* knockout mouse model. Germline disruption of *Srd5a2* results in smaller prostates which is consistent with the critical role of stromal-derived dihydrotestosterone in prostate development and morphogenesis. Single cell analyses revealed a discrete subpopulation of luminal epithelial-2 (LE2) cells, particularly in anterior prostate lobes, that highly express ESR1 and Wnt5a. There is a decrease in apoptosis and increase in proliferation among LE2 cells, indicating that in the absence of Srd5a2, stromal epithelial interactions via Wnt signaling promote prostate cell survival.

The sixth plenary session focused on confounding factors of urological pathologies. Drs. Natasha Kyprianou from Mount Sinai and Zhou Wang from University of Pittsburgh were discussion leaders for this plenary session. Dr. Kimberly Stietz from University of Wisconsin discussed the impact of developmental exposure to the environmental contaminant polychlorinated biphenyls (PCBs) on the establishment and regulation of urinary function. Exposure to PCBs through gestation and lactation causes many dose- and sex-dependent effects in the bladder, such as increased $CD45^+$ hematolymphoid immune cells and $F4/80^+$ macrophages, decreased collagen density in female bladders, and increased dividing cells in male bladders. These results reveal that exposure to PCBs can perturbate the immune system and affect bladder biology. Next, Dr. Wei Li from the University of California at Irvine discussed an innovative bioinformatic algorithm to identify

risk genes for diseases. Alternative polyadenylation (APA) plays an essential role in tissue development and cancer progression. However, current transcriptome-wide association studies (TWAS) usually do not consider APA while nominating susceptibility genes. Dr. Li focused on GWAS SNPs associated with post-transcriptional regulation mediated by 3'UTR length changes and developed a 3' untranslated region (3'UTR) APA TWAS (3' aTWAS). With this novel tool, they discovered ATXN3 as a 3' aTWAS-significant gene for amyotrophic lateral sclerosis. By focusing on the changes in APA, Dr. Wei Li also identified several 3' aTWAS-significant genes associated with the transition of castration resistant prostate cancer. Next, Dr. Jonanthan Barasch from Columbia University described their effort to test whether molecular biomarkers of tubular injury measured at hospital admission were associated with acute kidney injury (AKI) in patients with COVID-19 infection. A prospective cohort observational study consisting of 444 consecutive patients with SARS-CoV-2 was conducted. The study showed that in the patients with COVID-19, the level of urinary neutrophil gelatinase-associated lipocalin (uNGAL) was quantitatively associated with histopathologic injury, loss of kidney function, and severity of patient outcomes. Lastly, Dr. Omar Franco from Louisiana State University discussed research on tumor microenvironment mediators of racial disparities in prostate carcinogenesis. There is an increased stromal remodeling in the prostate tumor microenvironment of African American (AA) men with prostate cancer compared to Caucasian patients. Cancer associated fibroblasts (CAFs) of AA patients have augmented lipid storage due to increased expression of Diacylglycerol O-acyltransferase 1 (DGAT1) and microtubule-organizing center (MTOC) amplification. CAFs of AA patients also elevate BDNF/TrkB signaling, which enhances tumor aggressiveness.

In this meeting, 30 trainees were selected from 152 submitted abstracts to receive the travel awards. On the night of Nov. 9 after the Leland W.K. Chung lecture, 3 of the travel awardees presented to compete for the Eula and Donald S. Coffey Innovative Research Award. Dr. Shiqin Liu from the University of California at Los Angeles won the award. Nine travel awardees were selected for oral presentations within the six plenary sessions.

In support of SBUR's goal to train the next generation of urologic researchers, a mission supported by the NCI and NIDDK, Dr. John Lee organized a Trainee Affairs Symposium for all trainee attendees. The Trainee Affairs Symposium was held on the first day of the meeting and consisted of three parts: Part 1 was a 45-minute session on strategies to gain bioinformatic training, Part 2 was a 1-hour career panel discussion, and Part 3 was a 45-minute session on grant writing tips for trainees. The turnout for the Trainee Affairs Symposium was over 70 trainees. Part 1 started with a talk from Dr. Antonina Mitrofanova (Rutgers University) who reviewed the broad definition of bioinformatics, different types of biological datasets, the importance of a strong background in statistics, and several key training opportunities both free and at cost to enhance one's skills. This was followed by a Q&A session that touched on the value of pursuing a second post-doctoral fellowship in bioinformatics and the rapidly evolving landscape of technologies including single-cell sequencing. The career panel discussion included five panelists that spanned academia, government, and industry. The panelists included Drs. Mitrofanova, Paul Alan Lombard (UC Davis), Ayesha Shafi (Center for Prostate Disease Research), Magda Grabowska (BioSkryb Genomics), and Susan Lim (National Cancer Institute). Each panelist described their career path, key factors that affected their journey, and the nature of their current position. Questions related to these career paths including differences in the overall scope of duties, training requirements, the importance of mentorship, maintaining a work/life balance, and the most satisfying aspects of their positions were addressed. Part 3 began with a talk by Dr. Lim who reviewed multiple training grant mechanisms available from the National Institutes of Health and National Cancer Institute including F series and K series awards such as the F99/K00, K22, and early/standard K99/R00 awards. General strategies for effective preparation of grants including the avoidance of common pitfalls were discussed. The Q&A session that followed focused on several case-by-case scenarios regarding eligibility criteria, the increasingly competitive paylines, and the need for trainees to diversify their applications to multiple grant funding agencies.

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Detection of Vasopressin Receptors in Bat: A Novel Animal Model for Nocturia

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Abstract

Background: Nocturia, or frequent night-time urination, significantly impacts patients' sleep, quality of life, and health. Both age and biological sex contribute to nocturia. The water-regulatory hormone arginine vasopressin (AVP) controls urine production. AVP binds to arginine vasopressin receptor 2 (AVPR), a G-protein-coupled receptor (GPCR), in various organs, including the kidney and bladder. Desmopressin, an AVPR2-specific agonist, is a mainstay treatment for nocturia, but its mechanism of action in the bladder is not fully understood. A better understanding of vasopressin signaling in the bladder is crucial for refining treatment options. The fruit bat Carollia perspicillata closely resembles humans in its physiology and can be kept in captivity for over ten years, making it an attractive translational model to study aging, including nocturia. We aim to (1) identify antibodies recognizing AVPRs in a novel animal model for nocturia, the fruit bat C. perspicillata (2) quantify AVPR expression in the bladder of the fruit bat.

Methods: Total protein was isolated from mouse and bat kidney, liver, and bladder by homogenization in RIPA buffer with a homemade bead mill, centrifugation, and - for bladder - concentration by ultracentrifugation. 10 µg protein each, as measured by BCA assay, was electrophoresed with size standards in 4%-12% SDS/polyacrylamide gels and electroblotted onto PVDF membranes. AVPR1A and AVPR2 proteins were detected with commercial 1° antibodies followed by fluorescent 2° antibodies, while total protein was detected with Fast Green. Fluorescence was measured by scanning (Odyssey M, Li-Cor) and quantitated with Fiji/ImageJ software.

Results: AVPR2 and AVPR1A were detected in the kidney and liver of both mouse and bat at the same expected apparent molecular masses of ~44 and ~67 kDa, respectively. In the bladder of both species, the predominant apparent molecular masses were ~80 and ~151 kDa, indicating dimerization, a common phenomenon with GPCRs. Relative protein levels were similar in the kidney and liver.

Conclusions: Commercial antibodies against human and rodent AVPRs recognize the orthologous proteins from the fruit bat C. perspicillata. The sizes of AVPR1A and AVPR2 proteins observed for bats match those for mice and those reported in the literature, indicating the specificity of the antibodies. AVPR1A and AVPR2 are expressed in bat kidney and bladder, the organs responsible for urine production, and are known in humans to be involved in nocturia. C. perspicillata is thus a tractable model for studies of AVP signaling in nocturia. Our next step will be to determine the cellular location of AVPRs and to measure their levels in the kidney and bladder of young and old bats.

2

Fructose Promotes Metabolic Reprogramming in Prostate Cancer Cells

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Abstract

Title: Fructose Promotes Metabolic Reprogramming in Prostate Cancer Cells

Background: Positron Emission Tomography (PET) scanning using fluoro-deoxy-glucose (FDG) is one of the most used techniques for clinical localization of primary tumors and sites of metastasis. However, in prostate cancer (PCa), FDG-PET demonstrate a limited clinical applicability, implying that PCa cells may use hexoses other than glucose, such as fructose, as a potential energy source. In accordance with this observation, previous studies from our laboratory indicated that PCa cell lines (LNCaP and PC-3) and patient-derived xenograft (PDX) tumors from PCa tissues were capable to proliferate and growth under chronic fructose stimulation. However, the biological impact of fructose on the metabolic reprograming of PCa cells has not been fully unraveled.

Methods: Using LNCaP cells, we aimed to study the effect of chronic fructose stimulation on the expression of key enzymes of the pentose phosphate (PPP) and de novo lipogenesis (DNL), the main metabolic pathways for biosynthesis of nucleotides and fatty acids, respectively. QRT-PCR, western blot, lipid droplet, ATP and lactate level measurements were performed in LNCaP cells under stimulation with 5,5 mM fructose or 5.5 mM glucose (control).

Results: Our results indicated that the DNL enzyme, fatty acid synthase (FASN), and the oxidative PPP enzyme, glucose-6-P-dehydrogenase, were significantly upregulated at the mRNA and protein level in LNCaP cells under fructose stimulation. Remarkably, the non-oxidative PPP enzyme, transketolase, did not change between both conditions. Interestingly, a significant reduction in the intra- and extracellular lactate levels were observed under fructose stimulation. However, the intracellular levels of ATP did not change significantly between both conditions. Consistently with the overexpression of the key DNL enzyme, FASN, a significant increase in lipid droplet formation was observed in LNCaP cells under fructose stimulation.

Conclusion: Fructose might increase DNL pathway by enhancing FASN enzyme expression, which promotes lipid biosynthesis and cellular proliferation.

3

Clusterin knockout mice: A murine model of glomerular fibrillogenesis

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Abstract

Background: Both fibrillary glomerulonephritis (FGN) and immunotactoid glomerulopathy (ITG) are characterized by deposits of fibril proteins in the glomeruli in humans, but the pathogenesis of these diseases is poorly understood. Clusterin (CLU) is a multifunctional chaperone-like glycoprotein, and CLU knockout (KO) mice develop age-dependent glomerulopathy. Interestedly, the glomerulopathy in these CLU KO mice is also characterized by glomerular fibril protein deposit, but the clinical relevance of this disease is not investigated. We hypothesize that CLU-KO mice represent a murine model of FGN or ITG in humans. This study was designed to further characterize the pathology of the glomerulopathy in CLU KO mice.

Methods: Wild type (WT) C57BL/6 (B6) and CLU-KO mice in B6 background (both female and male, 10-24 months old) were used. The kidney specimens were sectioned for staining with hematoxylin and eosin (HE), Masson-Trichrome and Congo red. Immunohistochemistry (IHC) was used to detect the special antigens (such as DNAJB9) in the glomerulus. Electron microscopy (EM) was used to characterize the fibril formation of the electron-dense mesangial deposits.

Results: Mice deficient in clusterin developed a progressive glomerulopathy characterized by the deposition of immune complexes in the mesangium. Up to 75% of glomeruli in CLU-KO mice exhibited moderate to severe mesangial lesions by 22 months in males and 12 months in females. WT mice exhibited little or no glomerular pathology at the same age. Masson-Trichrome revealed that fibrosis and immunocomplex were present in the mesangial lesion, but the amyloid was not detected. IHC staining showed that the glomeruli were negative for DNAJB9. EM revealed the accumulation of electron-dense material in the mesangial matrix were tubulo-fibrillary structures.

Conclusions: Data show that histopathological changes in the kidney of CLU-KO mice were similar to ITG, implying that the CLU-KO mice may represent a murine model of glomerular fibrillogenesis in humans especially for ITG.

4

NEK1-Mediated Phosphorylation of YAP1 is key to Prostate Cancer Progression

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Abstract

Background: YAP1/TAZ are the main effectors of the Hippo pathway, which is involved in regulating organ size through multiple cellular functions, including cell proliferation, apoptosis and resistance to chemotherapy. The Hippo pathway responds to a variety of cellular cues, including cell-cell contact, mechanotransduction, and apico-basal polarity. When Hippo signaling is activated, the key kinases MST1/2 and LATS1/2 phosphorylate YAP1 (at S127) and inactivate/degrade it. In PCa, YAP1 has been identified as a binding partner of AR and co-localized with AR in an androgen-dependent manner and in an AI in CRPC and, when expressed ectopically in LNCaP, activates AR signaling and confers castration resistance, motility, and invasion. YAP1-activated AR signaling was sufficient to promote LNCaP cells from an AS state to AI. However, the activators of the Hippo/YAP1 in PCa remain unclear. The phosphorylation of S127 has dominated the literature to the detriment of important other sites revealed by MS, like Y407 which lies within the TAD and a known degron motif.

Results: We identified a novel axis of the Hippo pathway characterized by the sequential kinase cascade induced by ADT: AR->mTOR>TLK1B>NEK1>pYAP1-Y407, leading to CRPC adaptation. Phosphorylation of YAP1-Y407 increases upon ADT correlated with the known increase in NEK1 expression/activity, and this is suppressed in a YAP-Y407F mutant. Overexpression of wt-GFP-YAP but not Y407F resulted in dramatic morphologic changes. In fact, LNCaP expressing wt-YAP were AI whereas the Y407F mutant were AS, and LNCaP expressing wt-GFP-YAP underwent EMT transformation. This, largely reflecting transcriptional differences in both AR-dependent genes (FKBP5, PSA) and some involved in EMT (ZEB1, TWIST, E-CAD) We also found that J54, a pharmacological inhibitor of the TLK1>NEK1>YAP1 nexus led to degradation of YAP1, suggesting that the Y407 phosphorylation is critical for its transcriptional activation and stabilization. Specifically, Nek1-mediated phosphorylation of YAP-Y407 increased its productive interaction with transcriptional activators TEAD or AR, resulting in its nuclear retention and stabilization. This was further demonstrated by coIP of TEAD1 or the AR with GFP-YAP antiserum, as well as via LUC expression using ARE and Hippo reporter.

Conclusions: The phosphorylation of YAP-Y407 by NEK1 leads to its increased transcriptional activity and may be critical for the initial adaptation of AS cells to ADT.

5

Loss of AZGP1 promotes angiogenesis and fibroblast growth in prostate cancer

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Abstract

Background: AZGP1 has been involved in various biological processes, including lipid metabolism, regulation of cell proliferation, migration and invasion, and immune response. AZGP1 is associated with different cancers, including prostate cancer, where its expression levels have been related to clinical outcomes. The loss of AZGP1 is associated with worse clinical outcomes, and AZGP1 has been indicated as a potential biomarker for prostate cancer. However, the mechanisms of how AZGP1 function in prostate cancer remain unknown.

Methods: A lentiviral system was used to generate AZGP1 knockout and overexpressing prostate cancer cells. The effects of AZGP1 were evaluated by in vitro cell proliferation, migration, and invasion assays. AZGP1-/- mice were generated and prostate tissues were collected from different time points. To investigate the angiogenic effects of AZGP1, in vivo xenograft mouse models were utilized. Furthermore, proteomics analysis was conducted to assess the protein profiles of the tumors.

Results: Our findings indicate that AZGP1 does not exert any effect on prostate cancer cell proliferation, migration, and invasion. However, when AZGP1 was overexpressed in a xenograft mouse model, it exhibited a notable ability to inhibit tumor angiogenesis without affecting tumor growth. AZGP1-/- mouse prostates are morphologically normal; however, there was increased fibroblast growth in the periglandular stroma by 6 months. Interestingly, the overexpression of AZGP1 did not impact the growth of PC3 and DU145 tumors. Instead, PC3-AZGP1-OV and DU145-AZGP1-OV tumors displayed reduced microvessel density compared to the control cells, suggesting that AZGP1 possesses anti-angiogenic properties. Proteomic profiling revealed distinct differences in angiogenesis-related proteins between PC3-AZGP1-OV and PC3 control cells, particularly in proteins such as YWHAZ, EPHA2, SERPINE1, PDCD6, MMP9, GPX1, HSPB1, COL18A1, RNH1, and ANXA1.

Conclusions: We demonstrate that AZGP1 induces heterotypical effects on stromal cells, and loss of AZGP1 enhances angiogenesis within the tumor microenvironment. This study sheds light on the anti-angiogenic characteristics of AZGP1 in prostate cancer and suggests that AZGP1 could serve as a potential therapeutic target for prostate cancer.

6

Siglec-7/9 are novel glyco-immune checkpoints, with CD59 acting as a ligand specifically for Siglec-9 in prostate cancer

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Abstract

Background:

Prostate cancer remains a leading cause of cancer-related deaths in men worldwide. Although immune checkpoint inhibitors have shown significant success in treating various cancers, they are not effective for prostate cancer. Siglec-7/9, which are expressed in immune cells including dendritic cells and T cells, have been identified as potential immune checkpoints in various cancers. Recent studies have shown that disrupting the interactions between Siglec-7/9 and sialic acids can enhance immune responses and effectively inhibits tumor growth in multiple cancer types. Siglec-9 ligands have been identified in prostate cancer tumor tissues. Despite these findings, the understanding of Siglec-7/9-sialic acid interactions in prostate cancer remains limited.

Methods:

Cancer Genome Atlas Prostate Adenocarcinoma PRAD TCGA datasets were used to assess the Siglec-7/9 transcripts levels in 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 labelling on prostate cancer cells. Sialidase, and anti-Siglec-7/9 antibodies were used to block the Siglec-7/9 and their ligands interactions. In vivo humanized mouse model was further used to examine the inhibition of Siglec-7/9 and their ligand interactions. CRISPR/Cas9 sgRNA system was employed to achieve CD59 knockout in prostate cancer cells.

Results:

Tumor-infiltrating immune cells derived from prostate cancer patients exhibited high levels of Siglec-7 and Siglec-9. Prostate cancer tumors showed elevated levels of Siglec-7/9 ligands. Notably, prostate cancer cell lines, especially PC3 and 22Rv1, demonstrated significant expression of Siglec-7 and Siglec-9 ligands, along with observable cell surface sialic acids. Blocking the interactions between Siglec-7/9 and sialoglycans enhanced T cell-mediated cytotoxicity against prostate cancer cells. Treatment with anti-Siglec-7/9 antibodies effectively suppressed the growth of PC3 and 22Rv1 tumors in a humanized mouse model. Further analysis of anti-Siglec-7/9-treated prostate xenografts revealed reduced proliferation, decreased vascularization, increased apoptosis, and higher immune cell infiltration. CD59 was identified as a potential ligand for Siglec-9 based on CRISPR screen and mass spectrometry analysis. Moreover, knockout of CD59 led to reduced binding activity of Siglec-9-Fc chimera proteins and increased T cell-mediated cytotoxicity against prostate cancer cells.

Conclusions:

Our findings demonstrate that blocking the interactions between Siglec-7/9 and their ligands enhances immune cell-mediated suppression of prostate cancer. CD59 is identified as a potential ligand for Siglec-9 in the context of prostate cancer. These results provide valuable insights into the potential of targeting Siglec-7/9 and their ligands as a novel therapeutic strategy for prostate cancer treatment.

7

Molecular Characterization of Penile Squamous Cell Carcinoma Patient-Derived Xenograft Animal Models Based on HPV Status

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Abstract

Purpose. Penile squamous cell carcinoma (PSCC) is a rare but aggressive disease. Approximately half of all PSCC cases are positive for high-risk human papillomavirus (HR-HPV). We generated patient-derived xenograft (PDX) models of HPV-positive (+) and HPV-negative (-) PSCC to determine genomic, transcriptomic, and proteomic differences to define potential molecular targets for therapy.

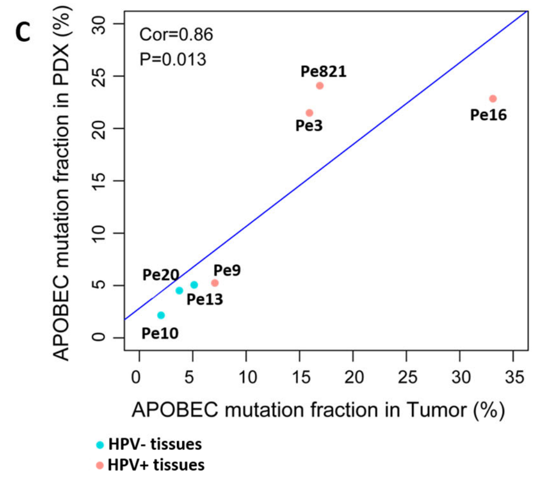
Experimental Design. Patient tumor tissue (donor tissue) was directly implanted into immunocompromised mice. With successful engraftment, PDX tumors were further passaged in mice up to five times. We compared PDX tissue to patient donor tissue by histology, STR fingerprinting, immunohistochemistry for (CDKN2A) p16, whole exome (WES) and RNA sequencing, and reverse phase protein array. Proteins observed to be differentially expressed based on HPV status were further studied using immunoblotting.

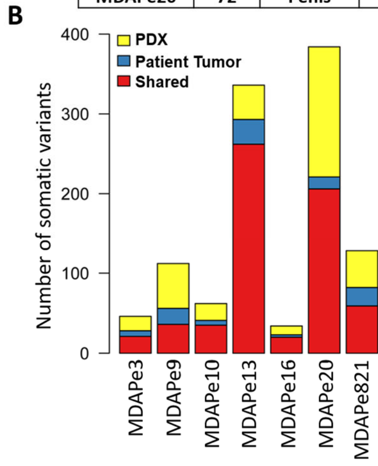
Results. We generated 4 HPV+ and 3 HPV- PSCC PDX animal models (Fig. A). The PDX tumor tissue was found to be similar to donor tissue both by histology and STR. Donor tissue mutations were largely preserved in PDX tissues; however, a higher number of somatic mutations were found in the PDX tissues (Fig. B). Similar APOBEC mutational fractions in donor tissue and the PDX tissue (Fig. C) were noted and, a higher APOBEC mutational fraction is found in HPV+ versus HPV- PDX tissues ($P = 0.044$). Significant transcriptomic and proteomic expression differences based on HPV status included p16 (CDKN2A), RRM2, and CDC25C (Fig. D).

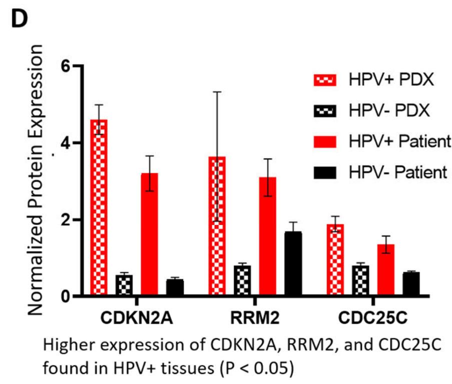
Conclusions. We generated HR-HPV positive and negative PSCC PDX animal models that resembled human donor tissue at histopathologic and molecular levels. We are now evaluating if the differential expression of certain cell cycle proteins (p16 (CDKN2A), RRM2, and CDC25C) based upon HPV status could be a possible therapeutic vulnerability.

A

Model	Age	Tumor Site	p16 status	HPV subtype	Pathological staging	Recurrence	Neoadjuvant Treatment
MDAPe821	68	Penis	-	16	rpT4NxM1	+	+
MDAPe3	67	Penis	+	16	rpT2Nx	+	
MDAPe9	33	Lymph Node	+	16	pT3N3Mx	+	+
MDAPe10	80	Penis	-		pT3N3Mx		+
MDAPe13	67	Penis	-		pT3Nx		
MDAPe16	82	Penis	+	16	pT3N0		
MDAPe20	72	Penis	-		pT3Nx		







Higher expression of CDKN2A, RRM2, and CDC25C found in HPV+ tissues (P < 0.05)

8

Androgen Receptor Reprogramming in Prostate Cancer among Men of African Ancestry with Shorter CAG Repeats

Dr. Dong Han PhD[1], Mr. Muqing Li BS[1], Dr. Maryam Labaf PhD[2], Ms. Lily Ting N/A[1], Ms. Songqi Zhang MS[1], Ms. Mingyu Liu BS[1], Ms. Susan Patalano BS[1], Dr. Jill Macoska PhD[1], Dr. Steven Balk MD/PhD[2], Dr. Changmeng Cai PhD[1]

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Abstract

Background: While men of European ancestry (EA) make up the majority of prostate cancer (PCa) patients, men of African ancestry (AA) experience higher prevalence and worse outcomes. The androgen receptor (AR) plays a crucial role in PCa development and therapy resistance, and genetic differences, in the AR gene have been established between EA and AA men. In the AA population, the AR protein exhibits shorter glutamine repeats (coded by CAG repeats) in its N-terminal domain. Although it has been demonstrated that these variants of AR may have higher transcriptional activity, their global impact on AR chromatin binding and transcriptional programs in PCa remains unclear. This study aims to investigate how the shorter CAG repeats affect AR global transcriptional activity and determine their significance in PCa response to different therapies.

Methods: To examine the impact of shorter CAG repeats on the AR, we utilized CRISPR-Cas9 gene editing to generate isogenic cell lines with a shorter CAG repeat of the AR protein in PCa cells. Immunoblotting and ChIP-qPCR approaches were employed to examine AR protein stability and chromatin binding. Furthermore, ChIP-seq and RNA-seq analyses were performed to determine AR chromatin binding and transcriptomes. The cells were subjected to different therapies to determine their differential responses.

Results: Two LNCaP PCa cell clones were created with a shorter CAG repeat of the AR. Interestingly, while AR transcript levels remained similar among these clones and the parental cells, there was a significant increase in AR protein levels, which were attributed to reduced protein degradation. As a result, enhanced AR chromatin binding occurred at androgen-responsive elements, along with increased recruitment of specific epigenetic cofactors. Global analysis revealed a significant upregulation of classic AR target genes and AR-regulated genes involved in fatty acid/cholesterol synthesis.

Conclusions: This study reveals that the AR protein with a shorter poly-Q track exhibits enhanced resistance to proteasome-dependent degradation, stronger chromatin binding, and enhanced recruitment of selected cofactors, contributing to enhanced activation of fatty acid and cholesterol synthesis pathways in PCa cells. These findings shed light on the importance of understanding AR genetic differences between EA and AA men and may have implications for tailoring therapies to individual patient populations.

9

The expression of PKM1 and PKM2 in developing, benign and cancerous prostatic tissues

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Abstract

The expression of PKM1 and PKM2 in developing, benign and cancerous prostatic tissues

Background: Neuroendocrine prostate cancer (NEPCa) is the most aggressive prostate cancer (PCa). The metabolic reprogramming, one of the cancer hallmarks, regulates PCa progression and therapy resistance. However, the energy metabolism in NEPC has not been well studied yet. Pyruvate kinase M (PKM), catalyzing the final step of glycolysis in most tissues, has PKM1, PKM2 isoforms due to alternative splicing. Previous studies have assessed PKM2 expression in prostatic tissue and found that PKM2 is expressed in prostate adenocarcinomas (AdPCa). However, the expression pattern of PKM1 and PKM2 in NEPCa remains unknown.

Methods: Immunofluorescence (IF), immunohistochemistry (IHC), and Western blot were conducted to examine the expression of PKM1 and PKM2 in both murine and human prostatic tissues. The bioinformatics analysis was done using the publicly available RNA-Seq data obtained from the cBioportal, the Cancer Genome Atlas (TCGA), and the Cancer Cell Line Encyclopedia websites (CCLE).

Results: First, using immunohistochemistry staining, we assessed the expression of PKM1 and PKM2 during murine prostate development. We found that PKM2 was expressed throughout prostate development. Its expression was detected in both embryonic prostate and adult prostate. In contrast, PKM1 was predominantly expressed in prostate stromal cells. Its expression was absent in the epithelial cells of developing prostate but was detected in the adult prostatic epithelial cells. In TRAMP mice, PCa mouse models, the highest PKM2 expression was detected in prostatic intraepithelial neoplasia (PIN) lesions. Its level was higher in PIN tumors than normal prostatic tissues. PKM2 level was also higher in PIN than NEPCa tumors of TRAMP mice. In contrast, PKM1 expression was detected in NEPCa but not PIN of TRAMP. In human specimens, PKM2 expression was detected in most human prostatic tissues including benign prostates, AdPCa and NEPCa. In a subset of NEPCa tumors, PKM2 level is slightly lower compared with AdPCa. PKM1, similar to its expression in murine prostates, is highly expressed in stromal cells of human prostatic tissues. Low to moderate levels of PKM1 expression was detected in the basal epithelial cells of benign prostates. However, its expression was absent in majority AdPCa cells but detected in a subset of NEPCa. Further, we characterized the transcripts of PKM gene in human PCa cell lines and patients' samples by analyzing RNA-seq data. We found that ten PKM transcripts including two PKM1 and one PKM2 isoforms were expressed in prostatic tissues with high abundance. The expression of these isoforms was quantified.

Conclusion: In conclusion, PKM2 is expressed throughout prostate development, in both AdPCa and NEPCa with slightly reduced expression in some NEPCa cases. PKM1 is not expressed in AdPCa, but low levels of PKM1 expression is detected in NEPCa. These data provide a foundation for studying

the role of PKMs in PCa carcinogenesis and NEPCa progression. These findings lay the groundwork for understanding PKMs' role in PCa carcinogenesis and NEPCa progression. The distinct expression patterns of PKM isoforms in different prostate cancer subtypes may offer insights into potential therapeutic strategies and precision medicine approaches.

10

Cellular Heterogeneity and Origin of Tissue-resident Macrophages in Prostate Organogenesis

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Abstract

Background: Dysregulation of the prostate immune microenvironment is hypothesized to contribute to prostate disease. Thus, additional knowledge on the function, cellular origin, and androgen dependence of immune cells in the prostate is essential to developing more effective treatments that target immune cell contributions to prostate diseases. Studies in organs including the lung, mammary glands, and testes have revealed significant tissue-specificity and diversity in the function of tissue-resident macrophages during development and disease, and the need to understand this heterogeneity for developing disease treatments. Recent published single-cell RNA sequencing data have revealed heterogenous and prostate-specific macrophage populations in the adult prostate. We hypothesized that heterogeneous populations of prostate macrophages with distinct developmental origins and tissue-specific properties regulate the organogenesis and function of the prostate.

Methods: We conducted histological and immunostaining analysis, high-resolution three-dimensional imaging and flow cytometry analysis of macrophages in mouse prostate tissues during prostate organogenesis. To determine the contribution of embryonic yolk sac-derived macrophages to prostate organogenesis, we conducted *in vivo* genetic-lineage tracing of yolk sac macrophages using a tamoxifen-inducible *Cre* line and YFP reporter.

Results: Our findings indicate that macrophages are abundant in the prostate prior to and during puberty. During puberty, macrophages were present in ductal and interstitial regions of the prostate. We found that *Cx3cr1* expressing interstitial macrophages surround the prostate and were associated with neurons. Through *in vivo* lineage-tracing experiments, we provide evidence for tissue-resident prostate macrophages originating from the yolk sac.

Conclusions: Our results increase our knowledge of macrophage heterogeneity during prostate organogenesis and suggest functions for prostate macrophages in prostate growth during puberty. These findings may have implications for understanding macrophage function in prostate disease.

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EZH2 regulates multilineage cell states in neuroendocrine-like prostate cancer that requires mTORC1 and the RNA binding protein Tristetraprolin (TTP), a novel mechanism for therapeutical target.

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Abstract

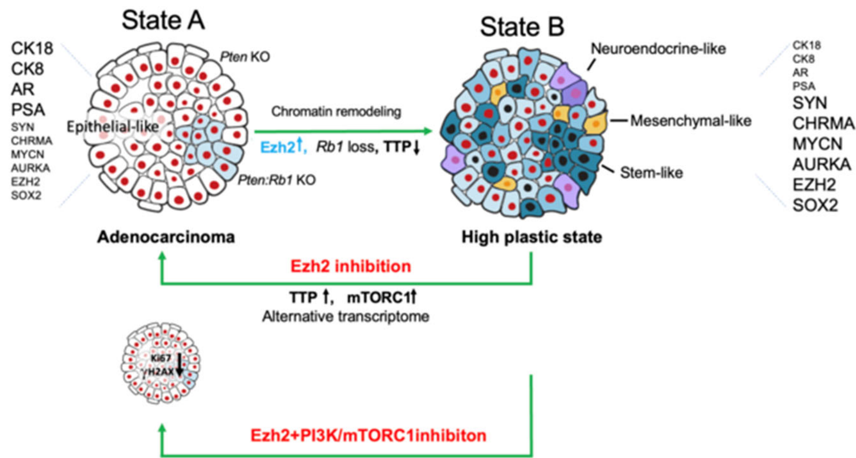
Background: Second-generation androgen deprivation therapies (ADT) have provided significant life-extension for patients with metastatic castration resistant prostate cancer (mCRPC), but unfortunately tumors will progress via therapy resistance. Currently no therapies provide durable response. Approximately 15-20% of these mCRPC are independent of AR activity via a mechanism termed phenotypic plasticity, which activates alternative transcriptomic programs associated with loss of function of the tumor suppressor genes RB1 and/or TP53. The RB1 genomic loss is predictor of poor survival, whereas alterations in RB1 and TP53 are associated with shorter response to ADT in mCRPC. These tumors often display altered kinase signaling and multilineage states including neuroendocrine, stem, and basal-like gene signatures. Using GEMMs devoid of *Pten* and *Rb1*, we previously demonstrated that the reprogramming factor EZH2 regulates alternative transcription programs promoting phenotypic plasticity. Here, our overall goal was to better understand EZH2 role in reprogramming and how this could be exploited therapeutically.

Methods: To investigate cell identity control by EZH2, prostate cancer GEMMs were treated with the EZH2 inhibitor EPZ011989. Analysis was performed using ChIPseq, CRISPR/Cas9 functional screen, RIME and snRNASeq. To validate these data, genetic and chemical tools were used with in vitro and in vivo prostate cancer models.

Results: EZH2 regulates alternate transcription programs leading to multilineage cell states that are associated with activation of the mTORC1. These data were further supported through RIME and functional genomic data indicating cross talk between EZH2 and activation of translation. Combined chemical inhibition of EZH2 and PI3K/mTORC1 resulted in superior anti-tumor activity in murine and human phenotypic plastic models and was most significant when this combination was used with castration. Moreover, we elucidated that the regulation of cellular state transition by EZH2 inhibition requires activation of TTP.

Conclusions: Together, these data indicate phenotypic plasticity dependence on coordination between EZH2, TTP and mTORC1 signaling and represent a novel therapeutic approach for this lethal prostate cancer phenotype.

Graphical abstract:



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Functional impact of androgen deprivation therapy on patients with castration-resistant prostate cancer

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Abstract

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Background

Androgens play a neuroprotective role in maintaining the normal physiological functions of the brain. It also plays a pivotal role in prostate cancer development and progression. Current prostate cancer treatment is majorly based on androgen deprivation therapy (ADT) that blocks the male hormone testosterone. Moreso, prostate cancer patients that undergo ADT have been shown to develop neuropathological conditions such as loss of memory, learning, reasoning, and decision-making. Several studies have shown a positive association between ADT and risk of cognitive impairment. Currently, there are no clinical markers to identify patients susceptible to ADT-mediated cognition. This demands a need for biomarkers that can help distinguish the vulnerability of patients toward cognitive impairment undergoing ADT. We utilized a mouse model to study the impact of ADT in different regions of the brain and to identify biomarkers of ADT.

Experimental design

Sixteen-week-old BALB/c mice were subjected to peroral intake with 50 mg/kg of enzalutamide (mimicking ADT) per day for 5 days per week (0.2 ml of vehicle consisting of 0.5% methylcellulose and 0.025% Tween 20) for a total of 8 weeks. The control group of animals received 0.2 ml vehicle per day for the same time period. Mice were observed for behavioral changes post-enzalutamide treatment. 18F-FDG-PET imaging was performed followed by termination of the experiment, and the brain was excised for the cortex, cerebellum, and hippocampus, and the protein lysate was

subjected to mass spectroscopy. Differentially expressed proteins were identified and validated using quantitative real-time polymerase chain reaction (qRT-PCR) in the mice's blood.

Results

Enzalutamide treatment to mice for 8 weeks resulted in a modest weight gain and lack of attention and lethargies in these mice. Examination of H&E sections of the mice brain from the control group by light microscopy showed normal morphology of neurons in the cortex. Enzalutamide treatment showed lower neuron density and signs of neuron injury, as well as cytoplasmic swelling of the astrocytes. 18F-FDG-PET imaging demonstrated a loss in glucose activity in the mouse brain after 8 weeks of enzalutamide treatment. The mass spectrometric analysis identified a few differentially expressed proteins that were validated in the blood. These molecules include ABCB10, CAB, DAZAP1, DCU, DERL1, FBXO, MBNL, PUM2, SLC8A3, SLC9A, TCF20, VIM, and ZYXIN. Among them, the expression of SLC8A3, PUM2, and DERL1, were significantly higher compared to others in the enzalutamide-treated group.

Conclusion

Our findings demonstrate that enzalutamide treatment in mice demonstrates cognitive impairment similar to that observed in men on ADT protocol. Our study highlights that blood-based markers can be developed for ADT-mediated cognition that can lead to the identification of new strategies for prevention and early intervention to improve quality-of-life for prostate cancer survivors.

Grant Support: Department of Defense grants W81XWH-18-1-0618 and W81XWH-19-1-0720 to SG.

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ESR1+ luminal epithelial cells contribute to androgen-independent prostate survival in the absence of SRD5A2

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Abstract

INTRODUCTION AND OBJECTIVE: Steroid 5α reductase 2 (SRD5A2) is an enzyme responsible for converting testosterone into dihydrotestosterone (DHT), a potent androgen that plays a crucial role in prostatic development. Among the medications used to manage benign prostate hyperplasia (BPH), 5α reductase inhibitors (5ARI) are the only class known to reduce prostate size. However, the varying responses of patients to 5ARI therapies remain largely unexplained. Previous research has shown that approximately 30% of adult human prostatic tissues do not express the SRD5A2 gene and protein due to epigenetic modifications, however, continue to suffer from symptoms associated with BPH. Therefore, we developed a novel Srd5a2-/- mouse model and utilized single-cell RNA sequencing (scRNA-seq) to investigate alternate prostate growth patterns in the absence of SRD5A2.

METHODS: We generated homozygous Srd5a2-/- mice alongside littermate control heterozygous SRD5A2+/- mice. Using single-cell RNA sequencing (scRNA-seq), we obtained differentially expressed gene profiles to analyze cellular diversity. Furthermore, we evaluated the spatial expression of luminal epithelial 2 (LE2) markers through RNA fluorescence in-situ hybridization (RNA-FISH) and assessed cell proliferation by detecting Ki67 expression using flow cytometry. Additionally, immunohistochemistry was performed on prostate biopsies obtained from the NIDDK supported clinical trial of 5ARI for Medical Therapy of Prostatic Symptoms (MTOPS). Data was provided by NIDDK CR, a program of the National Institute of Diabetes and Digestive and Kidney Diseases. The expression of SRD5A2 and LE2 markers were correlated with AUA Symptom Score (AUASS).

RESULTS: We found that the prostate weight of Srd5a2-/- mice was significantly less compared to Srd5a2+/- mice ($p<0.001$). The analysis of scRNA-seq data revealed cellular heterogeneity and highlighted lineage plasticity within the cell populations. Notably, the LE2 subpopulation exhibited significantly enriched estrogen target genes. Following an SRD5A2-independent ESR1 differentiation trajectory in the absence of SRD5A2, LE2 cells displayed a higher proportion and a proliferative phenotype. Through RNA-FISH with Esr1/Pvt1 marker genes, LE2 cells were predominantly found in the anterior lobe of the prostate. Importantly, human prostate biopsy samples demonstrated an inverse correlation between the expression of SRD5A2 and the expression of LE2 markers, Esr1 and PKCα. Additionally, there was an inverse correlation between SRD5A2 expression and the 5-year change of AUASS.

CONCLUSIONS: Our research provides valuable insights into the heterogeneity of LE subtypes in the mouse prostate and establishes connections between these subtypes and human prostate LE cells, with potential implications in drug-resistant BPH. The findings suggest that the estrogenic signaling pathway may play a pivotal role in facilitating prostate growth in the absence of SRD5A2.

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Aire deficient mice as an emerging model for understanding the relationship between inflammation and BPH

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Abstract

Aire deficient mice as an emerging model for understanding the relationship between inflammation and BPH

Background: Benign prostatic hyperplasia (BPH) is the most common urologic disease in aging men, resulting in significant morbidity. BPH is complex, presenting with stromal and epithelial proliferation, fibrosis, lower urinary tract symptoms (LUTS), and associated inflammation. This complexity presents challenges in developing models for studying aspects of BPH. For example, studying the relationship between inflammation and BPH/LUTS requires immune-intact *in vivo* models. Here, we characterize an Aire deficient murine model, in which the Aire transcription factor (which eliminates self-reactive T cells) has been knocked-out, for the study of prostatic inflammation. This chronic inflammatory model has, except for central tolerance, a normal immune response.

Methods: Aire -/- C57BL/6 mice were subcutaneously injected with prostate homogenate protein and Freund's Complete Adjuvant and boosted after 10 days. After 21 and 35d prostates were collected for histology, flow cytometry, and scRNA-seq and compared to human BPH data from the transition zone of 10 small (<40 g) and 10 large (>90 g) prostates.

Results: Aire -/- mice have increased localized prostatic inflammation relative to controls, whereas Aire +/- mice have a predominantly wild-type phenotype based on necropsy, histology, and flow cytometry. Tertiary lymphoid structures (TLS), which arise in response to chronic inflammation, are identified in the Aire -/- mouse and human BPH prostates. We identify similar populations of cells in the human and Aire -/- mouse prostates, including an aging-associated Gzmk+ CD8+ T cell subset (Taa) that correlates with BPH symptoms in human patients. While the cellular composition of the 21d and 35d Aire -/- mice are similar, the proportion of Taa cells in the scRNA-seq data suggests they increase with age. A ligand-receptor analysis suggests potential interactions between Taa cells and other cellular subsets, including fibroblasts expressing Fn1 and senescence-associated secretory phenotype components Il6, Ccl2, and Cxcl1. This fibroblast subset is similar to a human population in which GZMK induces proinflammatory phenotypes.

Conclusion: Aire -/- mice develop inflammatory characteristics of human BPH, including the presence of Taa cells, which may exacerbate prostate inflammation with age through interactions with senescent fibroblasts. We anticipate this model will be useful in understanding the interplay between aging, the immune system, and BPH. Ongoing work aims to characterize Taa cells in mouse and human prostates.

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Dual targeting of HSP70 and AURKA improves treatment in neuroendocrine prostate cancer

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Abstract

Background: Neuroendocrine prostate cancer (NEPC) is the most aggressive type of prostate cancer with no effective treatment. Therefore, there is an urgent need to develop novel therapeutic strategies. N-Myc plays a key role in driving NEPC progression, and Aurora kinase A (AURKA) prevents N-Myc from degradation by interacting with N-Myc and blocking the intervention of E3 ubiquitin ligase SCF^{Fbxw7}. The AURKA inhibitor alisertib inhibits NEPC tumor growth by disrupting N-Myc signaling; however, it has recently failed in a phase II clinical trial for prostate cancer. In this study, we tested the synergy between the novel HSP70 allosteric inhibitor JG231 and alisertib in NEPC cells and patient-derived xenograft (PDX) organoid models, which will provide new approaches for NEPC treatment.

Methods: RT-PCR, western blotting, and immunohistochemistry (IHC) were used to determine the expression of N-Myc and neuroendocrine markers in different prostate cancer cell lines and PDX tumors. After knockdown of HSP70 and AURKA by siRNA, cell proliferation and N-Myc expression were assessed using cell viability assay, RT-PCR, and western blotting, respectively. A co-immunoprecipitation assay was performed to determine N-Myc ubiquitination levels. RNA sequencing was employed to determine the changes in gene programs regulated by JG231 in NEPC cells. The effects of JG231 and alisertib on cell proliferation were examined in NEPC cell lines and PDX organoid models.

Results: PC3, CWR22Rv1, H660, LuCaP93, and LuCaP173.1 PDX tumors had significantly increased expression of N-Myc and neuroendocrine markers (NSE, SYP, CHGA). Knockdown of HSP70 by siRNA or using the HSP70 inhibitor JG231 significantly inhibited the growth of H660 and CWR22Rv1 cells, leading to a combination effect with alisertib ($p<0.001$). JG231 combined with alisertib also inhibited the growth and induced the death of LuCaP93 and H660 tumor-derived organoids in a dose-dependent manner ($p<0.001$). Mechanistically, JG231 blocked neuroendocrine-related signaling pathways in NEPC cells. JG231 treatment inhibited the protein expression of N-Myc through the ubiquitin-proteasome system and promoted STUB1 entry into the nucleus to bind to N-Myc.

Conclusions: JG231, a novel HSP70 inhibitor, improved the treatment efficacy of the AURKA inhibitor alisertib against NEPC by regulating N-Myc protein homeostasis.

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Decoding the Heterogeneity and Plasticity of Prostate Cancer at the Single-Cell Level

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Abstract

Background: Androgen deprivation therapy has improved patient survival. Nevertheless, treatment resistance inevitably emerges due to the complex interplay of tumor heterogeneity and lineage plasticity. Methods: R and Linux packages were used for bioinformatics. Immunohistochemistry, immunofluorescence, and western blot were used to access protein expression. The 10X genomic V3 kit was used for scRNAseq. SCID mice were used for xenografts. Results: We integrated scRNAseq data from billions of cells, including both public cohorts and data generated in our laboratory, and generated the HuPSA (Human Prostate Single cell Atlas) and MoPSA (Mouse Prostate Single cell Atlas) datasets. Through unsupervised clustering and manually annotation, both atlases not only validated previously known prostate adenocarcinoma (AdPCa), neuroendocrine prostate cancer (NEPCa), stromal, and immune cell populations but also unearthed the less described populations including MMP7+ normal prostate club cells and two novel lineage plastic cancerous populations, namely progenitor-like and KRT7+ cells. Immunostaining experiments confirmed the presence of these populations in both human and mouse tissues, solidifying their significance in PCa biology. To unravel the drivers of these distinct cell populations, we calculated the upstream regulators of the genes enriched in these cells. Furthermore, leveraging the power of state-of-the-art bioinformatics analyses, we scrutinized over one thousand human PCa bulk RNAseq samples. Employing HuPSA-based deconvolution, we reclassified these samples into different molecular subtypes, including the newly discovered KRT7 and progenitor-like groups. The HuPSA and MoPSA provide invaluable blueprints for analyzing and interpreting external PCa single-cell RNAseq datasets. Moreover, employing supervised dimensional reduction and label transferring techniques, we mapped the scRNAseq data derived from C4-2B xenograft tumor onto HuPSA. Our analysis effectively unveiled treatment-induced populational heterogeneity and neuroendocrine differentiation. Conclusion: Our data elucidates the roadmap of PCa progression, showcasing the development of heterogeneous populations through lineage plasticity. This understanding holds promise for guiding the development of precise medicine in the PCa field.

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Sulforaphane reduces BBN-induced clonal expansion in bladders from male mice and slows tumor progression in male and female mice

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Abstract

Background: Bladder cancer is one of the most common cancers in the US, with an estimated incidence over 82,000 in 2023. It is 3-fold more prevalent in men, but women are often diagnosed with more advanced disease and often have poorer outcomes. While androgen and immune contexts play a role in the sex disparities of bladder cancer, the effect of chemopreventive agents is not well understood in the context of sex disparities. Carcinogen exposure is the greatest risk factor for bladder cancer development in men and women. N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) is a cigarette smoke-mimicking carcinogen that can induce urothelial cell proliferation and invasive bladder cancer in preclinical models. Sulforaphane (SFN) is an isothiocyanate isolated from cruciferous vegetables that has shown chemopreventive effects. SFN acts primarily through the activation of enzymes involved in carcinogen detoxification and inhibition of enzymes involved in carcinogenesis.

Methods: C57/BL6Tac mice were exposed to BBN through drinking water for 4 or 12 weeks (wk) to evaluate sex differences in urothelial proliferation and bladder pathologies. In a second experiment, SFN or vehicle was given at the time of BBN exposure and bladder specimens were H&E stained, and pathologic analysis was performed. Slides were also stained for Ki-67 to assess urothelial cell proliferation rates, and Nrf2 to determine SFN activity.

Results: Bladders from male mice had increased proliferation and progressive pathologies with 4-wk BBN exposure compared to female bladders. BBN exposure for 4-wk in male mice and 12-wk in female mice had continuous Ki-67 staining patterns in the basal layer and some proliferative areas indicating clonal expansion. SFN reduced tumor progression in bladders from both male and female mice exposed to 12-wk BBN, with reduced basal cell layer proliferation. There was also increased Nrf2 activation in bladders from SFN-treated mice.

Conclusion: The similar advanced proliferation pattern found in the 12-wk female mice and the 4-wk male mice demonstrates increased bladder tumor progression in male mice and suggests that male mice may have intrinsic mechanisms that increase the progression of bladder tumors. SFN treatment increases the activation of the Nrf2 pathway in urothelium, indicating one possible mechanism of chemoprotection. Clonal mutation expansion analysis of bladders treated with SFN will be performed with targeted sequencing to further evaluate these results.

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Targeted engineering mRNA 3'UTR length enhances immunotherapy response in prostate cancer

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Abstract

Background:

Global mRNA 3'untranslated region (3'UTR) shortening through alternative polyadenylation (APA) has been widely observed in most cancers; however, it has not been demonstrated whether targeted interference of specific oncogenic mRNA 3'UTR lengths can inhibit tumor growth and potentiate immunotherapy response in prostate cancer.

Methods:

DaPars algorithm was applied for *de novo* identification of dynamic alternative polyadenylation (APA) during prostate cancer progression to the lethal phase; 3'UTR polyadenylation site (PAS) locations and usages were identified by Poly(A)-ClickSeq (PAC-seq) and 3'RACE; APA transcripts were quantified by RHAPA assay; a 3'UTR CRISPR/dCas13 Engineering System (3'UTRCES) was developed to manipulate the length of desired 3'UTRs; RIP-qPCR and PAR-CLIP-qPCR assays were used to determine the mechanistic basis of 3'UTRCES in APA editing; RNA-seq was utilized to evaluate the off-target effects and molecular mechanisms underlying 3'UTRCES-mediated cell growth inhibition; Quantitative proteomics and immunoprecipitation assays were conducted to investigate how major histocompatibility complex (MHC) class I substrates were recognized by SPSB1-containing ubiquitin ligase complex for degradation; Lipid-like nanoparticles (LLN) were applied for intratumoral delivery of 3'UTRCES RNA molecules for targeted 3'UTR therapy.

Results:

3'UTR globally shortens during prostate cancer progression to castration-resistance. Through blocking the proximal PASs, 3'UTRCES efficiently and specifically reverses the 3'UTR shortening of novel APA-linked, clinically-relevant prostate cancer oncogenic mRNAs, such as *SPSB1*, leading to reduced *SPSB1* mRNA translation and prostate cancer cell proliferation. Notably, downregulation of SPSB1 protein by 3'UTRCES disrupts SPSB1-associated ubiquitin ligase complex and subsequently increases MHC class I protein stability and abundance, with the potentials to sensitize prostate cancer to immune checkpoint therapies. Lastly, intratumor injection of LLN encapsulating 3'UTRCES RNA molecules effectively and safely inhibits prostate tumor growth in engrafted and transgenic mouse models.

Conclusions:

Our results establish the concept of "3'UTR targeted therapy"for treatment of prostate cancer with broad applications to other cancers and other 3'UTR-related diseases.

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NMR-based metabolomics discriminates between biopsy-positive and biopsy-negative patients with elevated PSA.

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Abstract

Background:

Early diagnosis of prostate cancer (PCa) is decisive for curative treatment and is usually triggered by first measuring prostate-specific antigen (PSA) in the blood. According to the European Association of Urology (EAU) guidelines, patients with a clinical suspicion of PCa based on an elevated PSA and multiparametric magnetic resonance imaging of the prostate are suggested for biopsy. In about 20-25% of patients, the biopsy is negative despite a persisting elevated PSA. In this study, we aimed to identify a metabolite profile that could discriminate biopsy-negative from biopsy-positive individuals.

Methods: NMR-based metabolomics analysis using a Bruker AVANCE NEO 600 MHz spectrometer and lifespin´s proprietary profiling software was performed for 1.078 archived serum samples collected from 1066 male patients with elevated PSA levels, who underwent prostate biopsy. Patients were categorized into biopsy-negative (n=649, benign group) and biopsy-positive (n=429, cancer group). Detailed statistics and machine learning procedures were applied for model building (state-of-the-art and lifespin´s proprietary algorithms).

Results: We identified 36 metabolites, which were significantly different between benign and cancer patients. Among these, a key metabolite of the urea cycle was significantly higher in cancer-positive patients with a cohen`s d of -0.50. Notably, four other metabolites annotated to the urea cycle were significantly upregulated in sera of cancer-positive patients with cohen`s d values ranging between –0.14 and 0.37. Furthermore, we discovered significant alterations in choline metabolism, with two metabolites being significantly regulated in PCa patients. Of note, we also found substantial changes in citrate cycle and lipid metabolism.

Using a random forest classifier, we obtained a model discriminating benign from cancer patients based on the measured metabolite concentration. Three times repeated 10-fold cross validation was performed to measure the performance of the model, which achieved an area under the curve (AUC) of 0.77 (± 0.039). Feature importance for the classifier confirmed the metabolic pathways mentioned.

Conclusions: Overall, our large dataset revealed major metabolic changes in urea cycle and choline metabolism and our model performed better than commonly used parameters in discriminating biopsy-negative from biopsy-positive patients.

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Intercepting Androgen Receptor addiction in prostate cancer

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Abstract

Background: Prostate cancer (PC) is a leading cause of cancer-associated deaths in men. It is notable for a unique dependence on androgen receptor (AR) signaling, a fact that has been exploited for therapeutic benefit. However, the precise nature of a PC cell survival and growth by the AR is unclear. The AR cistrome comprises of thousands of genomic regions, and several hundred genes are transcriptionally regulated by AR activation. The present study aims to identify the direct AR targets and downstream cellular network(s) regulated by the AR that mediate PC cell survival, and that could highlight therapeutically vulnerable nodes for new treatment strategies beyond mere AR targeting.

Methods: We identified genes which display androgen-dependent upregulation through RNA sequencing analysis of AR-positive prostate cancer (ARPC) cells and pooled them with previously published nascent-RNA sequencing data along with AR variant (ARv7)-associated genes to generate a comprehensive "androgen-upregulated/ARv7-associated" gene list of over 1200 cellular genes. A pooled CRISPR_Cas9 deletion library was then generated which contained 10 guide RNAs (gRNA) against each gene. We leveraged ARPC models of diverse genomic backgrounds and subjected publicly available ARPC lines, namely LNCaP_FGC, LNCaP_C4-2B, 22Rv1, VCaP and LAPC4 cells to the pooled CRISPR screen. Additionally, we established cell line models of 2 LuCaP-Patient derived xenografts (PDX), namely LuCaP-35CR_CL and LuCaP-189.4_CL and employed them toward the pooled CRISPR screening of the desired gene set. Each of the cell lines were harvested at a final population doubling of 6 (PD6), the gRNAs identified by next-generation sequencing and data analyzed for dropouts with respect to the reference PD0 time point using the Model-based Analysis of Genome-wide CRISPR/Cas9 Knockout (MAGeCK) algorithm.

Results: We identified several genes which display common dependencies across all the ARPC models. These genes encompass a wide range of cellular networks and processes including transcriptional regulation, translational control, organelle homeostasis, stress response, cholesterol or lipid metabolism, and a few genes of underexplored functionalities. Validatory efforts using cell competition assays highlighted differential temporal effects on an ARPC cell proliferation, where the knockout of a subset of genes strongly impinged on growth properties while the deletion of others displayed a modest, albeit gradual proliferative decline. Interestingly, the former class exhibit varied cellular functions and may suggest multiple dominant cellular nodes of dependence for survival.

Conclusions: We screened 7 ARPC cell lines and identified a common or "core" androgen-regulated gene set, the continued expression of which seem necessary for the survival of all the ARPC models under consideration. We noted that although the MAGeCK analysis suggested genetic dropouts within the temporal confines of the primary screen, namely PD6, the individual dependencies are relative in nature and the genes can further be sub-categorized based on the strength of their respective dependencies. We conjecture that this strength is rooted in the cellular function of the corresponding gene product. Future efforts are being directed toward mechanistic dissection of these cellular functions to better understand the fundamental choices an ARPC cell overwhelmingly pivots onto for proliferative advantages and determine ways for therapeutic targeting to allow for continued rescindment of tumor growth.

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PDEF restricts Epithelial to Mesenchymal Transition and Resistance to AR targeted therapies in Prostate Cancer

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Abstract

PDEF restricts Epithelial to Mesenchymal Transition and Resistance to AR targeted therapies in Prostate Cancer

Background: Prostate-derived Ets factor (PDEF) is a member of Ets transcription family and is highly expressed in normal prostate tissue and early stage PCa. Previously we have shown that in clinical cohorts, PDEF is decreased during PCa progression and that PDEF impedes aggressive phenotype in androgen-independent prostate cancer (PC-3 and DU145 cells). In the present study we evaluated the role of PDEF in LNCaP cells.

Methods: PDEF was silenced using lentiviral shRNA while Flag-PDEF cloned into pBABE was used for ectopic PDEF expression. Cell growth , migration assay, colony formation assay, western blotting and, qPCR for AR and epithelial-mesenchymal transition (EMT) signaling pathway were performed in LNCaP vector control and LNCaP sh-SPDEF cells. We performed GSEA analysis of PCa patients in TCGA data set with low and high PDEF expression.

Results: SPDEF knockdown in LNCaP cells resulted in increased cell growth and are resistance to enzalutamide treatment. PDEF knockdown also resulted in increased cell migration and clonogenic activity. We also observed that PDEF knock down decreased expression of AR signaling (AR, PSA and FOXA1) and epithelial markers (E-cadherin, cytokeratin 18, and EPCAM), and concomitant increase in the expression of mesenchymal markers (N-cadherin, snail, Twist1, vimentin), and Stemness markers (LGR5, Sox2). Moreover, androgen deprivation of LNCaP cells also resulted in cellular plasticity (viz. NEPC markers) which was limited by ectopic PDEF expression. In TCGA data set we observed that PCa patients with high PDEF vs low PDEF have distinct transcriptional profiles and in prostate cancer patients' loss of PDEF is also associated with enrichment of EMT gene set similar to our in vitro data.

Conclusions: Our results demonstrate that loss of PDEF results in cellular plasticity, emergence of EMT phenotype in PCa, and resistance to AR targeted therapies. We postulate that PDEF serves as a cellular plasticity restriction factor in prostate cancer and that PDEF expression could serve as an excellent prognostic marker for therapy resistant prostate cancer.

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The pioneer factor FOXA1 is a novel interferon-suppressed gene in muscle invasive bladder cancer

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Abstract

Background: Forkhead Box A1 (FOXA1) is a pioneer transcription factor (TF) which plays a critical role in urothelial cell fate determination. While highly expressed in luminal bladder cancers of urothelium and it is highly expressed in luminal molecular subtype of human bladder cancer (BCa), *FOXA1* expression is frequently lost in basal-squamous (BSQ) disease, which is highly aggressive. We previously showed DNA hypermethylation contributes to decreased FOXA1 levels in a minority of BSQ cases. However, DNA methylation is insufficient to explain the high percentage of BCa which fails to express FOXA1. Cell intrinsic and extrinsic mechanisms responsible for FOXA1 regulation are poorly understood. Because BSQ BCa is highly inflamed, we hypothesized that the proinflammatory cytokine interferon gamma (IFNg) is a cell-extrinsic repressor of *FOXA1* in BSQ BCa.

Methods: Following treatment with IFNg in vitro, we performed RNA-seq on three human luminal subtype BCa cell lines , and performed differential gene expression analysis. Genes identified as being significantly upregulated or downregulated following IFNg stimulation were validated via q-RT-PCR and western blotting. IFNg stimulation activates Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) signaling. Therefore, we determined the impact of genetic and pharmacologic inhibition of JAK/STAT pathway components on IFNg-induced *FOXA1* repression. STAT1 is the primary transcriptional effector following IFNg stimulation. Therefore, ContraV3 analysis was used to identify putative STAT1 binding sites within *FOXA1* cis-regulatory regions. DNA pulldown assays and ChIP-PCR were used to determine the degree to which STAT1 binds to cis elements following IFNg treatment.

Results: IFNg treatment of three human luminal cell lines resulted in an average of 13,843 genes which were significantly (FDR, q<0.05) differentially regulated. Of specific interest, *FOXA1* expression was significantly downregulated in all three models of luminal BCa, and these results were confirmed by q-RT-PCR and western blotting. Knockdown experiments and pharmacological inhibition shows IFNg-mediated FOXA1 repression occurs in a manner dependent upon JAK1/2 kinases, as well as STAT1. ContraV3 analysis identified 8 putative STAT1 binding sites within the FOXA1 gene. DNA pulldown assays and ChIP-PCR studies show significant increases in STAT1 binding to cis-regulatory elements within the *FOXA1* gene.

Conclusions: Collectively, our results identify *FOXA1* as a novel IFNg-repressed gene. To the best of our knowledge, this is the first report of a role for interferons in the regulation of FOXA1. By extension, our results suggest that active IFNg signaling within the inflamed tumor microenvironment is a significant driver of reduced FOXA1 expression in BSQ BCa.

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Identification and characterization of an AR-negative castration-resistant prostate cancer that displays a mixed basal, club, and hillock cell identity driven by KLF5

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Abstract

Background:

While the majority of localized prostate cancer can be cured with surgery or radiation, metastatic disease is lethal. Therapies targeting the androgen receptor (AR) are initially effective, but eventually men will develop castration resistant prostate cancer (CRPC). About 70-75% of CRPC displays restored AR signaling (ARPC), while the remaining 25-30% display lineage plasticity evidenced by the emergence of AR-negative subtypes including those that display neuroendocrine (NE) markers, or double negative CRPC (DNPC) lacking both AR and NE markers. AR independent manifestations of CRPC represent a clinical challenge because no effective therapies are available and therapeutic targets are largely unknown.

Methods:

We analyzed publicly available bulk and single-cell RNA-seq datasets derived from clinical CRPC specimens to characterize the cellular identity of AR-negative tumors. Using a database of all known transcriptional regulators, we identified potential drivers of these phenotypes. We performed siRNA-mediated knockdown experiments in two models of DNPC to explore the extent to which the candidate regulators maintain AR-negative cell identity and sustain growth of DNPC prostate cancer cells.

Results:

We identified a subset of CRPC tumors that co-express gene sets defining basal, club, and hillock cells in the benign prostate. This data supports the existence of epithelial cell lineage plasticity manifesting within CRPC tumors, as well as individual CRPC tumor cells, wherein AR-positive tumor cells transform to a phenotype encompassing basal, club, and hillock cell identities. Supporting this, prostate cancers display evidence of this cell lineage transformation after just 3 months of enzalutamide therapy. We nominated the stem cell transcription factor KLF5 as a regulator of basal, club, and hillock cell identities in CRPC. In cell line models of DNPC, knock down of KLF5 inhibited cell growth and reduced the expression of genes defining basal, club, and hillock cell identities.

Conclusions:

This work links a prevalent and poorly-defined subtype of AR-negative CRPC to AR-negative epithelial cell types in benign prostate tissue. Inhibition of KLF5 and its downstream effectors could represent a therapeutic strategy to prevent or delay lineage plasticity and thereby extend patient response to AR-targeted therapies.

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A TBX2-driven signaling switch from Androgen Receptor to Glucocorticoid Receptor confers enzalutamide resistance in Prostate Cancer

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Abstract

Background: Recent studies suggest that glucocorticoid receptor (GR) activation can cause enzalutamide resistance in advanced prostate cancer (PCa) via functional bypass of androgen receptor (AR) signaling. However, the specific molecular mechanism(s) driving this process remain unknown. In an effort to identify drivers of prostate cancer progression, in a previous study, we determined that TBX2, a developmental T-box transcription factor (TF) master regulator, is over-expressed in CRPC and drives bone metastatic progression. A recent report confirmed that TBX2 and GR are two of the four TFs that drive enzalutamide resistance in advanced PCa. Our current study demonstrates that TBX2 with known repressor and activator functions, is the molecular switch that represses AR levels while activating GR expression thereby resulting in the replacement of AR signaling to control tumor growth.

Methods: We genetically modulated TBX2 using multiple approaches: a) dominant negative, DN, to block TBX2 (TBX2DN), and b) overexpression, OE, to increase TBX2 expression (TBX2OE), c) shRNA mediated knockdown (shTBX2). RNA-seq was performed, and qRT-PCR, Western blot and immunohistochemical (IHC) analyses were used for validation. Further, we used chromatin immunoprecipitation (ChIP) and site directed mutagenesis (SDM) to confirm TBX2 binding on the AR promoter. We also used co-immunoprecipitation (Co-IP) to determine protein partners of TBX2.

Results: Mechanistically, our studies revealed that TBX2 bound to the promoters of both AR and GATA2, an AR coregulator, thereby resulting in a bimodal repression of AR expression. Conversely, TBX2 upregulated GR via direct GR promoter binding and TBX2-GR protein-protein interaction. Together, concurrent repression of the AR and activation of GR resulted in enzalutamide resistance. Importantly, we found that SP2509, an allosteric inhibitor of the demethylase-independent function of LSD1, a TBX2-interacting protein in the COREST complex, can disrupt both TBX2-LSD1 and TBX2-GR protein-protein interactions thereby uncovering a unique mode of SP2509 action in CRPC.

Conclusions: In summary, our study identifies TBX2 as the molecular switch that drives the AR to GR signaling bypass thereby conferring enzalutamide resistance. Further, our study provides key insights into a potential therapeutic modality for targeting the AR to GR signaling switch via disruption of the TBX2-LSD1 and TBX2-GR protein-protein interactions

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Tumor intrinsic lipid dysregulation induces immunometabolic reprogramming to create a suppressive prostate tumor microenvironment

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Abstract

Background: Dysregulated lipid metabolism and elevated lipogenic pathways are hallmarks of prostate cancer (PC) progression. However, the impact of the tumor intrinsic dysregulated lipid metabolism on the tumor microenvironment (TME) remains relatively unexplored. Previous studies from our lab and others have shown that increased synthesis of mitochondrial metabolite citrate a precursor molecule generating acetyl CoA required for de novo lipogenesis functions as a key regulatory step. PC utilizes glutamine derived alpha-ketoglutarate for reductive carboxylation to generate citrate catalyzed by mitochondrial enzyme aconitase 2 (ACO2). Genetic inhibition of ACO2 significantly reduced de novo lipogenesis and eventually regressed tumor growth in syngeneic PC mouse models. Since ACO2 loss reduces total lipid levels, we performed single cell RNA sequencing (scRNASeq) using wildtype (WT) and ACO2 depleted tumors to investigate the effects of dysregulated lipid metabolism on TME.

Methods: To identify the changes in the TME, scRNAseq was conducted on tumors isolated from syngeneic C57/B6 mice after injection of wildtype and ACO2 depleted TrampC2 cells. To study the effects on macrophages, bone marrow derived macrophages (BMDM) from non-tumor bearing mice were incubated with tumor conditioned media collected from MyC-Cap and TrampC2 control and ACO2 depleted cells. Macrophages (CD11b+/ F4/80+) were characterized by surface marker expression using MHCII and CD206 and gene expressions of Nos2, Il12a, Arg1 and CD206.

Results: ScRNAseq data revealed that the macrophages were significantly polarized, with pro-inflammatory M1-like macrophages being associated with the ACO2 depleted tumors, compared to WT tumors which displayed increased enrichment of pro-tumorigenic M2-like. By incubating BMDMs with tumor conditioned media, we observed a similar shift to a more M1 like population when incubated with ACO2 depleted media compared to WT. Our observations indicate that secreted factor/metabolite due to dysregulated lipid metabolism may be responsible for the polarization.

Conclusions: Current studies are directed towards identifying the secreted factors by LC/MS followed by deciphering the mechanisms involved in switching macrophage phenotype within the TME. Our studies will demonstrate that modulating lipid metabolism in PC may be sufficient to alter immune cell populations in the TME that could be therapeutically exploited in future.

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Development and evaluation of EZH2 PROTACs

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Abstract

Background: Enhancer of zeste homolog 2 (EZH2), a catalytic subunit of polycomb repressive complex 2 (PRC2), is significantly upregulated in advanced prostate cancer (PCa), with particularly high levels observed in castration-resistant prostate cancer (CRPC). However, inhibitors of EZH2 enzymatic activities, such as EPZ-6438, have limited efficacy in prostate cancer. Proteolysis targeting chimera (PROTAC) is a novel class of small molecules that recruits an E3 ubiquitin ligase to a specific protein, leading to its ubiquitination and subsequent degradation by the proteasome. Therefore, we hypothesize that EZH2 PROTAC can induce complete and sustained depletion of EZH2 proteins, thereby resulting in greater efficacy in suppressing CRPC in vitro and in vivo.

Methods: We performed mass spectrometry, Co-IP, and western blot analyses to assess the PROTAC degradation efficacy. We utilized Cell Titer-Glo, live-cell imaging, and Colony formation to investigate the effect of PROTAC on inhibiting PCa cell proliferation in vitro. We tested the EZH2 PROTACT Pharmacokinetic (PK) variability and maximum tolerated dose (MTD) on CD1 mice. RNA-seq was performed in LNCaP and 22Rv1 cell. Furthermore, we gave drug treatment to evaluate PROTAC effects on 22RV1 xenograft tumor growth and performed IHC assess changes in EZH2 and H3K27 trimethylation.

Results: We demonstrated that EZH2 PROTAC degrades EZH2 proteins in a dose-dependent manner and requires VHL expression. Cell viability and proliferation has been assessed in several PCa cell lines, the results showed that PROTAC had a modest inhibitory effect on LNCaP and C4-2B cells, while no significant effect was observed on PC3 and DU145 cells. Interestingly, PROTAC demonstrated a significant inhibitory effect on the proliferation of 22Rv1 and VCaP cells. RNA-seq data shows 141 PROTAC and EPZ up-regulated genes are significantly expressed more upon PROTAC treatment in 22Rv1 cells compare to LNCaP cells. Those genes are enriched in pathways that identified relating to arrest cell proliferation and promote apoptosis. PROTAC modestly reduced 22RV1 xenograft tumor growth in vivo.

Conclusion: In this study, we designed, synthesized, and evaluated a VHL-based EZH2 PROTAC 6272. The in vitro and in vivo results indicate that EZH2 PROTAC 6272 is a potential therapeutic approach for the treatment of some PC cells. The mechanism for this differential effect of the PROTAC in various cell lines is under investigation.

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SUMOylation of FLCN is Required for its Tumor Suppressor Activity

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Abstract

Germline mutation of the tumor suppressor *FLCN* (Folliculin) is associated with the rare inherited cancer syndrome Birt-Hogg-Dubé (BHD). BHD patients develop kidney tumors, pulmonary cysts, and benign skin tumors (fibrofolliculomas). Canonically, FLCN participates in the regulation of mTOR through its role as a GTPase-activating protein for the GTPase RagC. However, the precise mechanism of FLCN tumor suppressive function is unknown. Notably, it was previously shown that loss of FLCN leads to increased lactate dehydrogenase A (LDHA) activity. Our published work revealed that FLCN binds to and inhibits LDHA activity in normal cells to regulate the Warburg effect. Although mutations in FLCN generally lead to instability of the protein, the functional impact of these mutations, which are distributed throughout its coding sequence, remain elusive. Here, we engineered and exogenously expressed 19 pathogenic mutations of FLCN in the *FLCN*-null UOK257 cell line, which is established from a patient with BHD syndrome. The protein expression of these FLCN mutants were generally divided into two categories: stable and unstable. Treatment of cells expressing the unstable FLCN mutants with the proteasome inhibitor bortezomib stabilized the FLCN mutants. This stabilization restored FLCN interaction with LDHA and consequent inhibition of the enzyme's activity. Furthermore, we have discovered a lysine site within FLCN that facilitates FLCN binding to LDHA, which is subject to SUMOylation. Although pathogenic mutation of this lysine site does not impact the stability of FLCN, the lack of SUMOylation abrogates FLCN binding to LDHA and consequently leads to LDHA hyperactivity. Taken together, our data presented here provides a complex landscape of the impact of pathogenic mutations towards FLCN function and provides a strategy to restore the tumor suppressive activity of FLCN.

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TBX2 signaling drives prostate cancer bone metastatic phenotype through miR-375-3p/RBPJ signaling axis via exosomes

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Abstract

Background: Bone is the preferred site of metastasis in about 80% of advanced prostate cancer (PCa) patients. Recent studies have revealed that exosome secretion by prostate cancer (PCa) cells fosters multiple facets of tumor progression and metastasis, in part mediated by exosomal micro RNAs (miRs) that dictate gene expression changes. However, the molecular mechanism(s) of how PCa metastatic progression through which transfer of exosomal miRs orchestrates bone remodeling that in turn facilitates the establishment of a pre-metastatic niche (PMN) at the distant bone metastatic site remain elusive.

Methods: In this study we used Quantitative real time PCR (qRT-PCR) , Western blot Analysis, immunohistochemistry (IHC) for expression analysis. We also performed wound healing assay , Exosome isolation , Next generation sequencing and analysis, Genetic manipulation of miR-375-3p , Tumor Xenograft experiments in mice.

Results: We have identified that TBX2, a transcription factor that we previously reported plays a key role in PCa bone metastasis, downregulates a repressive - miR-375-3p - thereby resulting in de-repression/upregulation of its target RBPJ, an effector of Notch signaling and an established driver of PCa metastasis. Further, the unbiased identification of miR-375-3p as the topmost TBX2-downregulated miR in combination with experiments that genetically manipulate miR-375-3p in the context of TBX2 modulation - point to a crucial biological function of miR-375-3p as a key mediator in RBPJ regulation exerted by TBX2. Further, exosomes derived from human PCa cells blocked for endogenous TBX2 resulted in significant decreases in: a) proliferation and migration of human PCa cells, and b) osteoblast differentiation/bone remodeling - in an paracrine/endocrine manner. Further, using the c-bioportal human PCa database, we observed that TBX2 is positively associated with RBPJ.

Conclusions: Taken together, our study unravels the role of TBX2 in directing changes in the distant PCa bone microenvironment via a paracrine/endocrine mode mediated through the transfer of exosomal-miRs. In addition, the study highlights a dual level of RBPJ regulation by TBX2/miR-375-3p signaling through changes in gene expression at the: a) intra-cellular or autocrine level, and b) inter-cellular or paracrine/endocrine mode mediated via exosomal transfer.

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Molecular Profiling of Bone Marrow Neutrophil Immune response to Bone Metastatic Prostate Cancer Cells

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Abstract

[Background]

Bone metastatic prostate cancer (BM-PCa) remains a major cause of cancer-related mortality in men worldwide. Immunotherapy shows promise in cancer treatment, but cold tumors like prostate cancer exhibit limited responses due to low immunogenicity. We previously reported varying responses in the tumor-killing capability of bone marrow neutrophils against distinct BM-PCa cell lines, and that immature bone marrow neutrophils hinder T cell proliferation. In this study, we aimed to elucidate the underlying reasons behind the selective tumor-killing effectiveness of bone marrow neutrophils.

[Methods]

We evaluated the killing ability of bone marrow neutrophils against three human BM-PCa cell lines (C42B, PC3, and PCa2b). Neutrophils were isolated from bone marrow of a healthy 55-year-old male and co-cultured with PCa cells either overnight (for cytotoxicity assay) or for 3 hours (for gene expression analysis). After culture, remaining cancer cells were quantified using Trypan blue staining to measure neutrophil killing capacity. We investigated the genetic effects of BM-PCa cells on bone marrow neutrophil differentiation using single-cell RNA sequencing (scRNA-seq).

[Results]

After overnight culture, neutrophils were able to partially eliminate C42B, with 56% of cells remaining. Conversely, no significant impact on the survival of PC3 and PCa2b cells was observed. scRNA-seq analysis unveiled distinct gene expression patterns. Notably, neutrophils co-cultured with C42B are clustered into two distinct populations. One population showed heightened expression of CCL3 and CXCL8, indicative of an activated neutrophil subset, while the other exhibited elevated expression of MPO and ELANE, suggesting an immature subset. Gene ontology analysis pointed to an enrichment of processes such as cellular respiration and oxidative phosphorylation in the mature neutrophil population, both intricately linked to ATP generation. Notably, neutrophils cultured with PC3 formed two groups, but without the observable upregulation of genes associated with activated neutrophils. Interestingly, neutrophils co-cultured with PCa2b did not show significant changes in gene expressions compared to control neutrophils.

[Conclusion]

Bone marrow neutrophils demonstrated activation and proficient tumor-killing capacity of C42B, in contrast to their interaction with other cell lines. Understanding the mechanisms that define neutrophil anti-tumor immune response holds potential implications for cancer immunotherapy and the treatment of BM-PCa.

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Targeting undruggable transcription factor HOXB13 in metastatic prostate cancer by CRISPR/Cas13d-based nanoparticle therapy

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Abstract

Background:

The oncogenic transcription factor HOXB13, which is highly expressed in castration-resistant prostate cancer (CRPC), has been shown to promote CRPC growth and metastasis. However, HOXB13 is considered untargetable by traditional small-molecule-based drug design. Gene therapy is critical alternative strategy with potential to directly target such traditionally undruggable genes.

Methods:

Selective cell in organ targeting (SCORT) nanoparticles for precise delivery of nanoparticles to metastatic cancer cells in an organ were constructed and characterized by the NanoAssemblr Spark, Zetasizer Nano ZS, and transmission electron microscopy (TEM); Cas13d mRNA with pseudouridine modification was synthesized using in vitro transcription; Hemi-spleen injection of CRPC cells was performed to build CRPC liver metastatic models; RNA-seq was employed to evaluate off-target effects of Cas13d targeting.

Results:

By incorporating a prostate cancer-specific E3 aptamer to functionalized lipid-like nanoparticles (FTT5 LNP-E3), we have successfully constructed SCORT nanoparticles that enable preferential delivery of mRNAs to metastatic CRPC cells in the liver, as opposed to various normal cells in the surrounding liver tissue. We demonstrated that Cas13d-pre-gHOXB13 mediates highly effective and specific HOXB13 mRNA knockdown in CRPC cells. Significantly, systemic treatment of SCORT nanoparticles carrying Cas13d-pre-gHOXB13 mRNA decreased HOXB13 expression in the metastatic tumors, inhibited metastasis, and prolonged survival of mice bearing androgen receptor (AR)-positive (AR+) or negative (AR-) tumors. Notably, long term (6 and a half weeks) administration of SCORT nanoparticle-Cas13d-pre-gHOXB13 did not significant alter body weight, hepatic and renal function, chemokines and cytokines, and other factors, effectively highlighting its safety.

Conclusions:

This study is the first to demonstrate that undruggable oncogenic transcription factors can be targeted using nanoparticle-delivered gene therapy based on CRISPR/Cas13 RNA-targeting. The SCORT-CRISPR/Cas13d system is a highly flexible technology that would impact the larger field of translational science by allowing the design of Cas13d-based, cancer cell-specific delivered nanoparticles targeting other previously-undruggable oncogenic transcription factors in metastatic prostate cancer and other solid tumors.

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ASCL1 acts in concert with the chromatin remodeler SWI/SNF complex in prostate cancer lineage plasticity

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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). We have recently shown that an extensive epigenetic dysregulation underlies the conversion of CRPC to NEPC, governed by the neuronal lineage determinant transcription factor ASCL1. Importantly, loss of ASCL1 resulted in the collapse of the chromatin leading to reduced expression of neuronal and plasticity markers and overall abolishment of the NEPC program. It is still unclear how ASCL1 influences chromatin remodeling to bias cell fate toward a neuroendocrine lineage in prostate cancer and if the ASCL1-high population is vulnerable to targeting the chromatin remodeling factors. Thus, delineating the mechanism by which ASCL1 influences lineage plasticity could represent novel vulnerabilities that can be exploited for treating ASCL1-driven advanced prostate cancer. We hypothesized that ASCL1 acts in coordination with the chromatin remodeler SWI/SNF complex to induce a rapid lineage switch to drive the emergence of a highly plastic neuroendocrine state.

Method: To elucidate the reliance of ASCL1 pioneering function on chromatin remodeling activity of SWI/SNF complex and the role of SWI/SNF in shaping the chromatin landscape associated with ARPIs induced lineage plasticity and NEPC phenotype, we will utilize cell lines and patient-derived xenografts including CRPC with Dox-inducible ASCL1 or ASCL1 knockdown under the pressure of ARPIs, as well as NEPC following ASCL1 knockdown. We will perform RNA-seq, ATAC-seq, and ChIP-seq for ASCL1 and BAF155 (core SWI/SNF complex member). In addition, to assess the clinical relevance of our findings we will assess the effect of SWI/SNF inhibitor (FHD-286 currently in clinical trials) sequentially or in combination with γ-secretase inhibitor (MK-0752 used in multiple clinical trials) in ASCL1-high NEPC cell lines and patient-derived organoids *in vitro* and *in vivo* to establish synthetic lethality.

Result: We identified high-confidence ASCL1 binding sites in both accessible and inaccessible regions of NEPC, correlated with enhancers regulating stem and neuronal programs, reminiscent of ASCL1's pioneering function. We performed large-scale integrated genome analysis (GIGGLE) and revealed a correlation between SWI/SNF complex and ASCL1-dependent accessible regions, as well as ASCL1-bound regions. Unbiased proteomic (using proximity biotinylated-based approach) identified ASCL1 interacting with chromatin remodelers, specifically several subunits of the SWI/SNF complex. Interestingly, using a PROTAC degrader, we observed that targeting SWI/SNF ATPases downregulated ASCL1 expression, downstream neuronal programs, and proliferation. Interestingly, SWI/SNF inhibition resulted in a lineage switch to a non-neuroendocrine (NE) state, characterized by an increase in Notch signalling and enrichment of stemness pathways. Our observation is in concordance with previous studies reporting activation of the Notch pathway resulting in a switch from NE to non-NE in small-cell lung cancer. Our data support the notion that targeting the SWI/SNF complex in ASCL1-high NEPC is not sufficient to induce conditional lethality and supports the concept

of combination therapy. Hence, rationalizing co-targeting SWI/SNF and Notch signaling as potential therapeutic options for ASCL1-induced NEPC.

Conclusion: This project is centered on testing a new paradigm that involves the cooperation between ASCL1 and the SWI/SNF complex to facilitate lineage plasticity and the development of NEPC. Our goal is to target the epigenetic vulnerabilities of ASCL1-high NEPC tumors using patient-derived avatars and clinically available drugs. These drugs offer a potential therapeutic strategy and lay the groundwork for subsequent clinical trials.

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Prostate Cancer Susceptibility: Epithelial Stem Cell Changes by Arsenic and Lead

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Abstract

Background: The contamination of drinking water by inorganic arsenic (iAs) and lead (Pb) poses a significant public health crisis, with concerns also arising from open-burn-pit exposure for military personnel. Prostate cancer (PCa) stands as the second-leading cause of cancer-related death among American men. The International Agency for Cancer Research classifies iAs as a carcinogen, while Pb is considered a potential carcinogen in humans. Numerous epidemiological studies have indicated that increased exposure to either metal leads to higher PCa incidence and/or mortality rates in the general population.

Methods & Results: To explore this further, we conducted a small human study involving 40 PCa patients and 39 non-PCa patients attending a urology clinic. Analyzing a panel of 16 metals in their urine, we discovered that iAs and Pb levels were doubled in PCa patients compared to non-PCa patients. Employing a 2-hit animal model, where mice were exposed to metals for one month followed by chemical carcinogen treatment, we found that iAs and Pb significantly increased PCa incidence and pre-cancerous lesions in vivo.

Subsequently, we isolated prostate epithelial stem-like cells (PrESCs) from mice treated with iAs or Pb for one month, comparing them to PrESCs from untreated controls. Our observations indicated a significant increase in PrESCs, particularly the luminal P cell population, primarily found in the proximal ducts of the dorsal prostates (DP) in iAs-treated mice, with a similar trend in Pb-treated mice. Using single-cell RNA sequencing, we discovered that iAs-treatment disrupted the expression of genes in cancer- and organismal injury-pathways within the luminal P PrESCs of the murine DP. Notably, these genes are associated with beta-catenin, Erk1/2, and Tgf signaling pathways, suggesting a potential perturbation by metal exposure in PrESCs. This gene signature also predicts human PCa recurrence-free survival in the TCGA cohort.

Conclusions: Our findings suggest that iAs-induced neoplastic transformation of normal PrESCs may play a role in PCa initiation. This discovery holds significant implications for early detection of prostate cancer and identify new therapeutic targets.

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ceRNA Regulation of Prostate Fibrosis

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Abstract

Title: ceRNA Regulation of Prostate Fibrosis

Background:

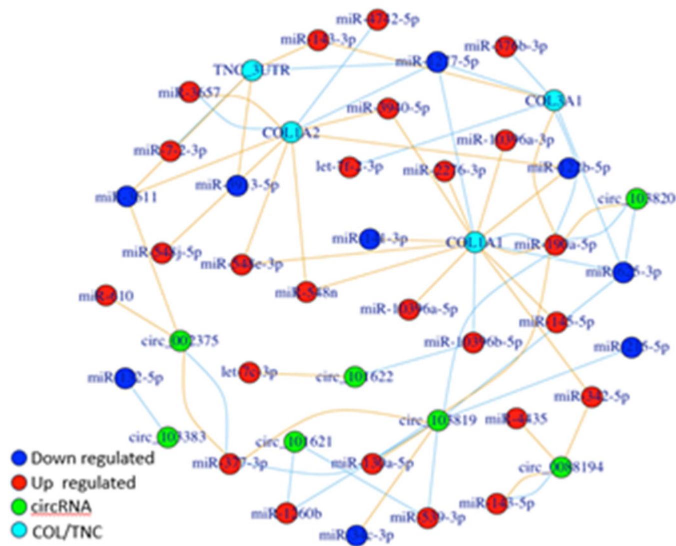
IL-4/IL-13 have been shown to increase fibrosis-related ECM proteins in prostate fibroblasts during LUTS development. Rather than focusing on signaling proteins that regulate ECM gene transcription, a new approach is to focus on understanding how regulatory RNAs control translation. While miRNAs are known to downregulate target transcripts and prevent translation, newly described circular RNAs (circRNAs) can inhibit this downregulation by “sponging” miRNA. This study aims to identify differential expression of miRNAs and circRNAs in prostate fibroblasts exposed to IL-4/IL-13 and elucidate miRNA/circRNA interactions that may contribute to overexpression of ECM proteins in LUTS development.

Methods:

ircRNA/miRNA expression from IL-4/IL-13 treated prostate stromal fibroblasts was identified via circRNA microarray / miRNA sequencing, and public databases and Qiagen CLC Genomics WB software were used to identify differentially expressed circRNA as well as potential miRNA binding partners. Expression of target circRNA/miRNA was validated using RT-qPCR and protein levels of bioinformatically-identified ECM protein targets were validated through immunoblotting.

Findings:

Several circRNA/miRNA were significantly differentially regulated in response to IL-4/IL-13. The mostly highly upregulated circRNAs were generated from transcripts of previously identified pro-fibrotic genes including circTNC (tenascin), circLIFR, and circCEMIP. miRNA sequencing showed significant enrichment of miRNAs identified as binding partners to these upregulated circRNAs including miR-190a-5p and miR-143-5p, which also have binding sites in the 3’ UTRs of COL1A1, COL3A1, and TNC transcripts. Western blot results show these ECM proteins are upregulated in response to IL-4 and IL-13.



Conclusions:

IL-4/IL-13 leads to significant increases in the protein level of several fibrosis-associated ECM proteins including COL1A1, COL3A1, and TNC in prostate stromal fibroblasts. Validated circRNA/miRNA analysis shows the potential for a regulatory circRNA-miRNA-ECM protein axis in prostate stromal fibroblasts. Further work showing direct binding as well as miRNA/circRNA knockdown and overexpression are required to demonstrate this.

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Role of ENTPD1 in Bladder Function

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Abstract

Introduction: Abnormalities in purine metabolism and purinergic contractility are commonly seen in patients with lower urinary tract symptoms (LUTS), but the underlying mechanisms remain incompletely understood. We have previously reported ENTPD1, a major ATP to ADP/AMP converting enzyme is strongly expressed in bladder smooth muscle (BSM), and mice BSM lacking of Entpd1 exhibits abnormal purine metabolism. However, the importance of Entpd1 in regulating bladder function is unknown.

Methods: Entpd1-/- mice were used to determine its role in regulating bladder function. Entpd1+/- mice were used to mimic the impaired ATP hydrolysis activity observed in LUTS patients. CD39TG mice, which express extra human copies of Entpd1 gene and exhibit increased ATPase activity were investigated on its bladder phenotype. Voiding spot assay (VSA) and cystometrogram (CMG) were used to evaluate the in vivo bladder function, and in vitro mygraphy and pharmacological studies were used to evaluate the impact of different Entpd1 expression levels on BSM contractility.

Results: VSA revealed that Entpd1-/- mice exhibited significantly increased number of voids and decreased size of individual voids. Entpd1+/- mice showed a phenotype similar to Entpd1-/- mice. On contrary, CD39TG mice have reduced number of voids and significantly larger individual voids. Consistently, CMG studies indicated shortened voiding intervals and reduced compliances in both Entpd1-/- and Entpd1+/- mice, but exhibited an opposite urodynamic changes in CD39TG mice. Both Entpd1-/- and Entpd1+/- mice have reduced BSM contractile forces in response to electrical field stimulation, and their purinergic contractions are particularly diminished. Interestingly, CD39TG BSM did not show contracile force difference compared to wild type controls, despite the significant difference in in vivo voiding phenotype.

Conclusion: Bladder function is finely regulated by Entpd1 expression levels, and deficiency of Entpd1 causes voiding frequency and small voids. Further molecular studies will reveal detailed underlying mechanism.

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Interplay Between Human Papillomavirus and Penile Cancer Microbiota

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Abstract

Background: Penile cancer (PeCa) is a rare but highly morbid disease. A significantly higher incidence and mortality rate among Puerto Rican men has been shown in comparison to other Caucasian, African American or Hispanic US populations. However, the cause for the disparity is unknown. Infection with human papillomavirus (HPV) has been identified as a risk factor for an average of 48% of PeCa cases in collected series. Tumor infection with high-risk HPV was shown to be a favorable prognostic factor for survival when compared to HPV negative PeCa. The molecular etiology of HPV+ and HPV- PeCa remains poorly understood, accounting for the limited treatment options. To date, the role of the microbiota in the pathogenesis of PeCa is unknown, as there are no studies that characterize the microbiome in association with penile cancer. Therefore, there is an urgent need to address this knowledge gap. Our objective was to determine the bacterial communities associated with HPV-positive and HPV-negative penile cancer.

Methods: Genomic DNA was extracted from biopsies of 51 patients, followed by HPV genotyping with the LiPA25 kit and microbiota analyses using 16S rRNA genes with the Illumina MiSeq platform. Demultiplexed data was deposited in QIITA for quality control and bioinformatic analyses and downstream analyses including alpha and beta diversity, taxonomic characterization, and biomarker analyses were done in R and Qiime2, highlighting the HPV status and tumor histology variables.

Results: We found that HPV positive samples with high grade histology have higher alpha diversity and abundance of Prevotella and Actiobacullum. HPV positive samples had higher levels of Actinobacteria, including Actinomyces europaeus, Mobiluncus sp. or Corynebacterium. HPV negative samples with high grade histology had higher levels of Sneathia, Gardnerella and even Lactobacillus. For the histology grade category alone, we found a reduction of Proteobacteria in high grade lesion, and an increase in Firmicutes and Actinobacteria in the intermediate and high-grade lesions, including Veillonella parvula, Corynebacterium kroppenstedtii and Actinomyces.

Conclusions: We found lipophilic and anaerobic bacteria associated with HPV and high-grade tumor lesions, that may be involved in triggering inflammatory responses and oncogenesis. Although many challenges must be overcome to dissect the specific interactions of coinfecting bacteria during the penile cancer infectious process, our findings demonstrate that microbes may be involved in these cellular processes.

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Activation of Pparg in the renal urothelium preserves parenchyma during 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. Patients undergoing ureteropelvic junction obstruction evaluation face subjective surgical indications and uncertain outcomes. No treatments can prevent CKD/ESKD; however, many patients evade surgical requirements and CKD/ESKD risk, indicating their kidney may have mounted a renoprotective adaptation. Future therapies may be identified by understanding how the kidney adapts to UTO. We previously discovered that congenital and acquired obstructions trigger the formation of protective uroplakin (Upk) cells, which contribute to renal parenchymal preservation. This suggests that efforts to increase Upk may have therapeutic utility for patients with UTO. Regulation of Upk cell formation in the renal urothelium is unknown, but in the bladder, Pparg drives urothelial differentiation and Upk formation. We hypothesize that Pparg drives UTO-induced Upk cell formation in the kidney.

Methods: Kidneys were collected from sham and unilateral ureteral obstruction (UUO) mice. Pparg was deleted (*Upk2*^{iCre};*Pparg*$^{fl/fl}$ -LOF;) or activated (*Upk2*^{iCre};*VP16-Pparg*$^{fl/+}$ -GOF) in Upk cells in vivo. Cre(-) mice served as controls. Immunofluorescent Analysis (IF-A) and renal ultrasound were used to evaluate the effect of Pparg pathway manipulation during UTO. Results were analyzed using One-Way ANOVA.

Results: Pparg was rarely detected in the renal urothelium of sham-operated mice; however, UUO triggered increased expression of Pparg (3.49% Sham, 22.78% UUO, Pparg index, P=0.0182) and its targets (Grhl3, Fabp4). At UUO post operative day 7, LOF mice had less Pparg (9.44% Cre(-), 1.35% LOF, Pparg index, P<0.0001), less Upk expression (17.57% Cre(-), 5.94% LOF, Upk positive area, P=0.0115) and reduced renal parenchyma (38.0% Cre(-), 25.9% LOF, P=0.0002), while GOF mice had increased Pparg (5.94% Cre(-), 16.35% GOF, Pparg index, P=0.0179), more Upk (17.19% Cre(-), 20.52% GOF, Upk positive area, P=0.0114) and exhibited parenchymal preservation (28.8 % Cre(-), 44.4% GOF, P=0.002).

Conclusions: Our results reveal that Pparg signaling promotes Upk cell formation and parenchymal preservation during UTO. Our findings advance our understanding of renal adaptation to UTO and reveal Pparg as a therapeutic target. Future studies will investigate whether Pparg activation supports functional preservation during UTO and whether pharmacological activation of Pparg mitigates UTO-induced kidney injury.

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Notch Signaling 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 CKD in children. Our lab has discovered that the renal urothelium undergoes a renoprotective adaptation during UTO, including the formation of differentiated Uroplakin (Upk) cells. However, little is know about renal urothelium, including signaling pathways that govern Upk expression and renal urothelial differentation. The Notch signaling pathway regulates bladder urothelial differentiation and single cell RNAseq data suggest Notch may play a role in renal urothelium development. Thus, we hypothesize that Notch signaling regulates renal urothelium differentiation and Upk expression.

Objective: To determine whether Notch signaling impacts urothelium differentiation using genetic loss of function models.

Methods: We generated *Hoxb7*^{Cre};*RBPJ*$^{fl/fl}$ (RBPJ$^{UB\text{-}KO}$) mice and *RBPJ*$^{fl/fl}$ (controls) to conditionally disrupt Notch signaling. We collected kidneys (focus of this study) and bladders (unaffected control tissue) from *RBPJ*$^{UB\text{-}KO}$ and *RBPJ*$^{fl/fl}$ (controls) at embryonic (E17.5, E18.5), neonatal (P1, P7), juvenile (P14, P21) and adult (P42+) time points. We confirmed tissue-specific depletion of RBPJ and *Notch* abrogation using immunofluorescent analysis (IF-A) and RNAscope, respectively. We used IF-A to evaluate urothelial differentiation (Upk, Pparg).

Results: At all time points, IF-A confirmed efficient RBPJ depletion in *RBPJ*$^{UB\text{-}KO}$ renal urothelium (81.5% v 3.86% P<0.0001) (and collecting ducts), but not bladder urothelium. RNAscope confirmed Notch targets, *Hes1* and *Hes6*, were significantly decreased in *RBPJ*$^{UB\text{-}KO}$ renal urothelium compared to controls (P<0.0001, and P=0.0307, respectively). IF-A showed that *RBPJ*$^{UB\text{-}KO}$ kidneys had significant decreases in Upk compared to *RBPJ*$^{fl/fl}$ starting at P7 (P<0.0001). Pparg, a transcription factor that regulates Upk expression in the bladder, and its targets were also significantly decreased in developing *RBPJ*$^{UB\text{-}KO}$ kidneys compared to controls.

Conclusions: Prior studies in our lab demonstrate that Upk-cells protect the kidney during UTO. Here, we demonstrate that Notch signaling is key to the formation and maintenance of Upk-cells in the renal urothelium. Our data also suggest that Pparg singaling in the renal urothelium may be regulated by the Notch signaling pathway. Future studies will investigate whether the Notch signaling directly regulates Upk expression or whether Upk expression is regulated by a Notch-dependent Pparg signaling axis.

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Citrate Transporter – A Potential Tumor Suppressor And Biomarker In Renal Cell Carcinoma

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Abstract

Background: Five-year survival of metastatic renal cell carcinoma (mRCC) patients is < 10% and African American (AA) males have the highest incidence. Identification of the molecular determinants of mRCC and racial disparity in RCC is critical for improving outcome. NaDC3 expressed in kidney epithelial cells is a succinate/citrate transporter, however its role has not been examined in any disease. We examined NaDC3 expression in normal and RCC tissues and correlated it with clinical outcome and racial disparity. We also evaluated biological functions of NaDC3 in RCC cells.

Methods: Differential gene expression in the matched normal and RCC tissues (n=6/category) was evaluated by microarray analysis; results were validated by QPCR and immunoblotting in tissues from 83 patients (White=46; Hispanic=24; AA=13). Tissues were sequenced for VHL mutations. NaDC3 was pressed in VHL+ and VHL- RCC cells and knocked down in normal kidney epithelial cells. Transfectants were characterized for cell proliferation, cell cycle, motility, succinate/citrate transport and reactive oxygen species (ROS) measurement assays under normoxia and hypoxia.

Results: NaDC3 was 63- and 100-fold downregulated in low- and high-stage RCC tissues. Q-PCR validation showed 40-fold downregulation of NaDC3 in RCC tissues when compared to normal kidney (P< 0.0001). Downregulation was 40-fold in White and Hispanic patients, but 198-fold in AA patients (P=0.0049) and correlated with tumor stage and metastasis (P=0.009), regardless of VHL mutations. Under hypoxia, NaDC3 expression caused 3-4-fold inhibition of proliferation, and increased SIRT activity in both VHL+ and VHL- cells (P<0.01); only VHL+ cells were inhibited under normoxia. Silencing of NaDC3 expression promoted cell proliferation. NaDC3 expression induced ROS levels and succinate transport by >3-fold (P<0.01) and activated p16INK4a-RB pathway and apoptosis (caspase-3 and PARP activation). NaDC3 expression in RCC cells inhibited tumor growth.

Conclusion: This is the first study on a functional biomarker in RCC, NaDC3, that is a possible novel tumor suppressor gene. NaDC3 loss promotes RCC growth, survival and inhibits cellular senescence and its downregulation correlates with metastasis and racial disparity.

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Aberrantly Activated JAK/STAT Signaling Drives Lineage Plasticity in Urothelial Carcinoma

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Abstract

Aberrantly Activated JAK/STAT Signaling Drives Lineage Plasticity in Urothelial Carcinoma

Shamara Lawrence, Hironobu Yamashita, Joshua I. Warrick, Vonn Walter, Lauren Shuman, David J. DeGraff.

Background: Muscle invasive bladder cancer (MIBC) is an aggressive disease with a propensity to progress to metastatic disease, which has limited treatment options. Lineage plasticity is defined as a shift in cancer cell transcriptional fate and can occur through tumor microenvironmental pressure or in response to treatment. While the vast majority of all MIBC begins as urothelial carcinoma, these tumors frequently develop morphologic variation. Squamous differentiation, the most common morphologic variant in MIBC, is almost exclusively associated with a basal-squamous (BSQ) gene expression signature and is associated with poor clinical outcome. A chronic inflammatory microenvironment has been linked with development of BSQ MIBC. However, it is unknown if an inflammatory tumor microenvironment drives BSQ lineage plasticity. Interferon gamma (IFNγ) is a major cytokine active within the MIBC tumor microenvironment. **We hypothesize IFNγ-activated JAK/STAT signaling drives BSQ lineage plasticity in MIBC.**

Methods: The Cancer Genome Atlas (TCGA) MIBC cohort was used to determine the association between molecular subtype and (1) genetic alterations in JAK/STAT pathway components, (2) immune classification, and (3) regulon activity. Using an in-house human MIBC cohort consisting of tumors with urothelial carcinoma and adjacent squamous differentiation, we performed immunohistochemistry for JAK/STAT pathway proteins. We performed RNA-seq and consensus subtyping of a panel of luminal human MIBC cell lines treated with IFNγ. In addition, the JAK/STAT pathway was pharmacologically and genetically inactivated in human BSQ MIBC cells followed by RT-qPCR, western blotting, and RNA-seq.

Results: We show BSQ MIBC is enriched for JAK2 copy number gains, exhibits high STAT1 regulon activity, and is predominantly classified as exhibiting an interferon-dominant immune signature. Immunohistochemistry confirms high levels of JAK1/2, total and phosphorylated STAT1 are detected in areas of squamous differentiation in MIBC. Treatment of luminal MIBC cells with IFNγ resulted in significantly decreased expression of luminal genes, yet significant increases in several BSQ markers, which was confirmed by western blotting and RT-qPCR. In addition, consensus subtyping shows IFNγ treatment results in a shift toward the BSQ transcriptional state. The JAK1/2 inhibitor Ruxolitinib significantly increased expression of *FOXA1* and other key markers of the luminal subtype, and decreased expression of an array of BSQ markers in *FOXA1*-deficient BSQ MIBC cells. Our results demonstrate that siRNA knockdown of JAK1/2 reproduces the Ruxolitinib-driven induction of the *FOXA1*. In addition, JAK1/2 inhibition by Ruxolitinib followed by RNA-seq and consensus subtyping confirms reversion to a luminal transcriptional state.

Conclusions: In summary, our work is the first to identify **IFNγ/JAK/STAT signaling as a driver of BSQ lineage plasticity in MIBC**. These results have important implications, as they suggest an inflammatory microenvironment may drive and support a stable lineage transition to a BSQ molecular subtype during disease progression.

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Selective Inhibition of PP5 as a Therapeutic Strategy in Renal Cancer

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Abstract

In the US this year, over 81,000 people will develop kidney cancer, also known as renal cell carcinoma (RCC), and almost 15,000 patients will die from this disease. Traditional radiation and chemotherapies are ineffective, and ~50% of patients develop metastatic disease, for whom the 5-year survival rate is only ~10%. Such grim statistics point to an urgent need for deeper understanding of the RCC biology and for developing better therapeutic strategies. Our previous work has shown that downregulation of Protein Phosphatase-5 (PP5) caused induction of apoptosis in clear cell renal cell carcinoma (ccRCC), the most common type of RCC. Here, we used an in silico approach to screen and develop a selective inhibitor of PP5. Compound P053 is a competitive inhibitor of PP5 that binds to its catalytic-domain and causes apoptosis in renal cancer. We further demonstrated that PP5 interacts with FADD, RIPK1 and caspase 8, components of the extrinsic apoptotic pathway complex II. Specifically, PP5 dephosphorylates and inactivates the death effector protein FADD. Our data suggests that PP5 promotes renal cancer survival by suppressing the extrinsic apoptotic pathway. Pharmacologic inhibition of PP5 activates this pathway, presenting a viable therapeutic strategy for renal cancer.

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Inhibition of synaptotagmin 4 reverses enzalutamide resistance in advanced CRPC

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Abstract

Background Most castration-resistant prostate cancers (CRPCs) are androgen receptor (AR) dependent. Next-generation anti-androgen therapies (NGATs), such as enzalutamide (Enza) can improve patient survival outcomes. However, while the blockade of the AR shows initial effectiveness, drug resistance still occurs frequently. The underlying mechanisms driving resistance to these treatments remains unclear. Synaptogamin 4 (SYT4), a membrane protein involved in membrane trafficking, is highly expressed in our established Enza resistant CRPC cells. Here, we explore the contribution of SYT4 in prostate cancer progression and enzalutamide resistance in CRPC.

Methods Differences in SYT4 expression were determined in Enza-resistant C4-2B-MDVR (MDVR) cells versus parental C4-2B cells by RNA-sequencing, real time PCR, and western blot. SYT4 expression was analyzed in GEO and cBioPortal databases from patients with prostate cancer. Specific siRNAs were used to inhibit SYT4 expression. Profile changes in expression were examined in MDVR cells with SYT4 knocked down compared to control cells by gene set enrichment analysis. The effect of SYT4 knock down on prostate cancer cell growth and sensitivity to Enza was assessed in MDVR cells.

Results We found that the SYT4 gene expression is significantly higher in MDVR cells than in parental C4-2B cells. Data analysis uncovered that SYT4 expression is significantly increased in patients with advanced prostate tumors. Furthermore, high expression of SYT4 correlates with worse overall patient survival. Transcriptomic profiling revealed enrichment in genes related to cell cycle checkpoint signaling and E2F transcription factors signatures in MDVR cells transfected with control siRNA compared to siRNA targeting SYT4; suggesting possible regulatory networks where SYT4 might be involved. Lastly, inhibition of SYT4 expression with siRNA suppresses prostate cancer cell growth and re-sensitizes MDVR cells to Enza treatment, highlighting SYT4 as a potential therapeutic target for treatment resistant prostate cancer.

Conclusions Our findings indicate that elevated expression of SYT4 promotes resistance to Enza and is associated with worse outcomes for prostate cancer patients. Future studies will link how SYT4 expression affects cell cycle checkpoint signaling and E2F targets. Unraveling these interactions holds promise for shedding light on the mechanisms that underpin resistance to NGATs. This may lead to advancements in better understanding the mechanisms of resistance to NGATs and the development of novel treatments for CRPC patients.

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Characterization of prostatic foam cells in steroid hormone imbalance

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Abstract

Background:

Benign prostatic hyperplasia (BPH) is an age-related disease associated with deteriorating urinary symptoms. The etiology of BPH involves an increase in estradiol-to-testosterone ratio and chronic inflammation but data is limited on how these processes are interconnected. In a mouse model reproducing steroid hormone imbalance (T+E2), we observed increased macrophage infiltration, accumulation in the prostate lumen and foam cell formation. However, the specific role of foam cells in the prostate is unclear. Therefore, to characterize the foam cell transcriptome and to gain a better understanding on macrophage populations and chemokine signaling in the prostate, we conducted single-cell RNA sequencing (scRNA-seq) on prostates from T+E2 mice.

Methods:

We implanted male C57BL/6J mice with pellets containing 25 mg testosterone and 2.5 mg estradiol and collected ventral prostates two weeks later. Cells were dissociated with cold protease and were loaded on Chromium Next GEM (7000 cells/sample). Samples were sequenced on a NextSeq2000 instrument at 100 million reads/sample. Confirmation of scRNA-seq results was performed using *in situ hybridization* (ISH) or immunohistochemistry (IHC) with specific probes for *Cd209a*, *Cxcl17*, *Folr2* and *Pmepa1* and an antibody against TGF-b1. Tissue samples were analyzed using Mantra II. Pathological Workstation and InForm software.

Results:

Our scRNAseq successfully identified five distinct macrophage clusters named after their corresponding marker genes: Mac^{Folr2+}, Mac$^{Pmepa1+}$, Mac^{Ear2+}, Mac$^{Cd209a+}$, and Mac^{Spp1+}, the latter representing foam cells. Further marker genes of Mac^{Spp1+} were *Gpnmb*, *Trem2*, *Fabp5*, *Ctsl* and *Mmp12*. Several cytokines and growth factors, including *Tgfb1*, *Vegf*, *Cxcl16*, and *Ccl6*, were significantly upregulated in Mac^{Spp1+} (confirmed via ISH and IHC). We also identified that Mac$^{Pmepa1+}$ cells were elevated in response to steroid hormone imbalance. We then conducted a chemokine screen in our scRNAseq data for all *Cxcl* and *Ccl* genes. This identified *Cxcl17* to be upregulated in epithelial cells which was then confirmed using ISH (13-fold, $p<0.001$).

Conclusion:

The upregulation of cytokines and growth factors in Mac^{Spp1+} foam cells suggest their potential pathological role in BPH. Mac$^{Pmepa1+}$ cells were also elevated highlighting their significance within the stroma. Furthermore, our cytokine screen identified *Cxcl17* as a potential driver of macrophage recruitment in the prostate. These results may promote the development of macrophage-targeting therapies for BPH.

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Identification of a Novel Transcriptomic Signature and a Therapeutic Target for Benign Prostatic Hyperplasia

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Abstract

Background: Benign Prostatic Hyperplasia (BPH) is a widely prevalent urologic disease in men aged >50 years. However, the molecular mechanisms that drive BPH pathophysiology remain elusive. The primary medical treatment for BPH uses 5-alpha-reductase inhibitors and/or alpha-blockers. However, failure of these treatments is common in BPH patients and surgical treatment is the only remaining option. Therefore, there is an urgent need to identify novel alternative molecular-based therapeutic approaches for BPH.

Methods: Datasets and expression profiles were downloaded from the Gene Expression Omnibus (GEO) and Genotypes and Phenotypes (dbGap) databases. Using multiple experimental approaches including bioinformatic analyses, we identified a signature BPH transcriptome consisting of common differentially expressed genes (cDEGs) among datasets obtained from three independent BPH patient cohorts.

Results: Based on our BPH signature, additional analyses that identified upstream regulators and potential therapeutic compounds led to the identification of TIAM1 as a common molecular target for BPH. Immunohistochemical analysis was utilized to validate the overexpression of TIAM1 in BPH specimens. Further, our analyses revealed NSC23766, a known inhibitor of TIAM1-RAC1 signaling, as the top therapeutic candidate compound for BPH. We found that treatment of human BPH epithelial and stromal cells with NSC23766 resulted in significantly decreased proliferation and reduced epithelial organoid budding and branching morphogenesis – a phenotype associated with BPH pathogenesis leading to the formation of nodules.

Conclusions: Our data suggest that TIAM1 is a potential therapeutic target for the treatment of BPH.

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Hematuria Suppresses the Toxicity of UTI

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Abstract

Background: Urine contains ~3,000,000 urinary RBC/day ("Addis Count"), each RBC containing a billion heme-iron rings. Hematuria is a metric of UTI and lysis of RBC by bacteria can provide nutrient heme. Heme is also a well known toxin for epithelia and may act similarly in the urothelium by oxidative mechanisms. We investigated the consequences of urinary heme in the setting of UTI.

Methods: The study utilized genetic knockouts of HMOX and Slc48a1, in situ hybridization and a novel method of labeling and extracting nascent RNA using Upk2Cre;Rosa-UPRTf/f mice which permits the retrieval of RNA specifically from urothelial cells at a designated time after infection.

Results: In the first part of our analysis, we demonstrate that UTI induces measurable urinary heme, and the bladder urothelium specifically responds by expressing heme importer (SLC48a1), heme metabolic genes (HMOX1), heme dependent regulators of the circadian clock CO (which we detected with a novel CO probe), BMAL and NPAS2. All of these genes were identified by inoculation of 4-Thio-Uracil in UPK2-Cre x floxed phosphoribosyltransferase mice and retrieving thio-label nascent RNA 4 hrs after inoculating bacteria. These data demonstrate an active heme-metabolic machine in the urothelium. To determine whether urothelial heme metabolism is critical, we examined HMOX knockout mice, and found that metabolism of heme is critical for urothelial survival. KO of HMOX caused urothelial shedding even at baseline, and even to a greater extent in the setting of UTI. To explore this pathway further we utilized Dr Iqbal Hamza's Slc48A1-/- heme importer knockouts and examined sheets of bladder mucosa, dissected from the mouse bladder and noted decreased levels of ferritin in mucosal implying that heme donates iron to urothelial cells at baseline via Slc48A1. To test whether the traffic of heme or iron is beneficial, we infused heme, which to our surprise completely abolished UTI induced caspase activation in infected urothelial cells, reduced neutrophil infiltration into the infected bladder, suppressed a full panel of cytokines and interleukins, and most surprisingly appeared to rescue CK20+ superficial urothelial cells long after UTI caused the desquamation of the urothelium. In contrast, heme did not affect the colony forming units obtained from the bladder.

Conclusions: Heme metabolism is active at steady state and during UTI, and the supply of heme or its iron or the production of CO secondary to heme metabolism is critical for bladder survival. Hence hematuria is a naturally occurring component of UTI that mollifies its cellular damage.

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PINK1 signaling augments mitochondrial function and modulates Olaparib sensitivity in CRPC Cells

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Abstract

Background: The PARP inhibitor (PARPi) Olaparib has shown great efficacy in treating castration resistant prostate cancer (CRPC) patients with deficiency in DNA damage response (DDR) genes. Despite general improvements in prognosis, some patients develop therapeutic resistance to Olaparib therapy. We developed an Olaparib resistant CRPC model (OlapR) to uncover potential mechanisms of therapeutic resistance as well as vulnerabilities associated with the resistant phenotype.

Methods: OlapR cells were generated from parental C4-2B cells with chronic exposure to Olaparib. RNA sequencing and gene expression analysis was performed on both lines. Mitochondrial Stress Tests using Seahorse XF Analyzer, MitoTracker staining, and ROS quantification assays were performed on both cell lines. The effects of PINK1 expression on sensitivity to Olaparib were also examined in OlapR cells.

Results: Pathway and gene enrichment analysis revealed a significant enrichment of pathways related to mitochondrial activity in OlapR cells, suggesting that OlapR cells demonstrate enhanced mitochondrial gene expression. We further demonstrate higher levels of basal Oxygen Consumption Rate, ATP production, Spare Respiratory Capacity, Maximal Respiration, and Coupling efficiency in OlapR cells. Furthermore, we detected twice as much active mitochondria in OlapR cells. Interestingly, higher non-mitochondrial oxygen consumption was also noted in OlapR cells, along with increased reactive oxidative species (ROS). Among genes associated with mitochondrial activity, PTEN-induced kinase 1 (PINK1) expression is significantly upregulated in OlapR cells. PINK1 acts as a master regulator of mitochondria-specific autophagy (mitophagy) through clearance of damaged mitochondria. Through knock-down (KD) of PINK1, recognition and subsequent clearance of damaged mitochondria is limited. This results in accumulation of dysfunctional mitochondria as well as increased cellular ROS. Furthermore, PINK1 KD limited OlapR cell growth, and sensitized OlapR cells to Olaparib supplementation.

Conclusions: These findings suggest that the activation of PINK1 signaling enhances mitochondrial function in Olaparib-resistant CRPC cells. The inhibition of PINK1 leads to the accumulation of damaged mitochondria that produce ROS, which in turn results in cell death and enhanced sensitivity to Olaparib.

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Urothelial insulin receptor signaling maintains bladder barrier integrity and promotes antimicrobial peptide expression

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Abstract

BACKGROUND

Urinary tract infections (UTIs) are 10 times more common in diabetics. While the factors that increase infection risk are not well defined, prior studies have identified suppressed insulin receptor signaling as a key factor. The bladder urothelium secretes antimicrobial peptides and acts as an impermeable barrier to prevent bacterial attachment and invasion. Here, we investigate the role of insulin receptor signaling on urothelial defenses by genetically deleting the insulin receptor (IR) in the basal or intermediate/superficial cells in the murine urothelium.

METHODS

IR knockout mice (IRKO) were generated by breeding mice with floxed *Insr* to mice expressing tamoxifen inducible Cre recombinase (Upk2-Cre and Krt5-Cre). Littermates lacking the Cre transgene served as controls (IRflox). To determine if IR deletion impacts host defense, female mice were transurethrally infected with uropathogenic E. coli (UPEC) and burden was enumerated in urine and bladder following infection. To assess bladder barrier permeability, uninfected bladders were mounted in Ussing chambers and transepithelial resistance (TER) and radioisotope permeabilities were measured. Junctional complex markers and antimicrobial peptides in isolated urothelium were assessed by qRT-PCR and Western blot. Murine studies were supplemented with in vitro studies in cultured human urothelial cells.

RESULTS

IRKO mice exhibit normal development, normoglycemia, and normal bladder histology. UPK2 IRKO mice have significantly greater UPEC burden in the urine and bladder following infection. Uninfected bladders from UPK2 IRKO mice have lower TER and increased water and urea permeabilities. No differences are observed in Krt5 IRKO in burden or permeability. Isolated urothelium from UPK2 IRKO mice expressed lower levels of many cell adhesion markers and antimicrobial peptides compared to controls. ChIP assays in cultured human urothelial cells suggest that NF-κB regulates AMP and cell adhesion marker expression downstream of insulin receptor.

CONCLUSIONS

These results suggest that insulin signaling is critical for UTI defense in the intermediate/superficial cells and not the basolateral cells of the urothelium. These findings support a role for insulin signaling in bladder barrier maintenance, perhaps by maintaining expression of cell adhesion molecules. Additionally, we show that insulin receptor signaling is required for antimicrobial peptide expression in the urothelium. Future studies will further define the molecular pathways downstream of insulin receptor that facilitate these innate immune functions.

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Prolonged inhibition of Androgen Receptor Signaling induces expression of Nuclear ErbB3 which renders Prostate Cancer cells susceptible to targeted inhibitors

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Abstract

Objective: Nuclear expression of the receptor tyrosine kinase (RTK) ErbB3/HER3 increases in highly aggressive prostate cancer cells, but its nuclear transport mechanism is currently unknown. Prostate tumors rely on the androgen receptor (AR), whose activation transcriptionally controls ErbB3 expression, but its role in ErbB3 nuclear localization had not previously been reported. Here, we investigated the mechanism by which subcellular localization of ErbB3 was altered in prostate cancer.

Methods: ErbB3 localization was investigated in the human prostate cancer tumor progression model LNCaP, C4, C4-2 and C4-2B. ErbB3 was stimulated with heregulin-1β (HRG). Nuclear translocation was tested with a nucleocytoplasmic transport inhibitor panel (chlorpromazine, filipin III, amiloride and Leptomycin B) LNCaP and C4 cells were continuously cultured with the AR activation inhibitor abiraterone acetate (AbiAc) or ethanol (VEH) or treated with the AR inhibitors enzalutamide, darolutamide and apalutamide. ErbB3 activity and subcellular localization were analyzed using confocal microscopy/subcellular fractionation/immunoblot. Cell viability was determined by MTT assay. Proliferation and apoptosis were determined by flow cytometry. Invasive potential was investigated by crystal violet staining of cell colonies. ErbB3 expression was reduced by siRNA technology.

Results: The ratio of nuclear to cytoplasmic ErbB3 increased in untreated cells from LNCaP<C4-2<C4<C4-2B. In all four lines, nuclear localization of ErbB3 peaked at 30 minutes after HRG treatment, with a rapid return to the cytoplasm in LNCaP cells (at 1 hour), slower in C4 cells (at 4 hours), and continued accumulation in the nucleus in C4-2 and C4-2B 8 hours following HRG treatment. Treatment with the transport inhibitor panel showed that nuclear accumulation of ErbB3 was prevented by the clathrin-dependent endocytosis inhibitor Chlorpromazine (CPZ) but not by the others. In LN-VEH and C4-VEH cells, HRG induced nuclear translocation of ErbB3 but not in LN-AbiAc or C4-AbiAc cells. Accordingly, HRG increased ErbB3 phosphorylation at Y1328 and nuclear Akt phosphorylation at S473 in VEH but not AbiAc cells. C4 cells, which had high baseline nuclear ErbB3, were more sensitive to the AR inhibitors and showed decreased viability and invasive potential compared to LNCaP cells.

Conclusions: These results indicate that (1) ErbB3 nuclear localization required clathrin-dependent endocytosis (2) ligand binding of ErbB3 as well as the presence of an active AR is necessary for ErbB3 nuclear localization and (3) the presence of nuclear ErbB3 increases sensitivity to AR inhibitors.

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Oleic acid ameliorates voiding dysfunction in an aged mouse model of lower urinary tract dysfunction (LUTD)

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Abstract

Title: Oleic acid ameliorates voiding dysfunction in an aged mouse model of lower urinary tract dysfunction (LUTD)

Introduction: Benign prostatic hyperplasia (BPH) and the associated lower urinary tract symptoms (LUTS) commonly develop as men age. Age is the greatest risk factor, making the hallmarks of aging potentially interesting mechanisms underlying the development of BPH/LUTS. Mitochondrial dysfunction, a hallmark of aging, is a potential driver of disease. We aim to investigate whether oleic acid, a mitochondrial modulator, can reverse urinary and mitochondrial dysfunction in an aged mouse model.

Methods: Aged (24 months) C57Bl/6J male mice were used for this study. A cohort of aged mice was treated with 30 mg/kg/day oleic acid in 100 mg of peanut butter daily for four weeks. Prior to treatment, baseline void spot assays were collected as well as immediately prior to euthanasia. Immunohistochemistry (IHC) was performed on prostate measuring NDUFS3, a protein in complex I of the oxidative phosphorylation (OXPHOS) pathway. IHC was also used to examine expression of NRF2, a possible pathway mechanism for oleic acid. Oleic acid was also used to treat human prostate stromal cells (BHPrS1), with and without the complex I inhibitor rotenone. Oxygen consumption rate was measured using a Seahorse Mito Stress Test, reactive oxygen species using the ROS-Glo assay, and collagen (a marker for fibrosis) was measured using qPCR and immunocytochemistry.

Results: Oleic acid treatment alleviated urinary dysfunction in aged mice. NDUFS3 expression, which decreases in aged mice, was improved with oleic acid treatment. In the cell line model, oleic acid improved respiration measures, decreased fibrillar collagen gene expression, and ameliorated an increase in collagen I protein deposition.

Conclusion: Oleic acid is a promising mitochondrial modulator and supplement for further investigation. While additional pre-clinical studies are needed, oleic acid has promise due to its antioxidant, anti-inflammatory, and potentially anti-fibrotic affects in targeting a current treatment gap in BPH/LUTS.

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OPEN MICROFLUIDIC PLATFORM FOR GENERATION OF HUMAN SPERM MICRODROPLETS

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Abstract

BACKGROUND:

Current approaches to cryopreservation for human sperm are adequate when sperm counts are normal. When sperm are rare (severe oligozoospermia, <5 million sperm/milliliter), it is a major technical challenge to cryopreserve sperm with any chance for reanimation upon thawing. Vitrification (ultra-rapid freezing) is ideally suited for rare cells in microliter volumes but has not been widely adopted for sperm due to challenges with manipulation of small fluid volumes containing viable cells. To address this challenge, our objective is to develop a user friendly platform for preparation of sperm microdroplet samples for vitrification, leveraging open channel droplet microfluidics.

METHODS:

Microchannel design was performed in Solidworks and devices were fabricated out of polytetrafluoroethylene and polystyrene on a Datron NEO mill. Motile sperm from subjects with normal sperm concentration and motility were isolated from semen samples using a swim out method. Purified motile sperm were suspended in Sperm Wash media and food coloring was used to aid droplet visualization. HFE-500 fluorinated oil was used as a carrier fluid to generate microliter droplets. Sperm motility was assessed before and after passage through the microchannel and with and without presence of food coloring with Integrated Visual Optic System.

RESULTS:

Using our open channel microfluidic device, we leverage spontaneous capillary flow and interfacial tensions between two immiscible liquid phases to generate microliter droplets containing human sperm. Microliter droplets can be easily transferred to droplet holder for eventual vitrification. Droplet visualization and passage of sperm through the device did not significantly impact sperm motility.

CONCLUSIONS:

We demonstrate application of a novel open microfluidic device to generate microliter droplets containing human sperm. Motility of sperm is preserved after passage through the microchannel and droplet visualization. Future work will focus on vitrification of sperm in microdroplets generated by the microfluidic platform.

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SYNCRIP, A Molecular Brake on APOBEC-Driven Tumor Mutational Burden, Heterogeneity, and Therapy Resistance

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Abstract

Background: Prostate cancer (PCa) is a prevalent malignancy often treated with androgen deprivation therapy (ADT). Despite advancements in androgen receptor (AR)-targeted therapies, resistance frequently arises, leading to poor outcomes. Tumor mutational burden and heterogeneity contribute to therapy resistance. APOBEC proteins are linked to mutational signatures in many cancers, but their role in therapy resistance remains unclear.

Methods: Utilizing an in vivo shRNA-based library screening, SYNCRIP emerged as the key candidate linked to AR therapy resistance. We generated SYNCRIP-deficient cells through CRISPR guide RNAs and shRNA systems. We conducted Co-IP, GST pull-down, IF, IHC staining, and FRET-based cytidine deaminase assays, revealing the SYNCRIP-APOBEC relationship and the mechanism behind SYNCRIP-loss mediated resistance. Furthermore, comprehensive analysis encompassing whole exome sequencing, bulk RNA-seq, single-cell RNA-seq, and FACS-based functional CRISPR screening was employed on wild-type and SYNCRIP-deficient PCa cells to pinpoint potential driver genes. We gauged clinical significance by scrutinizing patient cohorts from SU2C, TCGA, and CPGEA, and substantiated our findings through validation using patient-derived organoids, explants, and histological slides.

Results: We have identified SYNCRIP as a pivotal factor associated with resistance to AR therapy, utilizing both in vitro and in vivo systems. Deletion of SYNCRIP in enzalutamide-sensitive PCa cell lines not only induced therapy resistance but also intensified APOBEC-mediated mutagenesis. Our investigations revealed an interaction between SYNCRIP and APOBEC3B, with APOBEC3B-driven mutagenesis exhibiting a significant correlation with unfavorable clinical outcomes in patients. Through functional CRISPR screening, we pinpointed eight resistance drivers within SYNCRIP-deficient tumors. Moreover, our analysis of single-cell RNA-seq data unveiled the central role of APOBEC-driven mutagenesis in intratumoral heterogeneity and resistance. Notably, we identified dominant-resistant subclones bearing FOXA1 mutations.

Conclusions: This study uncovers SYNCRIP's role as a suppressor of APOBEC-driven mutagenesis in PCa. SYNCRIP loss activates APOBEC3B, leading to driver mutations and therapy resistance. Eight essential resistance drivers mutated by APOBEC3B were identified. These findings elucidate a cell-intrinsic mechanism driving APOBEC-mediated resistance to AR-targeted therapy in PCa.

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Alternative Polyadenylation as a Therapeutic Vulnerability in Prostate Cancer

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Abstract

Background:

Prostate cancer is the second leading cause of male cancer death in the United States. While localized disease can be cured by radiation or surgery, metastatic prostate cancer presents a clinical challenge. Metastatic prostate cancer can initially be controlled by endocrine therapies that target the androgen receptor (AR), however, these tumors will inevitably develop resistance. This stage of the disease, termed castration-resistant prostate cancer (CRPC), is responsible for the majority of prostate cancer-specific deaths. Current treatment options for CRPC patients are not curative and highlight the need to develop novel therapeutic options that will more effectively treat the disease. mRNA molecules undergo cleavage and polyadenylation downstream from a canonical 5’AAUAAA poly(A) site located within the 3’ untranslated region (UTR). Shortening of mRNA 3’UTRs through alternative polyadenylation has been observed in numerous cancers, which can lead to downregulation of tumor suppressor genes and upregulation of oncogenes.

Methods:

We conducted a loss-of-function screen of mRNA polyadenylation factors in hormone-sensitive LNCaP cells and hormone-insensitive LNCaP95 and 22Rv1 cells and conducted shRNA targeting of candidate trans-acting factors. We performed poly(A)-ClickSeq (PAC-seq) to determine global polyadenylation changes, and RNA-seq analysis to identify global gene expression changes. We inhibited cis-sequence elements involved in mRNA polyadenylation by employing antisense oligomer targeting.

Results:

We found that knockdown of the cleavage and polyadenylation specificity factor (CPSF) component, CPSF1, inhibited growth of all cell lines. Gene set enrichment analysis of the RNA-seq data revealed the Glycolysis Hallmark and Hypoxia Hallmark gene sets were positively regulated by CPSF1 across all cell lines. PAC-seq data revealed global induction of intergenic unannotated poly(A) sites distal to 3’ UTRs, indicative of 3’UTR lengthening, upon CPSF1 knockdown, and found 3’UTR lengthening caused decreased gene expression of metabolic factors.

Conclusions:

This work supports a role for mRNA polyadenylation in prostate cancer. We have shown CPSF1 regulates prostate cancer growth and the expression of metabolic factors, and nominates novel therapeutic targets in prostate cancer.

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Transcription Factor SOX2 as an Indicator of Progression in Early Stage Bladder Cancer

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Abstract

Background

Previous research has identified the transcription factor SOX2 as a potential driver of stage progression in bladder cancer. We thus set out to further investigate the role of SOX2 in driving aggressive behavior in bladder cancer using human samples and murine allograft models.

Methods

Immunohistochemistry (IHC) and RNA-sequencing were used to evaluate SOX2/*SOX2* expression in several cohorts of non-muscle invasive and muscle invasive human bladder cancers. In addition, we utilized a murine allograft model to compare wildtype and *SOX2* over-expressing MB49 cells to investigate the impact of SOX2 expression on progression *in vivo*.

Results

Evaluation of human bladder cancers via IHC demonstrated SOX2 expression is present and focal in both invasive and noninvasive bladder cancers. Additionally, analysis of publicly available RNA-sequencing data of UROMOL and TCGA cohorts identified increased *SOX2* expression as being significantly associated with higher tumor stage and stage progression in lamina propria-invasive bladder cancer. We then investigated the effects of SOX2 expression *in vivo* using murine allografts consisting of MB49 cells modified to stably overexpress human *SOX2*. Interestingly, allografts expressed SOX2 protein focally, much like human tumors. Additionally, *SOX2* over-expressing allografts demonstrated larger tumor size (p=0.025) and higher rates of local metastasis (p=0.009) compared to wildtype controls.

Conclusions

Our combined findings indicate that while SOX2 expression is focal within tumors, SOX2 expression drives aggressive features in bladder cancer including progression from early to advanced stage disease.

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Targeting SOX2 via the IGF1R-AKT-CREB1 Pathway Increases Enzalutamide Sensitivity in Castration-Resistant Prostate Cancer

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Abstract

Title

Targeting SOX2 via the IGF1R-AKT-CREB1 Pathway Increases Enzalutamide Sensitivity in Castration-Resistant Prostate Cancer

Background

SOX2 in the prostate has previously been identified as a mediator of resistance to AR-targeted therapies such as enzalutamide. Previous studies have shown SOX2 knock-out in castration resistant prostate cancer (CRPC) re-sensitizes cells to enzalutamide, resulting in decreased proliferation in vitro and increased survival in vivo. Identifying and pharmacologically targeting the regulators of SOX2 in CRPC could reduce enzalutamide resistance in patients, resulting in improved overall survival.

Methods

Using a genetically engineered nLuc-SOX2 fusion protein reporter system, we conducted a drug screen of over 400 compounds to identify commercially available drugs that decrease SOX2 but were not overtly cytotoxic. Drug screening results were validated by Western blot, and clinical implications for these drugs were assessed by co-treatments with enzalutamide in vitro and in vivo. To determine the intermediary signaling factors regulating SOX2, we utilized a kinase phospho-proteome profiler array and direct regulation of SOX2 by these mediators was assessed using Western blot and promotor/enhancer motif analysis.

Results

Of the 430 compounds screened, 39 compounds were identified to decrease SOX2 with minimal cytotoxicity. 10 of these compounds sensitized CRPC cells to enzalutamide, decreasing cell survival with co-treatment in vitro. Further validation by Western blot suggested targeting the IGF1R pathway decreased SOX2 most robustly and consistently. Clinical implications of co-treatment with IGF1R inhibitors and enzalutamide were assessed in vivo, where co-treatment reduced tumor volume vs. each treatment alone. Mechanistically, the kinase phospho-proteome profiler array implicated 5 proteins downstream of IGF1R as potential regulators of SOX2 (pAKT, pGSK3a/b, pPRAS40, pCREB1, pWNK1). Western blot validation and genomic assessment of the SOX2 promoter/enhancer implicated pAKT and pCREB1 as a regulator of SOX2 in CRPC.

Conclusions

Targeting SOX2 pharmacologically has revealed an upstream regulatory pathway through IGF1R-pAKT-pCREB1. Because SOX2 has been identified as a key factor in the development of CRPC and

anti-androgen resistance, a better understanding of the regulators of SOX2 could introduce new avenues of treatment for CRPC.

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Deciphering DNA Damage Repair in ATM Mutant Prostate Cancers

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Abstract

Background: Mutations in DNA Damage Response (DDR) genes, including Ataxia, Telangiectasia, Mutated (ATM), are common in advanced castration-resistant prostate cancers (PC). Poly (ADP-ribose) polymerase (PARP) inhibitors are approved in DDR mutant PC, but demonstrate limited clinical efficacy in PCs with ATM mutations. In this project, we sought to specifically define the impact of ATM loss on DDR pathways in PC, with the goal of identifying alternate therapeutic vulnerabilities.

Methods: ATM-KO PC cell lines were generated via CRISPR-Cas9 mediated knockout. ATM loss and abolishment of downstream ATM kinase activity was confirmed via western blot. We performed phospho-proteomic evaluation of DDR pathways in parental and ATM-KO cells after ionizing radiation (IR). Clonogenic survival assays were performed after treatment of cells with inhibitors and/or IR. Kinetics of DDR protein recruitment and resolution were interrogated with immunofluorescence (IF) staining for yH2ax, 53BP1, MDC1, and Rad51 foci.

Results: ATM-KO PC cells were able to effectively repair DNA damage following IR, as measured by recruitment and resolution of yH2ax, 53BP1, MDC1, and Rad51 foci. Unbiased phospho-proteomic studies demonstrated that ATM-KO cells maintain canonical DDR pathways through activation of ATR and DNA-PKcs kinases. Treatment of ATM-KO cells with either an ATR inhibitor (VX970) or a selective DNA-PKcs inhibitor (M3814) only incrementally affected DDR in ATM-KO cells compared to parental controls, as evidenced by clonogenic survival assays and maintenance of DDR foci. Importantly, combination treatment with VX-970 and M3814 prevented downstream DDR foci recruitment and radio-sensitized ATM-KO PC to a greater extent than parental controls. This suggested that activity of any of the trinity of kinases is sufficient to mediate DDR, and that blockade of both ATR and DNA-PKcs is required to effectively prevent DDR in ATM-KO PC. We then leveraged a RUVBL1 ATPase inhibitor Compound B, which has been shown to significantly attenuate the expression levels of these three kinases. We confirmed that Compound B treatment attenuated ATR and DNA-PKcs protein expression in ATM-KO PC cells, and demonstrated radiosensitivity of ATM-KO PC cells to Compound B.

Conclusions: Our phospho-proteomic data demonstrates that either ATR and DNA-PKcs are sufficient to mediate DDR following IR in ATM-KO PC cells. Dual targeting of ATR and DNA-PKC is necessary to block DDR following IR in ATM-KO PC cells. We have identified that the RUVBL1 ATPase inhibitor Compound B may effectively deplete ATR and DNA-PKc in ATM-mutant PC, enhancing their sensitivity to DNA damage. Our studies indicate the need for and the utility of a novel therapeutic strategy in ATM-mutant prostate cancer.

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Epigenetic Alterations in Chronic Prostatitis/Chronic Pelvic Pain Syndrome: Unraveling Theragnostic Potential in Peripheral Immune Cells.

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Abstract

DNA methylation, an essential epigenetic process controlling gene expression and cellular differentiation, has been implicated in various diseases. In this study, we investigated epigenetic aberrations in Chronic Prostatitis/Chronic Pelvic Pain Syndrome (CP/CPPS) and their impact on the local and systemic immune system. CP/CPPS is a challenging condition characterized by chronic pelvic pain without infection, affecting men of all ages. It has an elusive origin and no treatment. We explored the role of DNA methylation in regulating immune genes and its contribution to CP/CPPS pathogenesis.

Methods:

Peripheral blood samples were collected to isolate peripheral blood mononuclear cells (PBMCs) and CD4+ cells were isolated and subjected to DNA methylation analysis using bisulfite sequencing. Prostate localized cells from urine were purified to assess immune status through qPCR.

Results:

Our study in urine revealed significant alterations in CD4+ lymphocyte subpopulations, characterized by increased pro-inflammatory Th17 cells and reduced FoxP3 Tregs in CP/CPPS patients' prostates. Further examination of the PBMCs and CD4+ lymphocytes' epigenetic profile focused on inflammation-associated genes, particularly the promoter regions of IL-10 and ITGAL. We observed hypermethylation in the IL-10 promoter and hypomethylation in the ITGAL promoter in CPPS (Chronic Pelvic Pain Syndrome) patients compared to healthy donors. Additionally, we observed a diminished ability of the patient's immune systems to suppress immune reactions following LPS activation in in vitro experiments. These findings raise the possibility that changes in IL-10 expression might influence other local mediators, contributing to disease progression.

Conclusion:

Our study highlights the involvement of epigenetic modifications, specifically DNA methylation, in the dysregulation of immune genes associated with CP/CPPS. The observed alterations in CD4+ lymphocyte subpopulations and epigenetic patterns of IL-10 and ITGAL suggest potential theragnostic targets for further exploration. This intractable condition may be diagnosed and treated through understanding its epigenetic basis. These findings need to be validated and translated into clinical practice through further research.

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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-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 (known as TPA-responsive elements or TREs). 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

To examine antagonism between AR and AP-1, 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). Through combination of treatment with doxycycline and DHT, we can toggle the activity of AR and AP-1 via achievement of four distinct conditions: 1. AP-1 on/AR-off; 2. AP-1 off/AR-off; 3. AP1-on/AR-on; 4. AP-1 off/AR on.

RESULTS

Rigorous analyses of AP-1 controlled features including proliferation and apoptosis have proven that inhibiting AP-1 markedly increases proliferation, S-phase entry in cell cycle activity, AR target gene expression and luminal cell fate specification in AR-dependent prostate cells. Further work using chromatin accessibility and immunoprecipitation followed with high-throughput sequencing approaches will unravel the regulatory elements responsible for coordinating the shift between AP-1 occupied and AR-occupied chromatin states in prostate cells.

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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An epigenetic mechanism in driving tumor lineage plasticity and drug resistance

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Abstract

Background: Prostate cancer develops usually as adenocarcinoma (PRAD) and is treated with anti-androgen receptor (AR) signaling therapies. Progression from PRAD to neuroendocrine-like prostate cancer (NEPC) is now recognized as a major mechanism that confers resistance to most of the current therapies. The exact molecular events accompanying the aberrant cell lineage differentiation and plasticity in the tumor remain poorly defined. Epigenetic alterations have been strongly implicated. However, so far only a few drivers and therapeutic targets of NEPC diseases have been examined.

Methods: Knockdown and CRISPR knockout of G9a were conducted to evaluate the role of G9a in NEPC. RNAseq, ChIP-seq and ATATC-seq were conducted to study G9a in genomic wide regulation of NEPC. The efficacy of G9a inhibitors were tested in NEPC PDX models in vivo.

Results: G9a is overexpressed in NEPC tumors and associated with poor clinical outcomes. Knockdown and CRISPR knockout of G9a significantly repressed cell proliferation and expression of NEPC drivers and markers. Inhibitors of G9a displayed strong activities in suppression of growth and survival of NEPC cells and PDX organoids. Among them, CM272, a new first-in-class, reversible G9a/DNMT1 dual inhibitor, showed the highest potency. Furthermore, G9a antagonists displayed strong activities in inhibition of the growth of PDX tumors. Our RNA-seq and ChIP-seq analyses demonstrated that G9a directly activates the expression of genes involved in neuronal lineage differentiation and axonogenesis programs and that G9a inhibitor diminishes its occupancy at the gene regulatory sites.

Conclusion: Here we identified histone methyltransferase G9a as a strong candidate of therapeutic target in NEPC. Our study revealed an epigenetic regulator G9a as a major driver of NEPC and identified a new strategy for treatment of advanced prostate cancer.

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Deciphering Human Prostate Carcinoma-Associated Fibroblast Heterogeneity using scRNA-seq

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Abstract

Background: Carcinoma-associated fibroblasts (CAF) are a heterogeneous component of the prostate tumor microenvironment (TME) and have been demonstrated to regulate prostate cancer growth and progression in a variety of ways. The extent of CAF heterogeneity in prostate cancer tissues has not been well described. An evaluation of prostate CAF was conducted with the hypothesis that fibroblasts in the TME contribute to cancer proliferation and immunosuppression via altered production of secretory molecules compared to normal fibroblasts from the same patient.

Methods: Human prostate cancer and patient-matched benign tissues were digested and prepared for single-cell mRNA-sequencing (scRNA-seq) analysis. Cell sorting using exclusion of CD45 (immune), CD200 (endothelial), and EpCAM (epithelial) markers enriched for fibroblasts and smooth muscle cells. Digestion of prostate cancer-containing peripheral zone (PZ, n=5) and matched cancer-free transition zone (TZ, n=3) was followed by cell sorting of all viable cells plus additional CD45-CD200-EpCAM- cells for scRNA-seq analysis using the 10X Chromium System. Bioinformatic subclustering of the non-immune stromal cells from all samples was conducted to compare the gene expression between PZ versus TZ derived fibroblasts.

Results: We determined that, unlike epithelial or immune cells, enriching for fibroblasts requires a longer tissue digestion protocol. Bioinformatic analysis of PZ versus TZ fibroblasts indicates that 15 transcriptomically distinct fibroblast subclusters are present. Increased expression of secreted factors CXCL8, CXCL1, CXCL3, and HMOX1 in PZ versus TZ fibroblasts suggest an immunosuppressive regulation of the TME. SERPINE2, a serine protease inhibitor, is also upregulated in PZ versus TZ fibroblasts and is primarily expressed in the stromal regions of prostate cancer tissues.

Conclusions: Cell sorting resulted in a complete cellular landscape of the prostate and included significant populations of fibroblasts and smooth muscle cells/myofibroblasts for further characterization. Ongoing studies aim to identify consistently altered signaling pathways in the PZ versus TZ stromal cells of cancer patients. Further investigation of the unique CAF subpopulations present in prostate cancer tissue will provide defined markers of functional fibroblast subtypes and determine the metabolic or secreted factors with therapeutic potential.

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Aberrant function of a circadian regulator in control of tumor chromatin bivalency in prostate cancer progression

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Abstract

Abstract

Background: Chromatin bivalency plays an important role in cell lineage specification during development. Prostate cancer (PCa) therapy resistance involves an extensive reprogramming of gene expression in tumor lineage plasticity. However, the underlying mechanisms are poorly understood. Rev-erb-alpha, a member of the nuclear receptor transcription factor family, plays a key role in regulation of circadian rhythm and metabolism primarily through repression of its target genes.

Methods: We performed sequential ChIP-seq epigenome profiling of tumor tissues from the clinic and PDX models and integrated analysis of tumor epigenome and transcriptome. We also treated the PDX models with small molecule inhibitors of the receptor.

Results: We found that most of the tumor lineage plasticity genes, including those in programs of neurodevelopment, neural signaling, stemness and EMT, are controlled by bivalent promoters with concurrent marking of H3K27me3 and H3K4me3. Our further studies revealed that the chromatin bivalency resolves during the development of cancer therapy resistance. Interestingly, Rev-erb-alpha, a key circadian rhythm regulator, plays a critical role in promoting the bivalency resolution and activation of the gene programs. Its pharmacological targeting effectively reverses the bivalency resolution and blocks the lineage plasticity in anti-AR therapy-resistant tumors.

Conclusions: Therefore, our study establishes that resolution of chromatin bivalency is a major epigenetic mechanism of tumor lineage plasticity and demonstrates that therapeutic targeting of the aberrant function of Rev-erb-alpha represents a novel treatment of advanced PCa.

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The HOX/CUT Transcription Factor ONECUT2 is a Driver of Metabolic Plasticity in Lethal Prostate Cancer

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Abstract

The ONECUT2 (OC2) transcription factor acts to promote lineage plasticity in prostate cancer by attenuating androgen receptor (AR) activity and upregulating expression of multiple neuroendocrine drivers and other pathways. OC2 can be directly inhibited with a novel class of small molecules our group has developed. We have shown that OC2 can modify the epigenome, which is tightly coupled to metabolic state, suggesting that OC2 may trigger reprogramming of tumor metabolism. In this study, RNA-seq analysis indicated that overexpression of OC2 in human prostate cancer cells under conditions of lipoprotein deficiency activated both glycolysis and oxidative phosphorylation. RNA and protein expression measurements demonstrated that enforced OC2 causes robust over-expression of PDK4, an enzyme that promotes aerobic glycolysis, suppresses mitochondrial reactive oxygen species, and stimulates acidification of the tumor microenvironment from secretion of lactate. Enforced OC2 promoted a euchromatin state at the PDK4 promoter, as shown by ATAC-seq, ChIP-seq, CUT&RUN-qPCR and Hi-C data. OC2 chromatin binding at the PDK4 promoter increased in OC2-enforced cells under nutrient stress. Seahorse mito-stress test and lactate quantification assays demonstrated higher mitochondrial respiration and lactate secretion with enforced OC2. This extensive metabolic shift was suppressed by pharmacologic OC2 inhibition. A gene expression signature associated with these metabolic changes was found to be upregulated in human castration-resistant and neuroendocrine prostate cancers with high OC2 expression. Our findings reveal that OC2 promotes simultaneous activation of both aerobic glycolysis and oxidative phosphorylation, suggesting these metabolic effects of OC2 activation underlie some of its oncogenic effects on cell proliferation, malignant growth, metastasis, and drug resistance. We conclude that OC2 activates a “hybrid” metabolic phenotype and, consequently, is a targetable master regulator of tumor metabolism in prostate cancer.

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Impact of mouse genetics on steroid hormone response in models of lower urinary tract dysfunction

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Abstract

Background: Benign prostatic hyperplasia (BPH) is a multifactorial disease that, with a combination of smooth muscle dysfunction, prostatic proliferation, and fibrosis, can lead to lower urinary tract symptoms (LUTS). BPH/LUTS develops and affects a significant portion of men over the age of 50, significantly impacting quality of life. While several mouse models exist to examine the lower urinary tract dysfunction, how these mouse models recapitulate human disease is not well understood. C57Bl/6 mice are often used in pre-clinical studies due to the suitability for the development of knockout and transgenic animals. However, while C57Bl/6 mice are considered an inbred strain, a wide range of phenotypic and genetic differences exist between the substrains of mice. Between C57Bl/6N and C57Bl/6J, 51 coding variants, 34 coding SNPs, 2 indels, and 15 structural variants have been identified and characterized. These genetic alterations lead to phenotypic differences between these substrains including alterations in metabolism and behavior. We hypothesize that the genetic variations between C57Bl/6J and C57Bl/6N mice will alter the histological response within the prostate in the steroid hormone model of urinary dysfunction.

Methods: To control any differences in breeding, environment, and diet, we obtained all mice from The Jackson Laboratory. C57Bl/6N mice that were maintained at the NIH were rederived at The Jackson Laboratory and denoted C57Bl/6NJ. Both C57Bl/6J and C57Bl/6NJ mice were received at 6 weeks of age for acclimation, and 25mg testosterone (T) and 2.5mg 17β-estradiol (E2) were implanted subcutaneously to induce urinary dysfunction. Void spot assays were performed weekly and analyzed by VoidWhizzard. To assess for proliferations, mice were administered BrdU in drinking water for 5 days immediately following surgery. Mice were euthanized at 4, 8, and 16 weeks, and prostate and bladder tissues were collected. Proliferation was assessed by immunohistochemistry (IHC); fibrosis assessed by picrosirius red and Masson's trichrome staining. Senescence-associated β-galactosidase activity was assessed on fresh frozen tissues.

Results: Both C57Bl/6J and C57Bl/6NJ mice showed an increase in urinary dysfunction as measured by void spot assays. Bladder volume and mass were also significantly increased in both groups. There was a significant increase in fibrosis in C57Bl/6NJ mice in mice with dysfunction. Additionally, there was a difference in senescence-associated β-galactosidase in the prostate lobes of the substrains of mice. At later time points, C57Bl/6J exhibited an increase in mortality compared to age matched C57Bl/6NJ.

Conclusion: Although both substrains of mice exhibit a significant increase in urinary dysfunction, the mechanism by which the dysfunction occurs differs. This suggests that both models are valuable in modeling BPH/LUTS, a disease whose etiology and manifestation is varied within the patient population. However, careful consideration of genetic background will be critical in future experiments to mitigate the genetic drift seen within this inbred strain of mice.

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FOXA1 Loss Remodels the Tumor Immune Microenvironment in Late-Stage Prostate Cancer

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Abstract

Background: Castration resistant prostate cancer (**CRPC**) has shown a poor response to immune checkpoint inhibitors due to its immunosuppressive tumor immune microenvironment (**TIME**). A deeper understanding of the tumor intrinsic mechanisms shaping the TIME in prostate cancer (**PCa**) is needed to harness immunotherapies for PCa patients. Epithelial transcription factor FOXA1 is frequently downregulated in CRPC. Recently, we found FOXA1 loss induces tumor cell invasion and macrophage recruitment *in vitro*, mediated in part by HIF1A-CCL2 signaling. However, the extent to which FOXA1 loss regulates the TIME in PCa remains unclear, largely due to the lack of appropriate immune proficient mouse models.

Methods: We generated a novel genetically engineered mouse model (**GEMM**) with prostate-specific deletion of *Pten* and *Foxa1* (PbCre:$Pten^{f/f}Foxa1^{f/f}$) by crossing the PbCre:$Foxa1^{f/f}$ mouse with the *Pten*-null model (PbCre:$Pten^{f/f}$) that is known to develop invasive PCa as early as 9wk of age. We collected prostate tissues of intact mice at early (12wk) and late (18wk) timepoints of tumor progression for analysis by hematoxylin and eosin (**H&E**) staining, immunohistochemistry (**IHC**) staining, scRNA-seq, and spatial transcriptomics. We also castrated a cohort of mice to model castration-resistant disease. Finally, we performed multiplex immunofluorescence (**mIF**) and bioinformatics analyses of PCa patient samples to validate the clinical relevance.

Results: H&E and IHC analyses revealed that PbCre:$Pten^{f/f}Foxa1^{f/f}$ prostate tumors exhibit a more aggressive histopathological phenotype as compared to age-matched PbCre:$Pten^{f/f}$ mice. In addition, spatial transcriptomics analysis of the GEMMs showed a striking remodeling of the TIME upon *Foxa1* loss, with increased infiltration by immunosuppressive M2-like macrophages, Tregs, and exhausted T cells in PbCre:$Pten^{f/f}Foxa1^{f/f}$ tumors. Notably, scRNA-seq analysis revealed that *Foxa1* deletion induced the expression of immunosuppressive cytokine genes, such as *Tgfb3*, *Ccl2*, and *Ccl20*, which stand out as potential therapeutic targets whose blockade may promote anti-tumor immunity. Finally, mIF and CIBERSORTx analyses of PCa patient samples confirmed that FOXA1 expression negatively correlates with macrophage and Treg tumor infiltration.

Conclusions: We present a novel GEMM for the study of FOXA1 function in an immunocompetent setting. Our data supports FOXA1 as a critical tumor intrinsic regulator of the TIME. This study will shed light on potential immunotherapeutic approaches for CRPC with FOXA1 loss.

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Potential roles of fibroblast growth factor 5 and the YAP/TAZ pathway in prostate cancer progression

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Abstract

Background: In the aging male population, prostate cancer (PCa) is the second most frequently diagnosed malignancy and is driven by dysregulation of androgen receptor (AR) signaling. While current therapeutic options attempt to modulate this pathway, the vast majority of patients progress from androgen dependent PCa to castration resistant prostate cancer (CRPC). Current treatments for PCa and CRPC remain largely ineffective and the identification of new molecular targets is critical for alleviating the health burden of PCa. The fibroblast growth factor (FGF)/FGF receptor (FGFR) signaling axis has been implicated in prostate carcinogenesis, but focused studies on FGF5 are limited. Nevertheless, the literature indicates FGF5 overexpression is associated with stemness, metastatic potential, proliferative capacity, and increased tumor grade in a variety of cancer models. This study aims to evaluate the role of FGF5 in prostate cancer progression and implicate Yes Associated Protein 1 (YAP1) as a potential intermediate in FGF5/AR signaling.

Methods: Meta-analysis on FGF5 expression was completed using prostate cancer datasets. IHC staining and correlation analysis of FGF5 and AR was completed on non-tumorigenic, PCa, and metastatic tumor microarrays (pTMAs). Immunocytochemistry (ICC) studies were used to evaluate AR and YAP1 expression in AR positive PCa cell lines after FGF5 stimulation or overexpression. This was followed by ICC studies examining how YAP1 inhibition (verteporfin) impacts AR expression in PCa cell lines stimulated with rFGF5.

Results: Evaluation of gene expression arrays indicated that FGF5 significantly increases with disease progression. A potential link between FGF5 and AR was established from pTMA data demonstrating increased correlation between FGF5 and AR in PCa and metastatic pTMAs compared to non-tumorigenic samples. Results from ICC studies further validated the association between FGF5 and AR where AR and YAP1 expression significantly increased in PCa cell lines after stimulation with rFGF5. Analysis of FGF5 stimulated PCa cells treated with verteporfin demonstrated a significant reduction in AR signal intensity.

Conclusions: We propose that FGF5 has potential roles in prostate cancer progression and the transition to castration resistance. The mechanism of action by which FGF5 modulates AR may involve YAP1 as an intermediate. We propose that further investigation of FGF5 and its link to AR signaling may have therapeutic relevance in combination with AR targeting therapeutics.

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A New Regulatory Pathway Governing WNT Ligand Trafficking and AR-Independent Progression in Prostate Cancer

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Abstract

Background

The WNT signaling pathway, integral to biological processes, has been identified as a driver of prostate cancer progression. While this relationship is recognized, the intricate mechanisms of WNT signaling within prostate cancer are still not completely clear. Several WNT ligands have been discovered to propel tumor advancement and resistance to drugs, yet the aspect of cellular trafficking of these ligands has been minimally explored. Therefore, focusing research on key components in WNT ligands transportation could be a vital avenue in the quest to develop potent therapies for aggressive prostate cancer.

Methods

Utilizing an in vivo screening approach, the potential tumor suppressor neurolysin (NLN) was identified within prostate cancer cells. To further investigate its role, transcriptome profiling and co-immunoprecipitation mass spectrometry (CO-IP MS) were applied. These techniques led to the discovery of the NLN-KIF11 axis as novel regulators of WNT3A trafficking within prostate cancer cells.

Results

In a pioneering finding, my research has revealed new functions of NLN and KIF11 in prostate cancer. Under normal circumstances, NLN directly interacts with KIF11, aggregating it and inhibiting its capability to transport WNT3A into the extracellular tumor environment. When NLN is absent, however, KIF11 is freed, causing a marked elevation in WNT3A secretion. WNT3A activates WNT signaling in the neighboring cells, which enhances malignant cell growth even in the presence of androgen receptor (AR) signaling targeted therapies such as castration and enzalutamide. This leads to AR-independent tumor progression, highlighting a multifaceted and challenging form of resistance. Remarkably, this resistance can be countered with WNT signaling inhibitor (ICRT3) and KIF11 inhibitors (Filanesib and Ispinesib), offering promising avenues for the development of innovative therapeutic agents.

Conclusion

The discovery of the NLN-KIF11-WNT3A axis within prostate cancer introduces a previously unrecognized regulatory pathway. NLN's interaction with KIF11 controls the secretion of WNT3A, influencing tumor progression. This insight illuminates the intricate molecular mechanisms that contribute to resistance against AR-targeted therapies. The unearthing of this regulatory axis provides a promising avenue for the potential creation of innovative treatments or diagnostic tools, with the potential to substantially enhance both the management and comprehension of advanced prostate cancer.

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Models of Acquired Therapeutic Resistance for CRPC, Implication of Treatment Sequencing

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Abstract

Background: Common current treatments for castration resistant prostate cancer (CRPC) in general fall into 3 categories; next-generation anti-androgen therapies (NGAT) such as enzalutamide, abiraterone acetate, apalutamide, and darolutamide, taxane therapy such as docetaxel, and PARP inhibitors (PARPi) such as olaparib. Despite efficacy and improvement of outcomes, a portion of patients will not survive due to the development of therapeutic resistance. Further complicating the clinical landscape is limited knowledge regarding the effect of treatment sequence on the potential for therapeutic cross-resistance in CRPC. We have developed several CRPC models of acquired therapeutic resistance, to utilize for gene expression analysis as well as assessment of response among several treatments.

Methods: NGAT-resistant (NGAT-R) MDVR, AbiR, ApaR and DaroR, cells were generated from C4-2B cells through chronic exposure to enzalutamide, abiraterone acetate, apalutamide, and darolutamide respectively. Likewise, docetaxel-resistant TaxR cells and olaparib-resistant OlapR cells were also generated from the C4-2B background through chronic exposure to docetaxel and olaparib respectively. Parental C4-2B as well as resistant derivative lines were subjected to RNA-seq and expression analysis, as well as assessed for sensitivity to the therapeutics discussed.

Results: We find that NGAT resistance induces relative resistance to other NGATs, but not to docetaxel or olaparib. Docetaxel resistance induces cross resistance to olaparib, but not to NGATs. OlapR retained parental sensitivity to both docetaxel and NGATs. NGAT-R and OlapR exhibit increased gene expression relating to mitochondrial function while TaxR showed downregulation. TaxR cells had increased expression relating to drug efflux pumps. Genes associated with the G2/M checkpoint were downregulated in all resistant lines except for OlapR.

Conclusions: Our findings suggest cross-resistance across NGATs, but not resistant across therapeutics in other classes such as docetaxel and olaparib. Similar conclusions can be made for olaparib resistance, as OlapR cells displayed inter-therapeutic sensitivity. TaxR cells were the only cells to display inter-therapeutic resistance to olaparib but displaying sensitivity to NGATs. Furthermore, our data suggests resistance mechanisms differ between drug classes. These results may have clinical implications, as increased knowledge of resistance within versus across classes of therapies can inform treatment.

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Blocking PTH1R inhibits prostate cancer metastases

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Abstract

Background: Parathyroid hormone receptor 1 (PTH1R) is the only cell membrane receptor for parathyroid hormone-related protein (PTHrP). The causative effects of PTHrP in prostate cancer (PCa) bone metastases have been demonstrated. However, clinical trials on blocking PTHrP have not yet been successful. PTHrP secreted by PCa cells binds to PTH1R on osteoblasts, stimulates osteoblasts to express RANKL. RANKL further activates osteoclasts, which resorb the bone and release the growth factors and cytokines that stimulate the further growth of PCa cells. There is considerable interest in developing drugs to target PTH1R, a G protein-coupled receptor (GPCR). In general, GPCRs comprise 35% of the current clinical drug targets. Therefore, we explore the effects of blocking PTH1R in PCa metastases using both genetic and pharmacological approaches.

Methods: To determine the paracrine effect of PTH1R in PCa metastases, we crossed the floxed *Pth1r (Pth1r^{FloxE2})* mouse with the *Col1α2 CreERT* mouse. This mouse line was further bred into immunodeficient NSG background. No pathological changes were found in the *Pth1r^{ColCreERT}* knockout (KO) mice, compared to the *Pth1r^{FloxE2}* littermates or wild-type controls. We then xenografted the human castration-resistant PCa, PC3 (luciferase-labeled), intracardially into the *Pth1r^{FloxE2}* and *Pth1r^{ColCreERT}* KO littermates. Luminescent signals and bone lesions were monitored weekly.

PTH1R is also expressed in PCa cells. To determine the effect of blocking PTH1R on PCa cell growth, PTH1R peptide antagonist, (Asn^{10},Leu^{11},D-Trp^{12})-PTHrP(7-34) amide [PTHrP(7-34) amide], and a novel small molecule inhibitor, XC039, were both tested in vitro.

Results: We found metastases in multiple organs, including bone, liver, and kidney. The overall and organ-specific metastases, such as bone metastases, were significantly inhibited in the *Pth1r^{ColCreERT}* KO, compared to the *Pth1r^{FloxE2}* littermates. These data showed that blocking the paracrine effects of PTH1R inhibits prostate cancer metastases. To determine the autonomous effect of PTH1R inhibitors in PCa cells, we found that XC039, but not PTHrP(7-34) amide, significantly inhibited PCa cell growth, although both PTHrP(7-34) amide and XC039 significantly suppressed the ligand (PTH or PTHrP)-induced downstream cAMP signaling. Furthermore, our preliminary study showed a trend of bone metastasis inhibition of C4-2B-induced bone lesion development using the PTHrP(7-34) amide.

Conclusions: Taken together, these studies suggest and demonstrate a potential efficacy in blocking PTH1R for PCa metastases.

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Integrative analyses implicate cooperation of ONECUT2 and Kaiso in prostate cancer lineage plasticity

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Abstract

Prostate cancer (PC) health disparities among African American (AA) and European American (EA) men persist in screening, incidence, disease aggressiveness, and mortality. To address these disparities and improve outcomes for AA men, it is crucial to understand underlying mechanisms. Our lab identified the HOX/CUT transcription factor ONECUT2 (OC2) as a master regulator that drives PC metastasis and lineage plasticity. OC2 is expressed at highest levels in advanced disease, however it can also be active in primary PC, suggesting potential therapeutic opportunities for targeting this protein prior to the emergence of AR-independent lineage variants in castration resistance prostate cancer (CRPC). OC2 can be directly suppressed in vivo with novel small molecules developed by our group. ChIP-seq performed in 22Rv1 CRPC cells with enforced OC2 (OC2 OE) identified a high frequency of OC2 binding at sequence-specific (SS) motifs for the methyl-binding protein Kaiso (ZBTB33). Kaiso expression and activation are disproportionately linked to AA PC. Kaiso SS motifs were also highly ranked in ATAC-seq data derived from OC2-OE LNCaP cells. Integration of Kaiso LNCaP ChIP-seq data with OC2 LNCaP CUT&RUN data showed that Kaiso and OC2 co-binding was enriched in narrowly defined promoter regions of over 2,000 genes, suggesting the two proteins co-regulate a large gene expression network. OC2 and Kaiso OE and knockdown RNA-seq data were computationally integrated to produce OC2 and Kaiso activity signatures. Application of these signatures to multiple datasets showed a high correlation between Kaiso and OC2 activities in CRPC and NEPC patient datasets, lineage plasticity genetically engineered mouse models, and human PC xenografts. Analysis of single-cell RNA-seq data from human PC indicates a high correlation between Kaiso and prostate specific antigen expression (PSA/KLK3) in primary PC but not CRPC. Collectively, these findings suggest an AR-independent coordinate role for Kaiso and OC2 in advanced disease. These results may be relevant to novel therapeutic opportunities relevant to PC in AA men.

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The Alzheimer's Disease Associated Gene BIN1 Inhibits AR Activity Leading to Increased Levels of EGFR and cMYC

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Abstract

Background: Androgen-receptor signaling inhibitors (ARSI) have been shown to significantly alter the natural history of castration-resistant PCa (CRPC). Unfortunately, resistance to ARSIs is inevitable. Unraveling the underlying mechanisms to identify potential therapeutic interventions to treat these lethal tumors has become a critical challenge in the post-ARSI era. Recent genomic sequencing has revealed a subclass of PCa harbors deletion of bridging integrator-1 (BIN1) and is associated with increased AR activity. Notably, BIN1 deletion frequently co-occurs with SPOP mutation and deletion of SPOPL, collectively suggesting BIN1 may function as a tumor suppressor.

Methods: To elucidate the role of BIN1 in prostate cells, we generated a prostate-specific BIN1 knockout murine model (PB-CreBIN1FL/FL). This model revealed increased prostate mass at 6 months of age, accompanied by increased cell proliferation. We next crossed this strain to SPOP mutant (SPOPF133V) model to generate a prostate specific BIN1 knockout, SPOP mutant model (PB-CreSPOPF133VBIN1FL/FL) and found increased proliferation and greater prostate mass compared to animals harboring SPOP mutation or BIN1 deletion alone.

Results: We generated a doxycycline inducible BIN1 overexpressing PCa model (22Rv1BINOE) to evaluate AR signaling in vitro. We found that overexpression of BIN1 modestly reduced prostate cancer cell proliferation but profoundly suppressed androgen receptor protein level. To evaluate the transcriptional activity of AR and ARv7, we also utilized luciferase reporter system harboring ARE consensus sequences found in the KLK3 gene (AR-FL target) promoter or in the EDN2 gene (ARv7 target) promoter, respectively. These reporter assays illustrated suppression of both AR-FL and AR-v7 driven gene transcriptional program upon BIN1 overexpression. These observations were further confirmed using global RNAseq. Notably, inhibition of AR activity under BIN1 overexpression conditions was accompanied by increased cMYC and EGFR protein expression, suggesting an important role for MYC/EGFR signaling axis in potential cell-survival mechanism.

Conclusions: As a member of the amphiphysin family, BIN1 has been previously illustrated to bind to the GTPase dynamin and has been shown to play a critical role in clathrin mediated endocytosis. We postulated BIN1 mediated regulation of endocytosis is important for increased EGFR signaling and are currently evaluating this hypothesis. Our studies help elucidate the clinical significance of BIN1 deletion in PC patients and highlight important crosstalk between EGFR and AR-signaling axis governed by BIN1.

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Next-Generation Anti-Androgen Therapies Enhance PARP Inhibitor Efficacy in Advanced Prostate Cancer

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Abstract

Background: PARP inhibitors (PARPi) have significantly improved management of advanced prostate cancer. However, questions remain regarding their optimal clinical integration including 1) where do PARPi's best fit in the treatment sequencing paradigm? 2) what is the phenotype of PARPi resistant tumor cells? and 3) what agents can be combined with a PARPi to enhance efficacy? In this study, we sought to better understand PARPi treatment sequencing and to further define the mechanism of action underlying combinations of a PARPi with a next-generation anti-androgen (NGAT).

Methods: LN-OlapR and 2B-OlapR olaparib resistant cell lines were generated from LNCaP and C4-2B cells through chronic exposure to increasing doses of olaparib. Cell viability assays were used to assess response to androgen receptor directed therapies and combination treatments with a PARPi. RNA-sequencing, gene set enrichment analysis (GSEA), and qPCR were used to assess the PARPi resistant phenotype and treatment response. Western blots were performed to assay for DNA-damage response.

Results: Cell viability assays show PARPi resistant models respond to NGAT treatment similarly as parental cells suggesting a lack of cross-resistance between PARPi's and NGAT's. However, RNA-sequencing of the 2B-OlapR model suggests the advent of lineage plasticity with neuroendocrine features. Given that early evidence of a lineage switch may preclude a durable NGAT response, we hypothesized that a combination of a NGAT with a PARPi upfront may be more beneficial. We found that combining the NGAT abiraterone with either olaparib or rucaparib was significantly more effective than monotherapy. Mechanistically, we present evidence that synergy between these drug classes may depend partly on a class effect of NGAT's to reduce DNA repair and replication capacity. In line with this hypothesis, we show that the additional NGAT's, enzalutamide, apalutamide, and darolutamide, all enhance efficacy of olaparib.

Conclusions: Our data support recent successes of the PROpel and TALAPRO-2 clinical trials and add insight into the mechanism underlying the efficacy of combining PARPi's with NGAT's. Our data suggest all four approved NGAT's may work with a PARPi, and that there may be significant clinical flexibility in the design of these treatment regimens. Further work to understand tumor cell response to these combinations promises to improve their clinical impact.

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A PROSPECTIVE OBSERVATIONAL STUDY ON FACTORS AFFECTING EARLY URINARY CONTINENCE FOLLOWING ROBOT ASSISTED RADICAL PROSTATECTOMY

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Abstract

Introduction

Prostate cancer is the second most frequent cancer diagnosis made in men and the fifth leading cause of death worldwide. The preferred treatment option for localized prostate cancer with curative intent is radical prostatectomy (RP). In the modern era minimally invasive surgery techniques like laparoscopy (LARP) and robotic surgery (RARP) have become the standard of case. Despite advances in surgical technique and methodology, post prostatectomy urinary incontinence (PPI) remains a significant adverse event that negatively affects the quality of life. Preservation of post-RP continence and fluctuations in continence rates is multifactorial. These factors encompass not only anatomical and surgical aspects, but patient characteristics also. This study will be aimed at identifying and analyzing various preoperative, intraoperative as well as post-operative factors affecting the continence after RARP.

Objectives

To analyse various preoperative, intraoperative and postoperative factors affecting urinary continence outcomes at 2 weeks, 1, 3, and 6 months of follow up in patients undergoing robotic assisted radical prostatectomy.

Materials and methods

Data from 102 patients operated by a single surgeon from June 2021 to June 2022 with a maximum 6 month follow up were collected. Patient features (age, Body mass index-BMI, Prostate specific antigen-PSA, hypertension, diabetes mellitus, International prostate symptom score-IPSS score, comorbidity, Gleason's score, median lobe, preop Kegels, Charlson's comorbidity index, prior Transurethral resection of prostate-TURP, prostate size), surgical technique used (Rocco stitch alone/ anterior reconstruction with Rocco stitch, level of nerve preservation, bladder neck width), and post operative factors (T stage of the tumour, margin status) were assessed. Patients who received post-op RT were excluded. Patient's urinary continence was seen at follow-up of 2 weeks, 1 month, 3 months and 6 months. Complete urinary continence was defined as use of 0 pads.

This is a single institute experience with the cases operated by a single surgeon from June 2021 to June 2022. All the patients of localized and locally advanced carcinoma prostate undergoing RARP and consenting for the study were included in the study. Patients who were lost to follow up, who didn't give consent for the study, and those who underwent post op Radiotherapy were excluded from the study.

Patient diagnosis and work up

Patients with raised serum prostate specific antigen or hard nodular prostate had undergone a multiparametric magnetic resonance imaging (mpMRI) or MRI pelvis and transurethral ultrasound guided (TRUS) 12 core or more systematic biopsy depending upon the number and location of suspicious lesion. When the TRUS guided biopsy showed prostate adenocarcinoma, the Gleason score was noted. Prostate specific membrane antigen positron emission tomography (PSMA PET) or a Bone scan was done as imaging modality to see for locoregional and metastatic spread if any. Patients diagnosed with localized and locally advanced disease were selected for the surgery after explaining pros and cons of the other available modalities.

Preoperative pelvic floor muscle training

Patients were educated about the pelvic floor muscle exercises preoperatively 1 week before the scheduled surgery and its role in continence achievement. They were told to continue the exercise, about 20 pelvic contractions every 4 hours, postoperatively till continence was achieved.

Patient factors

Patient preoperative data like age, co-morbidities, body mass index (BMI), prior transurethral resection of prostate, prostate volume, presence of median lobe of prostate, serum PSA, preop international prostate symptom score (IPSS), Charlson's comorbidity index, Gleason's score was documented.

Technical aspects

The patient was positioned in steep Trendelenburg position after inserting perurethral catheter. Pneumoperitoneum would be created using CO_2 gas and ports would be placed and robot (da Vinci Xi platform) was docked. 6 ports were placed – 4 for robotic arms including a robotic camera and 2 as other assistant ports. Posterior dissection was done first. Bilateral seminal vesicles were dissected and bilaterally vas was divided. Bladder drop followed by anterior dissection was done. Periprostatic fat was dissected followed by lateral dissection.

Nerve preservation

Levels of nerve preservation was defined into 4 grades as grade 1 being- incision of the Denonvilliers and lateral pelvic fascia (LPF), representing the greatest degree of nerve sparing (NS) possible, only

for patients with no-to-minimal risk of EPE taken just outside the prostatic capsule. Grade 2 being incision through the Denonvilliers (leaving deeper layers on the rectum) and LPF - taken just outside the layer of veins of the prostate capsule. Grade 3 (partial/incremental) NS – Incision is taken through the outer compartment of the LPF (leaving some yellow adipose and neural tissue on the specimen), excising all layers of Denonvilliers ' fascia. Grade 4 (non-NS) NS – a wide excision of the LPF and Denonvilliers' fascia containing most of the periprostatic neurovascular tissue. The maximum nerve sparing done on either of the side was labelled as the overall grade of nerve sparing.

Nerve preservation attempt was done in all cases wherever feasible depending upon the extraprostatic extension of the lesion and intraoperative findings of surgical planes and bleeding. Bladder neck was then be divided and attempt was made to preserve the bladder neck width as much as possible. Bilaterally pedicles were clipped and divided. DVC was then identified, divided and secured. Periapical dissection was done and urethra divided distally. Rocco stitch was taken which is a running continuous absorbable suture used to reapproximate the remnant Denonvillier's fascia, posterior detrusor and posterior rhabdosphincter prior to completion of the vesicourethral anastomosis. Vesico-urethral anastomosis was done over 20 Fr urethral catheter. Bilateral extended pelvic lymphnode dissection was done depending upon the Gleason score and local stage of the disease. Anterior support reconstruction was performed by suturing the puboprostatic ligament to detrusor apron at anastomotic site. Specimen would be removed from the pfannenstiel incision.

Post operative factors

Postoperatively, depending upon the drain output and the generalized condition of the patient the abdominal drain was removed on postoperative day 1,2 or 3. Those patients having a high drain output, the drain fluid was tested for fluid creatinine. Any postoperative complication was noted.

Discharge and follow up

All patients were discharged with urethral catheter in situ with oral prophylactic antibiotics till catheter removal. According to our institutional protocol, all patient had undergone a cystogram on post operative day 14 on follow up which if normal, urinary catheter was removed. In case the cystogram showed any urinary leak, the catheter was kept for 1 more week and reassessment done. Patients were reviewed at 2 weeks, 1 month, 3 months and 6 months post-surgery to assess their urinary continence and pad usage was noted each visit. Achievement of urinary continence was strictly defined as usage of 0 pads. Patients requiring adjuvant RT/salvage RT were noted and excluded from the study. The primary end point of analysis was the determination of variables that influence urinary continence within 6 months after RARP. All patients were followed up via mail or telephone interview, and patient responses were recorded.

Statistical analysis

All the qualitative parameters were represented with frequencies and percentages. All the quantitative parameters were presented with mean and standard deviation. To find the association between different parameters and continence status at different time points we used Chi-Square test for measure of association. The data entered in M.S. Office and the analysis performed by using SPSS 23.0v. P value less than 0.05 considered as significant. All the information derived from the data was represented with relevant graphs. Multivariate logistic regression analysis was used to see the effect of multiple independent variables on the dependent outcome of urinary continence.

Results

Initially, there were a total of 119 patients enrolled in our study who underwent RARP during the defined study period, out of which 16 patients were lost to follow-up and 1 patient expired in the post-op period (due to cardiovascular event). So, the study was completed with 102 patients. The mean and standard deviation of the quantitative parameters are given in Table 1. The demographic details and the univariate analysis with the individual parameters is shown (Table 2).

TABLE 2- Demographic details and univariate analysis of factors

Table 1. Mean and SD of quantitative parameters of the patients.

Statistics

		Age (Years)	Pre Operative PSA	BMI	IPSS Preop	Prostate size
N	Valid	102	102	102	102	102
	Missing	0	0	0	0	0
Mean		67.83	17.373333	26.485686	15.28	37.491176
Std. Error of Mean		.625	1.5729154	.3622112	.834	1.8193086
Median		69.00	11.500000	26.300000	15.50	35.000000
Std. Deviation		6.314	15.8856668	3.6581534	8.427	18.3741160
Range		29	83.9900	22.2000	30	108.0000
Minimum		51	4.0100	16.7000	0	4.0000
Maximum		80	88.0000	38.9000	30	112.0000

UNIVARIATE ANALYSIS

No.	Variable	Category	2 weeks			1 month			3 months			6 months		
			C	IC	p value	C	IC	p value	C	IC	p value	C	IC	p value
1	**Age (years)**	<60(n=9)	0	9	0.433	1	8	0.525	4	5	0.59	6	3	0.248
		60-70 (n=56)	2	54		7	49		18	38		47	9	
		>70 (n=37)	0	37		2	35		10	27		33	4	
2	**BMI (kg/m²)**	<25 (n=35)	0	35	0.375	4	31	0.872	14	21	0.398	29	6	0.794
		25-30 (n=52)	2	50		5	47		14	38		45	7	
		>30 (n=15)	0	15		1	14		4	11		12	3	
3	**HTN**	Yes (n=66)	1	65	0.66	8	58	0.287	19	47	0.446	58	8	0.18
		No (n=36)	1	35		2	34		13	23		28	8	
4	**DM**	Yes (n=41)	1	40	0.775	6	35	0.117	13	28	0.952	35	6	0.811
		No (n=61)	1	60		4	57		19	42		51	10	
5	**Preop kegel's > 1 week**	Yes (n=8)	0	8	0.677	0	8	0.331	4	4	0.237	8	0	0.204
		No (n=92)	2	92		10	84		28	66		78	16	
6	**Median Lobe**	Yes (n=6)	0	6	0.721	0	6	0.405	1	5	0.424	6	0	0.276
		No (n=96)	2	94		10	86		31	65		80	16	
7	**Prior TURP**	Yes (n=14)	1	13	0.132	1	13	0.718	3	11	0.388	12	2	0.877
		No (n=88)	1	87		9	79		29	59		74	14	
8	**Prostate Size (cc)**	<30 (n=39)	0	39	0.375	4	35	0.564	14	25	0.614	31	8	0.537
		30-60 (n=52)	2	50		4	48		14	38		45	7	
		>60 (n=11)	0	11		2	9		4	7		10	1	
9	**Charlson's Comorbidity Index**	Mild (n=24)	0	24	0.587	1	23	0.545	7	17	0.902	18	6	0.334
		Moderate (n=67)	2	65		8	59		22	45		58	9	
		Severe (n=11)	0	11		1	10		3	8		10	1	
10	**IPSS**	Mild (n=24)	0	24	0.142	1	23	0.184	5	19	0.29	20	4	0.918
		Moderate (n=43)	0	43		3	40		13	30		37	6	
		Severe (n=35)	2	33		6	29		14	21		29	6	
11	**Gleason score**	6 (3+3) (n=22)	0	22	0.470	1	21	0.142	9	13	0.382	19	3	0.5634
		7 (3+4) (n=31)	1	30		6	25		11	20		28	3	
		7 (4+4) (n=31)	0	31		1	30		9	22		24	7	
		8 or more (n=18)	1	17		2	16		3	15		15	3	
12	**Preop PSA (ng/ml)**	4-10 (n=43)	1	42	0.832	6	37	0.458	16	27	0.659	38	5	0.138
		10-20 (n=31)	1	30		2	29		8	23		28	3	
		20-50 (n=23)	0	23		1	22		6	17		17	6	
		>50 (n=5)	0	5		1	4		2	3		3	2	
13	**Nerve preservation**	Level 1 (n=13)	1	12	0.416	4	9	0.021	5	8	0.65	12	1	0.712
		Level 2 (n=59)	1	58		6	53		20	39		50	9	
		Level 3 (n=29)	0	29		0	29		7	22		23	6	
		Level 4 (n=1)	0	1		0	1		0	1		1	0	
14	**Technique used**	R only (n=65)	1	64	0.683	3	62	0.02	22	43	0.475	54	11	0.649
		Rocco + Anterior reconstruction (n=37)	1	36		7	30		10	27		32	5	
15	**Margin status**	Free (n=81)	2	79	0.467	8	73	0.961	25	56	0.828	70	11	0.251
		Involved (n=21)	0	21		2	19		7	14		16	5	
16	**Bladder neck width preservation**	< 1 cm (n=29)	2	27	0.07	9	20	<0.01	13	16	0.111	26	3	0. 038
		1-2 cm (n=70)	0	70		1	69		19	51		59	11	
		>2 cm (n=3)	0	3		0	3		0	3		1	2	
17	**T Stage**	pT2N0 (n=32)	0	32	0.934	3	29	0.970	8	24	0.168	28	4	0.216
		pT2N1 (n=1)	0	1		0	1		1	0		1	0	
		pT3aN0 (n=35)	1	34		4	31		15	20		31	4	
		pT3bN0 (n=28)	1	27		3	25		8	20		22	6	
		pT3aN1 (n=1)	0	1		0	1		0	1		0	1	
		pT3bN1 (n=5)	0	5		0	5		0	5		4	1	

Only 1 patient had complication of delayed catheter removal due to abdominal to high abdominal drain output. Postop histopathology showed free margins in 81 (79.4%) patients. Most patients belonged to T3aN0 stage.

Chi square test was used for univariate analysis to see for significance of each individual parameter affecting the continence at various time period. At 1 month follow up, nerve preservation (p=0.021), bladder neck width preservation (p<0.01) and reconstruction technique used (p=0.02) were found to affect the continence significantly. At 6 months only bladder neck preservation (p=0.038) was found to affect significantly where no other factor showed any significant effect on the outcome. No factor was significantly affecting at 3 months of follow up.

Using the multivariate logistic regression analysis, nerve preservation was shown to significantly affect the urinary continence at 1 (p=0.044) and 6 months (p=0.027), whereas only bladder neck width preservation (0.030) and preop Gleason score (p=0.038) was found to significantly affect the outcome at 3 months. No other factor was statistically significant at any time period of follow up. Hence, bladder neck width preservation and nerve preservation maintained have their significant in multivariate analysis also.

The percentage of continence achieved at the end of 6 months was 84.31% using 0 pads a definition for complete urinary continence and was 95.09% for patients using 1 security pad. The continence at other follow up time period is mentioned in Graph 1 and 2.

Conclusion

Nerve preservation (at 1 and 6 months), bladder neck width preservation (at 1 month) and Gleason

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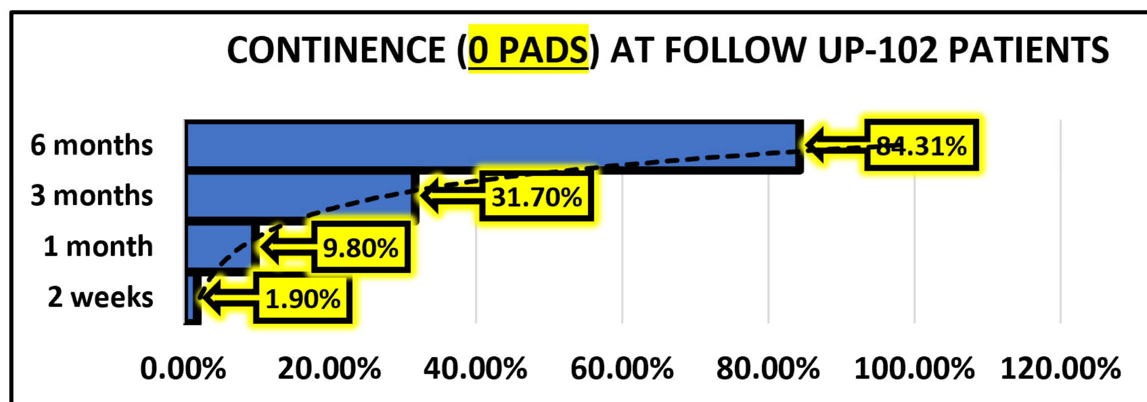
Nerve preservation level | Technique Used | Bladder neck preserved

% of Continent

Graph1. Overall continence rate at follow up with 0 pads use



<-1cm
1-2cm
>2cm
Months
value=0.038

3 MONTHS	Preop Gleason score	0.0386	0.5635	(0.3272, 0.9705)
6 MONTHS	Nerve preservation	0.0274	0.2137	(0.0542, 0.8421)

score (at 3 months) significantly affects the urinary continence post RARP.

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Raloxifene alleviates lower urinary tract dysfunction (LUTD) in mouse models of benign prostatic hyperplasia

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Abstract

Raloxifene alleviates lower urinary tract dysfunction (LUTD) in mouse models of benign prostatic hyperplasia

Background. Benign prostatic hyperplasia (BPH) impacts the majority of the aging male population, with associated lower urinary tract symptoms (LUTS) costing the healthcare industry around $4 billion annually. Estrogen signaling via receptors (ERs) within the prostate have been shown to play opposing roles, with ERα associated with increased proliferation while ERβ with apoptotic processes. Current BPH therapeutic strategies target androgen biosynthesis without considering that androgens are regularly converted to estrogenic ligands in the steroid hormone signaling pathway. Here, we investigated ERa as a potential therapeutic target in BPH/LUTS via raloxifene in a mouse model of lower urinary tract dysfunction (LUTD).

Methods. Twenty male C57Bl/6J mice were obtained from Jackson Laboratories. Prior to surgery, two baseline void spot assays (VSAs) were obtained to measure urinary function. Mice were surgically implanted with compressed hormone pellets consisting of 25 mg testosterone and 2.5 mg 17b-estradiol (T+E2) or given sham surgery between 8-10 weeks of age and allowed to develop dysfunction over 4 weeks. VSAs were collected weekly to measure voiding capacity. At 4 weeks of hormone treatment, those mice who received T+E2 pellets were randomly subdivided into two groups, one receiving vehicle injections and another receiving 10 mg/kg/day raloxifene intraperitoneally. Injections were administered daily over the course of 5 days, with VSAs performed 24 hours prior to the initial injection and 24 hours after the completion of the injection period. Upon experiment end, the lower urinary tracts were collected and masses obtained for hemi-prostate lobes in addition to bladder mass and volume measurements.

Results. Mass was significantly increased between sham and T+E2 groups as expected. No significant differences were found between anterior, ventral and dorsolateral (AP, VP, DLP) masses treated with or without raloxifene. Similarly, bladder mass was not significantly altered. Current experiments are underway to measure ERa expression in collected tissues as well as collagen deposition. Further experiments will investigate effects of extended raloxifene administration on lower urinary tract function.

Conclusions. Therapeutic targeting of ERa in hormone-induced LUTD leads to minimal changes in bladder mass and voiding ability.

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Elevated miR-196a-2 and novel HOXC variant gene expression in Castration-Resistant Prostate Cancer

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Abstract

Elevated miR-196a-2 and novel HOXC variant gene expression in Castration-Resistant Prostate Cancer

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Background: Androgen deprivation therapy or Androgen Receptor targeted therapies are used to treat metastatic Prostate Cancer (PCa). However, nearly all cases eventually progress to Castration-Resistant Prostate Cancer (CRPC). The molecular pathways associated with CRPC development and progression remain incompletely defined. This study aims to identify CRPC drivers and delineate the underlying mechanisms.

Methods: Three isogenic castration-sensitive and castration-resistant prostate cancer cell lines were analyzed by RNA sequencing and small RNA sequencing. Gene transcripts associated with castration resistance were further characterized by RT-PCR, Rapid Amplification of cDNA Ends (RACE), and bioinformatic analyses.

Results: We discovered a significant miR-196a upregulation in castration-resistant cell lines. To further identify the origin of miR-196a expression, we explored the transcriptional profiles of the cell lines using the sequencing data and detected an activity upstream of miR-196a-2 on chromosome 12. miR-196a expression in CRPC patients was further defined using clinical samples and secondary datasets. In addition, we performed 5'-RACE and 3'-RACE to identify the transcription start site of pri-miR-196a-2 and define splicing isoforms. Additional transcripts from the HOXC gene cluster were found to be associated with castration resistance and metastasis.

Conclusions: A subset of castration-resistant prostate cancers activate *MIR196A2* and *HOXC* gene transcription.

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A novel GRK3-HDAC2 regulatory pathway is a key direct link between neuroendocrine differentiation and angiogenesis in prostate cancer progression

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Abstract

Background: Treatment-related neuroendocrine prostate cancer (NEPC) is an aggressive subset of castration-resistant prostate cancer (CRPC), found in ~20% of lethal CRPC. The mechanisms underlying NEPC progression are still poorly understood, and new drug targets are desperately needed. Two prominent phenotypes in NEPCs are elevated NE marker expression and heightened angiogenesis, and thus they are expected to be linked. However, key direct molecular links between these two phenotypes are elusive, whose elucidation will substantially expand our knowledge in NEPC and enable the development of effective treatments for NEPC. Epigenetic regulation has been implicated in drug resistance, neuroendocrine differentiation and prostate cancer progression. Through kinase shRNA and cDNA screenings, we previously discovered G protein-coupled receptor kinase 3 (GRK3) is a key regulator of prostate cancer progression, however, its mechanisms in prostate cancer were largely unknown.

Methods: To identify new critical epigenetic drivers of NEPC progression, we examined the expression patterns of 147 epigenetic regulators in several prostate cancer datasets. We also assessed GRK3's expression patterns in patient samples. Using molecular and cell biology methods, we determined the impacts of GRK3 and HDAC2 overexpression and silencing on NE marker expression and angiogenesis in prostate cancer cells. Through biochemistry and mass spectrometry, we investigated the nature of GRK3-HDAC2 relation. By ChIP-PCR, we measured the impacts of GRK3 modulations on HDAC2's epigenetic activity, especially in repressing potent anti-angiogenic factor thrombospondin 1 (TSP1) and master NE-repressor REST. Through compound library screening and biochemical characterization, we searched for novel GRK3 inhibitors to study GRK3 biology and to evaluate whether GRK3 is a suitable drug target for prostate cancer.

Results: First, we found that HDAC2, whose role in NEPC has not been reported, is one of the most upregulated epigenetic regulators in NEPC. We hypothesized that HDAC2 is a critical regulator of the two prominent phenotypes of NEPC. Indeed, HDAC2 promotes both NE differentiation and angiogenesis. GRK3 is also upregulated in NEPC and is a critical promoter for the two phenotypes too. Of note, GRK3 phosphorylates HDAC2 at S394, which enhances HDAC2's epigenetic repression of TSP1 and REST. Intriguingly, REST suppresses angiogenesis while TSP1 suppresses NE marker expression in PCa cells, indicative of their novel functions and their synergy in cross-repressing the two phenotypes. Furthermore, the GRK3-HDAC2 pathway is activated by androgen deprivation therapy and hypoxia, both known to promote NE differentiation and angiogenesis in prostate cancer. Our GRK3 inhibitor reverses GRK3's action in regulating the HDAC2-REST-TSP1 pathway, reduces the survival and proliferation of NEPC cells in culture and in mouse xenografts.

Conclusions: NE differentiation and angiogenesis during prostate cancer progression converge on GRK3-enhanced HDAC2 suppression of REST and TSP1, which constitutes a key missing link between two prominent phenotypes of NEPC.

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Thalidomide Attenuates Lower Urinary Tract Dysfunction in Aged Mice.

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Abstract

Background: Benign Prostatic Hyperplasia (BPH) is a prevalent disease characterized by the overgrowth of prostatic tissue, resulting in urethral lumen narrowing and chronic urine outflow obstruction. Lower urinary tract symptoms (LUTS) such as urinary frequency, urgency, and nocturia are commonly observed in BPH. Recent research suggests that fibrosis may contribute to BPH pathogenesis and resistance to medical treatment. However, no therapies specifically targeting prostatic fibrosis currently exist, highlighting the need for further research. This study aims to evaluate the effects of thalidomide on lower urinary tract dysfunction in an aged mouse model of BPH/LUTS.

Methods: Aged (24-month-old) C57Bl/6J mice were assessed for baseline lower urinary tract dysfunction using the void spot assay (VSA). Mice with dysfunction were then treated with daily i.p. injections of 10 mg/kg thalidomide for 6 weeks. Weekly VSAs were performed to track changes in lower urinary dysfunction. At the end of the treatment period, mouse urogenital tracts were harvested, and fibrosis was assessed using histopathological methods. Collagen levels in the prostate lobes and prostatic urethra were quantified using picrosirius red (PSR) staining. PSR images were analyzed using CTFire and CurveAlign software to assess changes in collagen fiber alignment, width, straightness, and orientation.

Results: Thalidomide treatment resulted in a significant decrease in the number of void spots compared to baseline in aged mice. PSR staining showed a downward trend in total collagen within the dorsal and lateral prostate (DLP) in treated mice compared to controls. Moreover, treated mice exhibited a significant decrease in thicker collagen fibers in the DLP and anterior prostate (AP). PSR analysis of the prostatic urethra revealed a significant reduction in total collagen levels and thicker collagen fibers in thalidomide-treated mice. Analysis of individual collagen fibers around the prostatic urethra using CTFire and CurveAlign showed overall less collagen fiber alignment.

Conclusions: Our findings demonstrate that thalidomide attenuates lower urinary dysfunction in aged mice and this change may occur secondary to antifibrotic effects in the prostate lobes and prostatic urethra. These results provide valuable insights into the mechanisms of BPH/LUTS progression and suggest that antifibrotic agents could be used as monotherapy or in combination with existing treatments for BPH/LUTS patients.

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Elucidating the role of SPOPL in prostate cancer progression

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Abstract

Background: Recent clinical trials have shown that intensifying anti-androgen therapies before prostatectomy can induce pathologic complete responses. Importantly, both SPOP mutation and SPOPL copy-number loss (independent of each other) were found to be exclusive to exceptional responders. The Speckle-Type POZ protein (SPOP) mutant subclass of PC accounts for 10% to 15% of all primary PC cases. SPOP is an adaptor for Cullin3/Ring (CUL3-RING)-type E3 ubiquitin ligase complexes and provides substrate specificity. Previously we and other have shown that SPOP mutations results in increase AR and cMYC activity, and SPOP mutation exerts a dominant negative effect. However, very little is known about SPOPL, a paralog of SPOP. SPOPL deletions are found in 7% of The Cancer Genome Atlas (TCGA) PC cases.

Method/Results: To investigate whether SPOPL can regulate AR signaling similarly to SPOP, we first co-expressed AR and SPOPL in 293T cells and observed that SPOPL could effectively co-immunoprecipitate with AR, demonstrating a direct interaction between SPOPL and AR. Next, we evaluate whether SPOPL is capable of ubiquitinating AR. Intriguingly, akin to SPOP, our findings revealed that SPOPL can also facilitate AR ubiquitination and promote degradation via the proteasomal pathway. Notably, we co-immunoprecipitated SPOP and SPOPL, reinforcing the direct interaction between the two proteins as published previously. Lastly, we found overexpression of SPOPL resulted in suppression of AR target genes.

Conclusion: These observations offer important insights into the frequent deletion of SPOPL in PCa patients. Furthermore, they highlight an intricate dynamic between SPOP and SPOPL in regulating AR signaling. The novel finding of AR-SPOPL interplay introduces a new layer of complexity to our comprehension of PC progression, potentially offering fresh avenues for innovative therapeutic interventions.

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Upregulation of Sphingosine-1-Phosphate Receptor 3 Contributes to Olaparib Resistant Prostate Tumor Cell Proliferation

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Abstract

Progression of therapeutic resistance is one of the biggest challenges in prostate cancer. Despite significant advances, castration-resistant prostate cancer (CRPC) remains incurable. Poly (ADP-ribose) polymerase (PARP) inhibitors (PARPi) are clinically approved drugs designed to exploit synthetic lethality by capitalizing on cancer-specific DNA repair defects to provide therapeutic relief. Olaparib, FDA-approved PARPi for treating metastatic CRPC, represents a pivotal breakthrough in precision medicine. However, clinical resistance to PARPi's has been documented, and underlying mechanisms remain unclear. LNCaP and C4-2B cell lines are excellent preclinical models to study CRPC progression. To study acquired resistance, we developed olaparib-resistant LN-OlapR and 2B-OlapR cell lines generated through chronic olaparib treatment of LNCaP and C4-2B, respectively. Sphingosine 1-phosphate (S1P) is a lipid mediator that promotes tumor cell survival, proliferation, angiogenesis, and migration through G-protein coupled receptors (S1PR). RNA-sequencing revealed that S1PR3 was increased 18-fold and 10-fold in LN-OlapR and 2B-OlapR, respectively. The S1P/S1PR3 signaling axis is known to increase EGFR expression and regulate the downstream MAP kinase (MAPK) signaling pathway, which could have implications for Olaparib sensitivity. We will utilize the OlapR models to test our hypothesis that S1PR3 overexpression enhances EGFR/MAPK signaling to promotes cell proliferation and survival. Transcriptomic profiling revealed that S1PR3 is highly expressed in resistant models. We verified increased levels of S1PR3 RNA in the resistant models. We will perform western blots to confirm the increased protein level of S1PR3. The protein levels of Phospho-Spingokinase-1 (SphK1), Phospho-EGFR, and Phospho-ERK1/2 will be determined by Western blot analysis. ELISA will be used to determine S1P secretion. RNAi was used to inhibit S1PR3 expression. We found that RNAi inhibition of S1PR3 re-sensitized the OlapR models to Olaparib treatment. Our findings demonstrated that inhibiting S1PR3 aids in Olaparib sensitivity in the resistant setting. Future work will utilize OlapR models to study how the S1PR3/EGFR signaling axis promotes the development of resistance.

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Hyperglycemia Regulates Expression of Zinc Efflux Transporter, ZnT1, in Prostate Cancer

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Abstract

Background: For prostate function, normal prostate glandular epithelium has higher zinc levels compared to other tissues, but this level is decreased in prostate cancer (PCa). Zinc accumulation in PCa cells appears to be detrimental in some studies. Zinc efflux transporter (ZnT1) has a crucial role in regulating intracellular zinc by exporting zinc into the extracellular space, and it has a single N-glycosylation site (N299). Moreover, ZnT1 expression is upregulated in PCa tissues. Many studies have shown that hyperglycemia is associated with an increased risk of PCa progression, and interestingly, acute high glucose levels enhances zinc secretion in normal prostate epithelial cells and slightly in PC-3 cells. The mechanism by which high glucose might affect ZnT1 and intracellular zinc homeostasis is unknown. In this study, we examined the effect of high glucose level on ZnT1 expression, membrane localization, and glycosylation state in PCa cells.

Methods: LNCaP and PC-3 PCa cell lines were maintained in low glucose media, plated overnight, then incubated for 48 h in low glucose media (5.5 mM; control) or high glucose media (25 mM). Total cell lysate was collected and a biotinylation assay for cell surface proteins was performed. Samples were analyzed by Western blot using anti-ZnT1 antibody.

Results: In PC-3 cells, high glucose treatment significantly upregulated total ZnT1 expression ($p=0.032$) and ZnT1 cell membrane expression ($p=0.007$). In contrast, there was no significant difference in total ZnT1 level between high glucose treated LNCaP cells and the control. Analysis of the biotinylated ZnT1 in LNCaP showed that high glucose treatment significantly decreased nonglycosylated ZnT1 dimer ($p=0.032$), and glycosylated monomer ($p=0.04$). However, there was no significant difference in the total ZnT1, total glycosylated and ZnT1 dimer levels (glycosylated and nonglycosylated) on the cell membrane between high glucose treated LNCaP and the control.

Conclusion: These data support that high glucose levels might contribute to PCa progression via ZnT1 upregulation as shown in PC-3 cells. Further studies are needed to determine the effect of high glucose on intracellular zinc concentration and glycosylation state of ZnT1 in normal prostate epithelium and PCa cells.

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Impact of microRNA Depletion on Development and Maintenance of Bladder Urothelium

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Abstract

Background: MicroRNA (miRNA) serve essential roles in epithelial cell development, maintenance, and response to injury by regulating mRNA and protein expression, but their functions in urothelium remain largely unknown. To address this knowledge gap, we engineered mice with urothelium specific inactivation of Dicer, an exonuclease required for miRNA biogenesis.

Methods: We generated urothelial Dicer conditional knockout mice by crossing Dicerfl/fl and Upk2Cre or Upk2CreERT2 animals. A tdTomato (tdT) fluorescent protein was expressed in a Cre/LoxP dependent manner from the Rosa26 locus to identify cells in which Dicer inactivation had occurred. Urothelial lineage markers were evaluated by immunofluorescence microscopy, Western blotting, and QRT-PCR.

Results: Bladders from control animals displayed the expected urothelial morphology with distinct layers of Krt5+;Krt14+ basal (B) cells, Upk+ intermediate (I) cells, and Upk+; Fabp4+ superficial (S) cells. By 3 weeks of life, bladders from Upk2Cre;Dicerfl/fl mice exhibited rounded and exfoliating S cells and patchy/discontinuous Fabp4 expression. This coincided with a 1.5-fold increase in Krt5 protein, and a 3.4-fold increase in Krt14 protein (P=0.0040 and P<0.0001, respectively), despite unchanged Krt5 and Krt14 mRNA levels The urothelial phenotype was exacerbated at 6 and 9 weeks of life and associated with urothelial thickening (1.6-fold, P=0.0054, and 1.7-fold, P<0.0001, respectively). Inducible inactivation of Dicer in adult Upk2CreERT2;Dicerfl/fl mice recapitulated the aberrant phenotype observed in Upk2Cre;Dicerfl/fl mice, with S cell loss preceding B and I cell expansion.

Conclusion: Dicer serves essential roles in urothelial structural integrity by promoting S cell maintenance. This justifies further studies to identify specific miRNA responsible for this role.

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Manzamine A mitigates androgen receptor transcription via E2F8 and effectively inhibits prostate tumor growth in mice

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Abstract

Manzamine A mitigates androgen receptor transcription via E2F8 and effectively inhibits prostate tumor growth in mice

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Background: The androgen receptor (AR) is the main driver in the development of castration-resistant prostate cancer, where the emergence of AR splice variants leads to treatment-resistant disease. Therefore, developing new therapeutic tools targeting the mechanisms of AR activation and AR-regulated genes remains critical to block the development and progression of CRPC.

Methods: Using a marine alkaloid, manzamine A (MA), we investigated the antiproliferative and proapoptotic effect in androgen-responsive (LNCaP and 22Rv1) and androgen-resistant (PC-3 and DU145) prostate cancer cell lines. In vivo therapeutic efficacy and toxicity studies of MA were performed using tumor xenografts in mice.

Results: MA significantly inhibited the growth of the tested cell lines in a dose- and time-dependent manner, and was highly effective in inhibiting the xenograft tumor growth in mice without any pathophysiological perturbations in major organs. MA altered the cell cycle regulating proteins, and exerted proapoptotic activity in various assays. MA suppressed the full-length AR (AR-FL), its spliced variant AR-V7, and the AR-regulated PSA and hK2 genes. RNA-seq analysis revealed E2F8 as a potential novel target of MA suppressing AR transcription and its chemo-resistant variant AR-V7.

Conclusions: Identification of E2F8 as a novel target of AR transcription repression provides new avenues targeting CRPC via E2F8. Manzamine A is a small molecule inhibitor of E2F8 and may serve as a potential lead compound to overcome therapy resistance prostate cancer caused by AR remodeling.

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UCHL1 is a Molecular Indicator and a Therapeutic Target for Neuroendocrine Prostate Cancer

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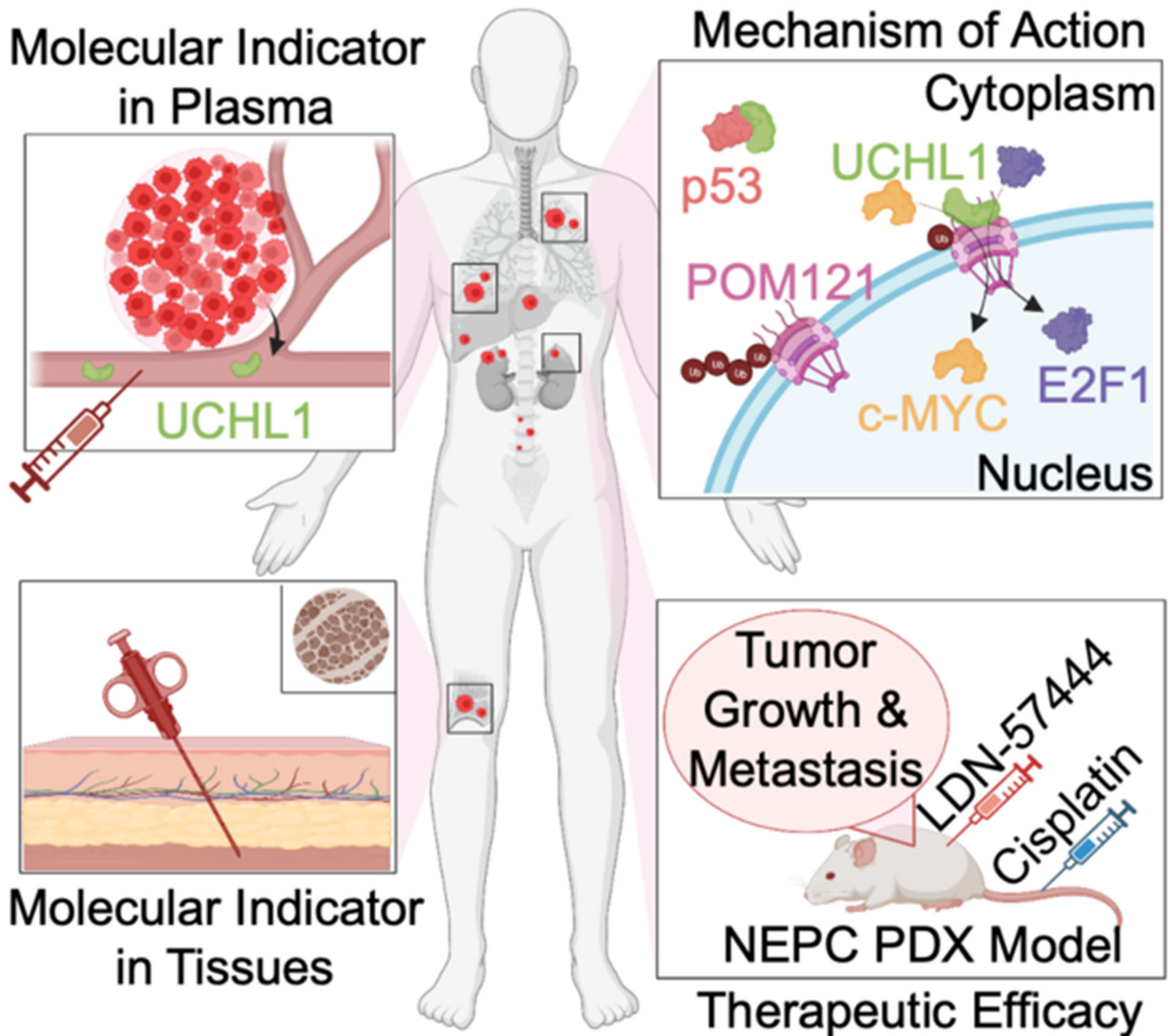
Abstract

Background: Neuroendocrine prostate cancer (NEPC) is a highly aggressive subtype of prostate cancer that is commonly observed in advanced prostate cancer after multiple rounds of hormone therapies. NEPC typically presents with distant metastasis and is associated with poor outcomes. Currently, there are no durable therapies for improving survival outcome for NEPC patients. Therefore, there is a critical clinical need to identify new drivers, therapeutic targets, and effective therapeutic strategies.

Methods: Ubiquitin carboxy-terminal hydrolase L1 (UCHL1) protein level and mRNA levels were analyzed in tissues from benign prostate, localized PC, castration-resistant PC (CRPC), and NEPC patients. Stable cell lines were generated by knockdown/knockout of UCHL1 in Trop2-driven NEPC (TD-NEPC) cell line or overexpression of UCHL1 in 22Rv1 and C4-2 cell lines. Colony formation and 3D-matrigel dot invasion assay were used to test cell growth and invasion in vitro. The functional role of UCHL1 on NEPC tumor growth and metastatic colonization in vivo was evaluated by subcutaneous xenograft implantation and intracardiac injection. Mass spectrometry was performed on TD-NEPC UCHL1 knockdown cells to define UCHL1 targets. LDN-57444, a UCHL1 inhibitor, was tested on NEPC tumor growth and metastasis as a single reagent or in combination strategy in vivo.

Results: We demonstrated that UCHL1 is significantly elevated in tissues from patients with NEPC. Loss of UCHL1 decreases tumor growth and inhibits metastasis of these malignancies. UCHL1 maintains neuroendocrine differentiation and promotes cancer progression by regulating nucleoporin, POM121, and p53. UCHL1 binds, deubiquitinates, and stabilizes POM121 to regulate POM121-associated nuclear transport of key transcriptional factors, E2F1 and c-MYC. Conversely, UCHL1 binds p53 and promotes p53 degradation. Importantly, treatment with UCHL1 inhibitor LDN-57444, significantly reduces tumor growth and metastasis of NEPC. The combination of UCHL1 inhibitors with the standard of care used for NEPC, cisplatin, halts tumor growth in pre-clinical settings.

Conclusions: Our study reveals new mechanisms of UCHL1 function in NEPC tumorigenesis and identifies UCHL1 as a therapeutic target and potential molecular indicator for diagnosis and monitoring treatment responses in NEPC.


Neuroendocrine Prostate Cancer
Molecular Indicator in Plasma
UCHL1
Mechanism of Action
Cytoplasm
p53
UCHL1
POM121
c-MYC
E2F1
Nucleus
Tumor Growth & Metastasis
LDN-57444
Cisplatin
NEPC PDX Model
Therapeutic Efficacy
Molecular Indicator in Tissues

81

Shed Trop2 Extracellular Domain is a Regulator of Prostate Cancer Metastasis

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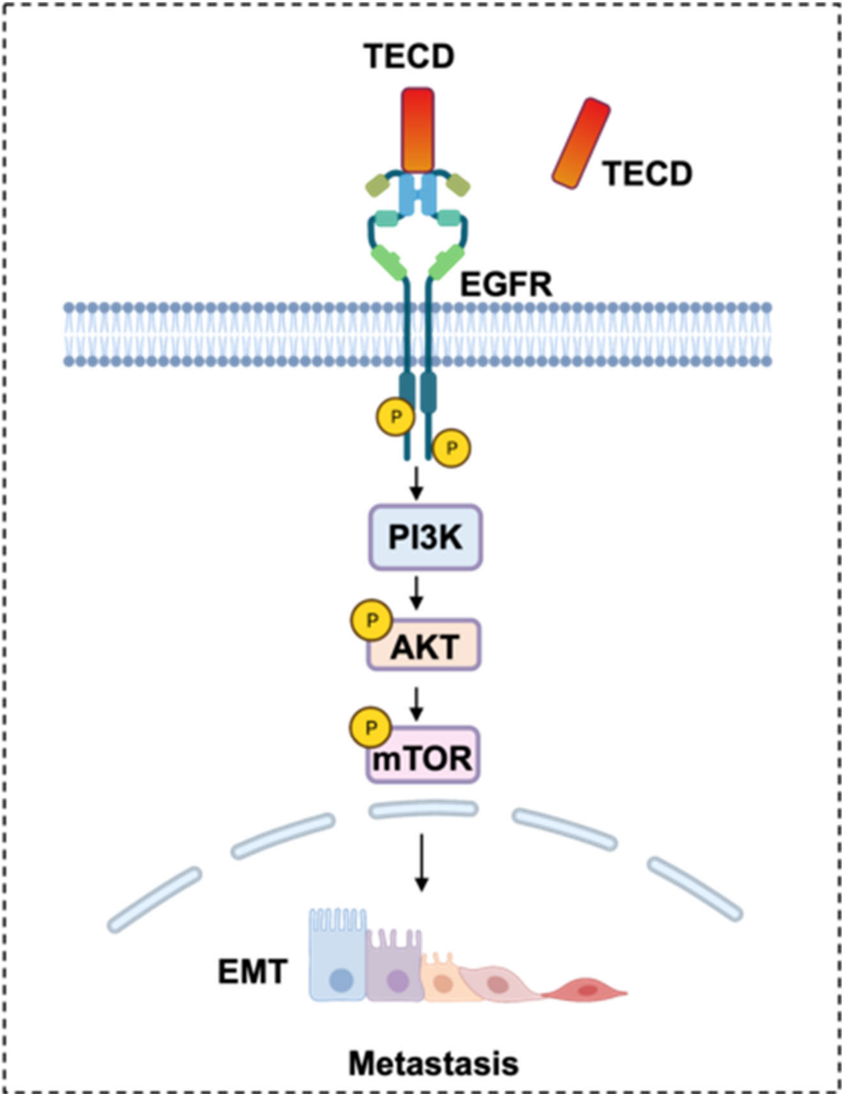
Abstract

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 extracellular Trop2 in tumor growth and metastasis in prostate cancer, and further identify the mechanisms underlying extracellular Trop2 in driving 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 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 vehicle or TECD (treated i.v. TECD 250 ng/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.

Results: We report that naturally occurring cleavage of Trop2 releases TECD into the extracellular environment, which can be detected in media from prostate cancer cells and serum from patients with clinically significant prostate cancer. Our results demonstrate that overexpression of TECD and treatment with TECD significantly increase cell migration, invasion, metastatic colonization, and spontaneous metastasis in vitro and in vivo. Proteomic profiling reveals that TECD modulates a set of proteins associated with invasion, migration, mTOR signaling, and epithelial-to-mesenchymal transition. TECD directly binds to EGFR and increases the phosphorylation of EGFR, resulting in the activation of the EGFR-PI3K-AKT-mTOR pathway in prostate cancer. Moreover, treatment with EGFR inhibitors suppresses prostate cancer invasion driven by TECD.

Conclusions: Our study reveals a new function of TECD in driving prostate cancer progression. Furthermore, our study suggests that TECD could be potentially used as a liquid biomarker for diagnosing and predicting prostate cancer.


TECD
TECD
EGFR
P
P
PI3K
P
AKT
P
mTOR
EMT
Metastasis

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Management Of Wunderlich Syndrome By Laparoscopic Partial Nephrectomy: A Case Series

Dr AMISH WANI MBBS, MS (general surgery), DNB (general surgery), Pursuing DrNB urology), Dr C Mallikarjuna MBBS, MS, Mch, Dr Syed Md Ghouse MBBS, MS, DNB (urology), Dr Rajesh Kumar Adapala MBBS, MS, DNB, Fellow in uro oncology

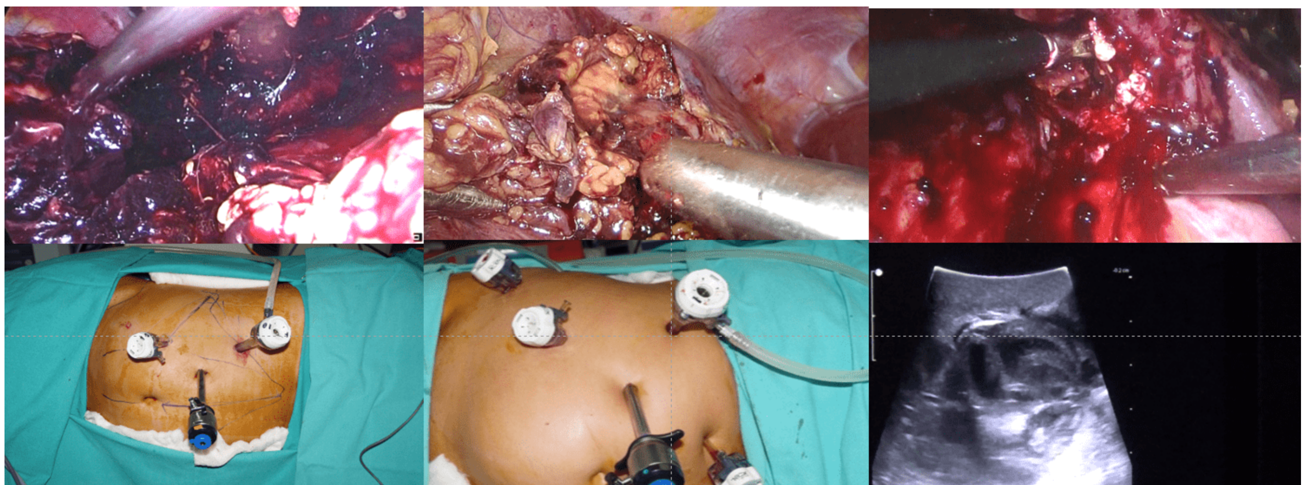
ASIAN INSTITUTE OF NEPHROLOGY AND UROLOGY, HYDERABAD, TELANGANA, India

Abstract

Introduction & Objectives:

Wunderlich syndrome (WS) is a clinical syndrome of spontaneous nontraumatic renal subcap

sular and retroperitoneal hemorrhage with the most common cause being renal angiomyolipoma (AML). Treatment depends on the clinical condition of the patient and underlying cause of the hemorrhage. We present 4 cases of WS due to AML which were managed by laparoscopic partial nephrectomy (LPN).



Materials & methods:

Out of the 4 cases of AML, 1st case was a 22-week primigravida, diagnosed previously with tuberous sclerosis, 2nd presented in acute kidney injury and sepsis, 3rd presented with AML with perinephric hematoma and the 4th had a post-selective renal artery angioembolization for left spontaneous hemorrhage with renal AML 6 months back. The technical difficulties were:1)Due hemorrhage and perinephric hematoma the laparoscopic field of vision is generally obscured, and dark; 2) Dense desmoplastic reaction around the tumor results in difficulty for enucleation especially following angioembolization; 3) Identification of the tumor location due to overlying hematoma will be difficult, hence intraoperative ultrasound is of paramount importance, 4) Delineation of the perinephric fat is difficult because of inflammation; 5) In case of pregnancy, as in the 1st case scenario, the intra-abdominal pressure while creating pneumoperitoneum cannot exceed more than 10 mm Hg, and the ports are placed 5-6 inches laterally to the midline because of reduced space due to the enlarged uterus inside the abdomen.

Results:

The mean operative time was 145 minutes, and warm ischemia time of 28 minutes. Intraoperative blood loss was around 350 ml. Postoperative transfusion were given in 3 patients. Histopathology of all the specimens showed AML with no signs of malignancy. All 4 patients showed no residual tumor and perinephric collections on imaging at a mean follow-up of 16 months.

Conclusions:

LPN is technically challenging laparoscopic surgery for treating WS due to AML by an experienced surgeon in selected patients only.

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A Retrospective Cohort Study to Identify Discrepancies in Urine Volume Between Two Devices and to Determine their Consistency for accuracy.

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Abstract

Introduction

It is well known that monitoring accurate urine volume in patients has been a challenge, mostly due to issues with accuracy and the lack of an established gold standard device. There have been several devices that are capable of recording urine volume as the first modality of hydration status. In our phase I clinical research facility we used two methods to measure urine: a glass measuring cylinder and a plastic bed pan. 84 data points collected from multiple different research subjects showed that there was a statistically significant variation in urine volume in milliliters measurement between the two devices.

Objectives

1. To determine if discrepancies in urine volume measurement exist amongst the two methods.
2. To determine if the discrepancy is of statistical significance.
3. To establish a simple, yet efficient method to reduce error in recording urine volume for future research and clinical purposes.

Methods

We retrospectively reviewed urine collection and processing data from six subject. The urine samples were provided multiple times to have at least a sum of 12 entries per subject. Each data entry was an addition of either one or multiple urine samples stretched over a twelve-hour period. Subjects were carefully instructed to urinate in a standard plastic bed pan/ hat and to avoid any spillage. The volume of urine in the plastic hat was confirmed and verified by two clinical researchers and an eyeball value was determined from the lower meniscus of fluid. Further, all the urine collected from throughout the twelve-hour period, was measured using a measuring cylinder, and the volume value was confirmed by two clinical researchers with the help of lower meniscus. Of the two methods, the measuring glass cylinder was presumed to be more accurate than the other.

After tabulating the difference amongst the two methods, a Breusch-Pagan test was utilized to confirm heteroskedasticity. A one sample t-test was used to confirm that the difference of readings between the two methods was of statistical significance.

Results

Of the 84 readings of urine volumes, the lowest and the highest difference in readings among the two methods was 0 ml and 732 ml respectively. The mean calculated difference was 63.76 ml and the median value was 50 ml. A Breusch-Pagan test with odds ratio of 111.39 and a p-value=0.000

confirmed that the variance of inaccuracy in the measurements of plastic containers was of statistical significance.

Further, the absolute differences were measured and a one sample t-test was used to determine the statistical significance of the difference in reading values of urine hat over glass measuring cylinders(OR 6.95 C.I. [45.51,82.01], p=0.000).

Conclusion

From our results, it is conclusive that plastic urine bed pans/ hats are not as precise as glass measuring cylinders for most cases. There could be other presumed sources of error, not limited to, medical personnel inspecting samples, but our statistical analysis shows that a plastic bed pan cannot alone be relied upon to evaluate urine output volume.

One-Sample Test						
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Absolute value of diff	6.949	83	.000	63.762	45.51	82.01

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Elevating PLK1 Overcomes BETi Resistance in Prostate Cancer via Triggering BRD4 Phosphorylation-dependent Degradation in Mitosis

Dr. Xiaoqi Liu PHD, Dr. Yanquan Zhang PhD

University of Kentucky, Lexington, KY, USA

Abstract

Elevating PLK1 Overcomes BETi Resistance in Prostate Cancer via Triggering BRD4 Phosphorylation-dependent Degradation in Mitosis

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Abstract

Background: Besides AR, bromodomain-containing protein 4 (BRD4) has been widely studied as an attractive therapeutic target of prostate cancer. Currently, a number of BRD4 inhibitors or degraders have been developed. However, recent studies revealed that loss-of-function SPOP mutations of prostate cancer patients lead to BRD4 stabilization, thus resistance to BRD4 inhibitor JQ1. As such, dissecting the mechanism underlying regulation of BRD4 stability may improve the clinical response and efficacy of BRD4-targeted therapy in PCa patients.

Methods: Standard biochemical and cellular biology approaches were used to identify BRD4 as a new substrate of Polo-like kinase 1 (Plk1). Both cell culture and xenograft mouse model were used to demonstrate a new approach to enhance the efficacy of BRD4 inhibitor JQ1.

Results: We discovered that BRD4 is significantly decreased in mitosis in a PLK1-dependent manner in PCa cells. Mechanistically, we show that upon mitotic entry, BRD4 is phosphorylated at T1186 by CDK1/Cyclin B complex. Priming phosphorylation of BRD4 at T1186 creates a docking site to recruit PLK1, which subsequently phosphorylates BRD4 at S24 and S1100. The later event leads to enhanced BRD4 binding to APC/CCdh1 complex, which drives its degradation via proteasome pathway. Accordingly, we found that overexpression of PLK1 lowers SPOP mutation-stabilized BRD4 in PCa cells, consequently rendering cells to be re-sensitive to BRD4 inhibitors. Intriguingly, we unveiled that sequential treatment of docetaxel and JQ1 resulted in significantly inhibition of prostate cancer cell proliferation, colony formation and patient-derived xenograft tumor growth.

Conclusion: Collectively, our results suggested that PLK1 phosphorylates BRD4 and consequently triggers its phosphorylation-dependent degradation in prostate cancer. Sequential treatment of docetaxel and JQ1 overcomes SPOP mutation-associated resistance to BETi, which may shed light on development of new strategies to treat prostate cancer.

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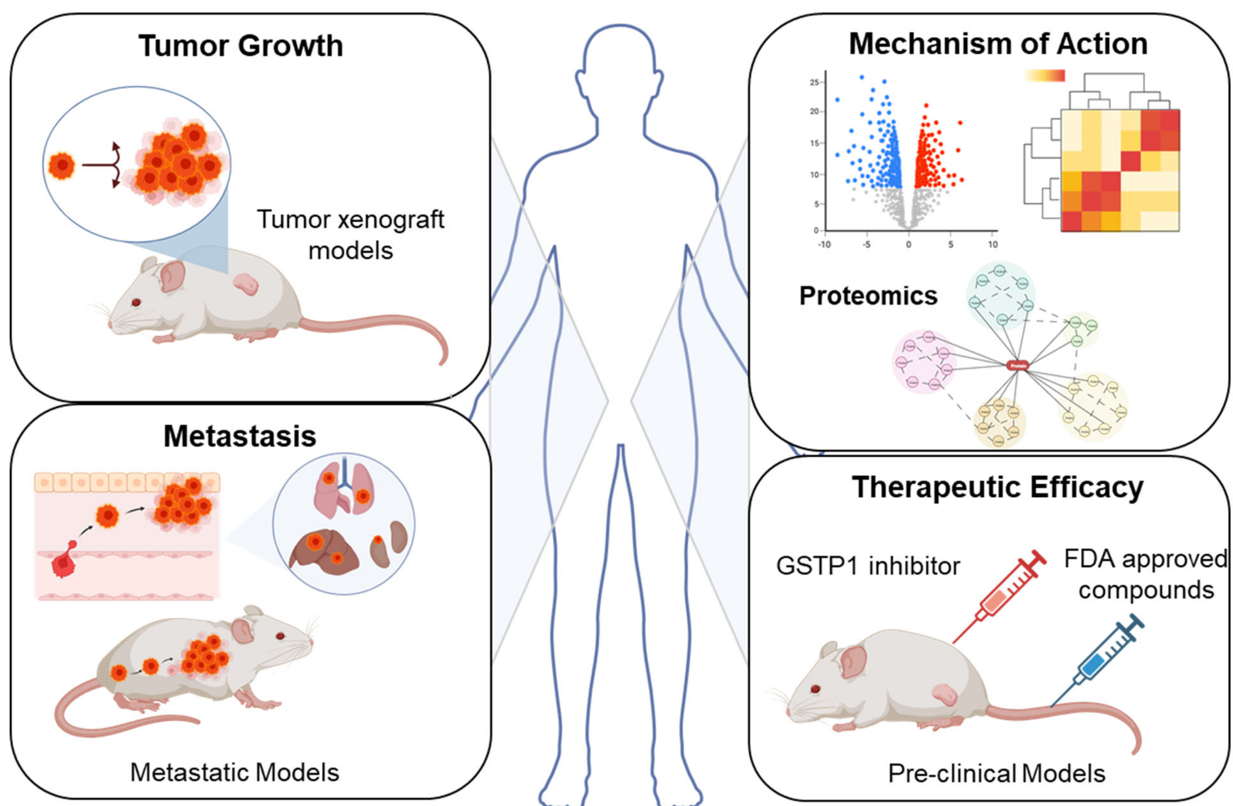
GSTP1 is a Regulator of Prostate Cancer Progression and a Therapeutic Target for Advanced Prostate Cancer

Associate Professor Tanya Stoyanova PhD

University of California, Los Angeles, Los Angeles, CA, USA

Abstract



GSTP1 is a Regulator of Prostate Cancer Progression and a Therapeutic Target for Advanced Prostate Cancer

Shiqin Liu1, Merve Aslan2, Fernando Jose Garcia Marquez2, En-Chi Hsu2, Abel Bermudez2, Michelle Shen1, Rosalie Nolley3, Christian Kunder4, James D. Brooks3, Eva Corey5, Sharon Pitteri1, and Tanya Stoyanova1,6*

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Background: Prostate cancer is the most common malignancy among men in the United States. While the initial hormone deprivation therapies are effective for most men, the disease relapses as castration-resistant prostate cancer (CRPC) phenotype leading to an advanced incurable state. With secondary hormone therapies, about 10-20% of heavily treated CRPC tumors acquire a neuroendocrine phenotype (neuroendocrine prostate cancer, NEPC). Here we report that GSTP1, a phase II detoxification enzyme, is upregulated in advanced prostate cancer including NEPC and we further define the functional role and therapeutic potential of GSTP1 in advanced prostate cancer.

Methods: GSTP1 protein level was analyzed in tissues from benign prostate, localized PC, castration-resistant PC (CRPC)-patient-driven xenografts (PDXs), and neuroendocrine prostate cancer (NEPC) PDXs. Colony formation and 3D-matrigel dot invasion assay were tested on GSTP1 knockdown/knockout cells or GSTP1 overexpression cells. The functional role of GSTP1 on prostate cancer tumor growth and metastatic colonization was evaluated by subcutaneous xenograft implantation and intracardiac injection in vivo. Mass spectrometry was performed on prostate cancer with GSTP1 knockdown xenografts to define whole protein changes upon GSTP1 modulation. Piperlongumine (PPLGM), a GSTP1 inhibitor, was tested on prostate cancer tumor growth and metastasis as a single reagent or in a combination strategy.

Results: We demonstrate that GSTP1 is highly expressed in NEPC patient samples, NEPC xenografts, NEPC PDX models, and CRPC with low levels of androgen receptor (AR). Overexpression of GSTP1 enhances cell proliferation and migration while downregulation or loss of GSTP1 in advanced prostate cancer suppresses tumor growth, migration, invasion, and metastases. Inhibition of GSTP1 suppresses prostate cancer cell growth, migration, and invasion in vitro. Proteomic profiling identifies PGK1 as the most upregulated druggable protein upon GSTP1 knockdown. The combination of PPLGM with a PGK1 inhibitor significantly decreases prostate cancer cell growth and invasion ability in vitro.

Conclusion: GSTP1 drives prostate cancer progression. GSTP1 is a potential druggable target for suppressing prostate cancer tumorigenesis and metastases as a single or combination strategy

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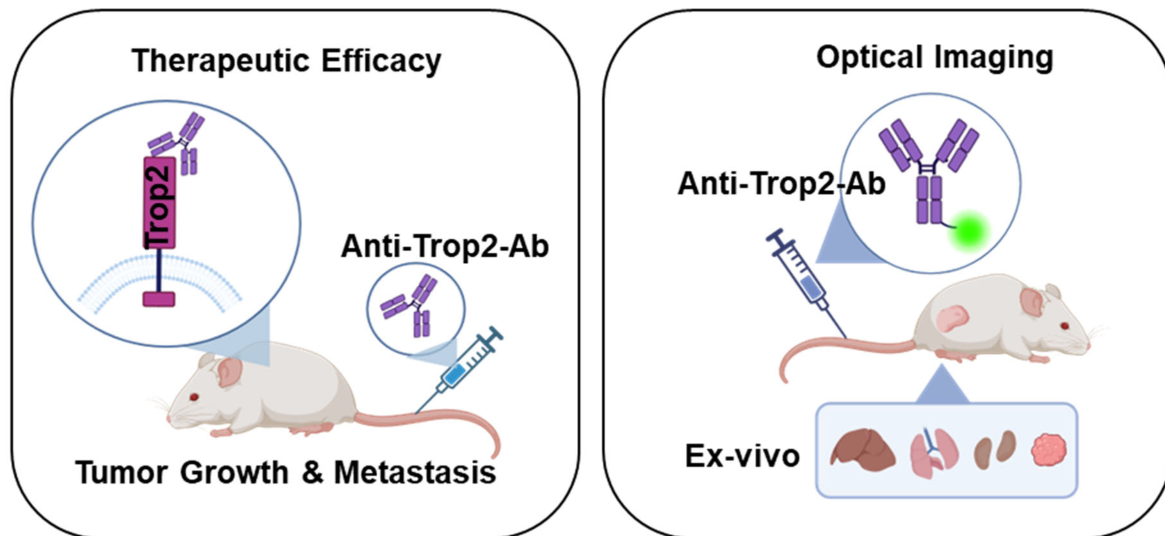
Developing New Antibody-based Therapy and Optical Imaging for Epithelial Cancers

Dr. Tanya Stoyanova PhD

University of California, Los Angeles, Los Angeles, CA, USA

Abstract



Developing New Antibody-based Therapy and Optical Imaging for Epithelial Cancers

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*Corresponding author. Email: tstoyanova@mednet.ucla.edu

Background: Carcinomas are cancers of epithelial origin and account for 80 to 90 percent of all cancer cases. Trophoblastic cell surface antigen-2 (Trop2) is a transmembrane glycoprotein and is overexpressed in various epithelial cancers, including prostate, breast, lung, and others. Trop2 is associated with tumor metastasis and poor prognosis across multiple epithelial cancers, making it a promising therapeutic and imaging target for epithelial cancers. This study aims to develop new and more effective Trop2-targeted therapy and imaging modalities for prostate cancer and other epithelial cancers.

Methods: Trop2 protein expression was accessed in prostate, breast, and lung cancer tissues and tissue microarrays (TMAs) by immunochemistry (IHC) staining. Dot plot and immunofluorescence staining were used to evaluate the binding ability of anti-Trop2 antibody fragments with Trop2. The binding kinetics of anti-Trop2 antibody fragments to Trop2 antigen was measured by Biolayer interferometry (BLI). The therapeutic effect of anti-Trop2 antibody fragments was tested in subcutaneous tumor models and metastasis models across multiple epithelial cancers including prostate, breast, and lung cancer in vivo. Anti-Trop2 antibody fragments-indocyanine green (ICG) optical imaging probes were tested in vivo to detect Trop2-positive epithelial cancers.

Results: Trop2 was elevated in advanced prostate cancer, triple-negative breast cancer (TNBC), and non-small cell lung cancer (NSCLC). Anti-Trop2 antibody fragment bound to Trop2 and had high binding affinities to Trop2 with Kd of 0.78 nM. Anti-Trop2 antibody fragments significantly delayed tumor growth and suppressed metastasis of epithelial cancers in vivo. No toxicity was observed in mice treated with anti-Trop2 antibody fragments assessed by animal body weight. Anti-Trop2 antibody fragment-ICG conjugates were able to detect and differentiate Trop2-positive tumors with a high Trop2-positive tumor-to-Trop2 negative tumor ratio.

Conclusions: Our study developed a new anti-Trop2 antibody-based therapy and optical imaging tool for prostate, breast, and lung cancers. Due to the altered expression of Trop2 in other malignancies, we believe our reagents will be applicable to other epithelial cancers.

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Cell Plasticity in a Mouse Model of Benign Prostate Hyperplasia Drives Amplification of Androgen-Independent Epithelial Cell Populations Sensitive to Antioxidant Therapy

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Abstract

Background. Current medical treatments of benign prostate hyperplasia (BPH) are unsatisfactory in many patients. Oxidative stress has been proposed to contribute to BPH development. Addressing the efficacy of new therapeutic strategies requires relevant and well characterized preclinical models of BPH. Probasin-prolactin (Pb-PRL) transgenic mice recapitulate many histopathological and urodynamic features of human BPH. To progress in our understanding of the mechanisms driving BPH, the aims of this study were to elucidate the complex cell composition of hypertrophied Pb-PRL prostates, and to explore the therapeutic benefit of antioxidant therapy in this animal model.

Methods. The cellular profile of hyperplastic Pb-PRL prostates was determined by single cell RNA-sequencing (scRNAseq) using 10x genomics technology, and compared to public scRNAseq datasets of WT prostates. Antioxidant treatment involved daily gavage with Anethole trithione (ATT), a specific inhibitor of mitochondrial ROS production, versus vehicle. Urodynamic parameters were determined using specialized metabolic cages (Shinfactory, Japan). Human epithelial BPH-1 cell line was used for mechanistic in vitro studies.

Results. The epithelium of Pb-PRL mouse prostates mainly comprises low-androgen signaling cell populations, including LSCmed (Lin-/Sac-1+/CD49fmed) cells equivalent to Club/Hillock cells enriched in the aged human prostate. Trajectory analyses predict LSCmed cells to result from the reprogramming of androgen-dependent luminal secretory cells through intermediate cell states. Hyperplastic Pb-PRL prostates exhibit increased vulnerability to oxidative stress due to reduction of antioxidant enzyme expression. One-month treatment of Pb-PRL mice with ATT by oral route reduced prostate weight and improved urodynamic parameters (reduced voiding frequency & increased volume per voiding). ATT treatment of BPH-1 cells altered mitochondrial metabolism, cell proliferation and stemness features. According to the latter, ATT prevented the growth of organoids generated from sorted Pb-PRL basal and LSCmed cells, the two major BPH-associated, androgen-independent epithelial cell compartments in Pb-PRL mice.

Conclusions. These data support cell plasticity as a driver of BPH progression towards reduced androgen sensitivity, and identify ATT, a drug already in clinical use for xerostomia, as a promising antioxidant therapy for BPH.

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MED12 promotes prostate cancer (PCa) cell proliferation through c-MYC and androgen receptor (AR) signalling

Dott. Mag. Chiara Andolfi Master´s degree[1], BSc Caterina Bartolini Bachelor´s Degree[1,2], BSc Elisa Morales Bachelor´s Degree[1,3], Dott. Martin Puhr PhD[1], Dott. Juan Guzman PhD[4], Dr. rer. nat. Sven Wach Doctor of Science[4], Prof. Helge Taubert PhD[4], Prof. Achim Aigner PhD[5], Assoc. Prof. Iris E. Eder PhD[1], Dott. Florian Handle PhD[6], Prof. Zoran Culig PhD[1]

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Abstract

Background: the Mediator complex is a multi-subunit protein that regulates gene expression in a genome-wide manner. The aberrant expression of some of its subunits promotes prostate cancer (PCa) by activating androgen receptor (AR) signaling. Preliminary data showed that its MED12 subunit knockdown downregulates PCa cell proliferation. Based on this evidence, we aimed to explore the role of MED12 in the promotion of PCa and in its response to AR-targeted therapy (e.g. enzalutamide).

Methods: we downregulated MED12 expression in LNCaP (AR+, enzalutamide-sensitive), 22Rv1 (AR+, enzalutamide-resistant), and PC3 (AR-) cell lines, and analysed its effects on gene and protein expression (RNA-sequencing, Western Blotting), formation of cell spheroids (IncuCyte analysis), cell proliferation, and cell adhesion (fluorescent-based nuclei counting). To investigate MED12 involvement in cell response to enzalutamide, we co-treated these cells with enzalutamide and evaluated AR activity (PSA secretion quantification).

Results: MED12 knockdown significantly inhibited AR and c-MYC pathways, two major promoters of PCa cell proliferation. Concordantly, MED12 inhibition significantly reduced cell proliferation in all our PCa models (30-50%) and slowed down the generation of 22Rv1 spheroids. LNCaP spheroid morphology and cell adhesion assays suggested that MED12 expression inhibition untightens cell-to-cell contacts. MED12 knockdown strongly decreased protein expression of the ligand-independent AR-variant 7 (AR-V7) (60%), a major promoter of enzalutamide resistance, in 22Rv1 cells. The combination of MED12 downregulation and enzalutamide treatment showed an additive effect in reducing PSA secretion (i.e. AR signaling) in both LNCaP and 22Rv1 cells, suggesting their combinatory effect in inhibiting PCa.

Conclusions: our data reveal that MED12 downregulation inhibits PCa cell proliferation in both AR-dependent and AR-independent ways. Its effect on AR signaling and AR-V7 protein expression suggests its involvement in enzalutamide resistance.

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BCL2 reprograms Androgen receptor signaling and augments castration resistance in CSPC

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Abstract

Background: Although 80-90% localized prostate cancers (PCs) exhibit initial sensitivity to ADT (castration sensitive PC/CSPC), however the duration of response is variable and relapse invariably occurs in the transition to castration-resistant prostate cancer (CRPC) within 2-3 years of initiation of ADT. Therefore, it is essential to discover early resistance mechanisms that initially protect castration-sensitive prostate cancer (CSPC) cells from ADT in order to develop novel combination therapies that block or delay the ADT-induced shift of CSPC to mCRPC.

Methods: We analyzed the tumor transcriptomic profile of a neoadjuvant randomized phase II trial (NCT00924469) of LHRH with abiraterone acetate ([AA]; was conducted in patients with localized high-risk PC (N=58). (Taplin et al, 2014, Sowalsky et al, 2018). Expreimntally, we subjected human CSPC cells and genetically engineered mice (GEM) prostate tumors to ADT exposure. Subsequently, we analyzed the oncogenic signaling pathways that exhibited enrichment following androgen/ADT treatment, comparing them to untreated samples in both clinical dataset and our experimental setup.

Results: We discovered ~10-fold increased mRNA expression of BCL2 ($p<0.001$) in ADT-treated PC patients compared to untreated patients in NCT00924469 samples. Our experimental study showed that syntenic AR-agonist R1881 (even at very low concentration) strongly reduces BCl2 expression in CSPC cells compared to untreated cells. We also observed that BCL2-overexpressed PC cell (LNCaP) exhibited relative resistance to ADT (Enzalutamide). We performed RNA sequencing followed by pathway analysis in BCL2 overexpressed and control LNCaP cells. Our preliminary RNA-sequencing data reveal a striking induction of cell metabolism pathways (fatty acid and xenobiotic metabolism), including activation of mTOR/PI3K/AKT signaling in BCL2 over-expressing CSPC cells in response androgen. Our data collectively emphasize, for the first time, the impact of BCL2 expression on reprogramming of AR transcriptional activity in PC cells, along with the biologically relevant pathways and castration resistance linked to this reprogramming occurrence.

Conclusions: Collectively, our data suggest a noncanonical function of BCL2 in the reprogramming of the AR-signaling and its influence on castration resistance. Our findings indicate that there is a significant interplay between the AR and BCL2 signaling pathways in CSPC biology, and this complex interplay may play a critical role in the early transition from CSPC to lethal CRPC.

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Bacteria Trigger Calcium Oxalate Crystal Formation

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Abstract

Background

Kidney stone disease prevalence is increasing worldwide. Approximately 80% of kidney stones are composed of calcium oxalate (CaOx). Recent studies suggest bacteria play an active role in CaOx nephrolithiasis. We hypothesize that uropathogenic bacteria trigger nephrolithiasis via biofilm formation. We aimed to identify bacteria within calculi and to investigate the effect of biofilm-forming bacteria on the formation and propagation of CaOx crystals.

Methods

A novel combination of high-resolution X-ray microscopy (microtomography / micro-CT) and X-ray fluorescence microscopy (micro-XRF) visualized the 3-dimensional morphology of human stones. Separately, CaOx crystals were formed in-vitro in the presence and absence of E. coli and P. aeruginosa, biofilm-forming bacteria frequently observed in stones collected from patients. Confocal and scanning electron microscopy (SEM) assessed crystal (1) size, (2) morphology, and (3) formation kinetics. Raman (RS) and Energy Dispersive X-ray Spectroscopy (EDAX) identified the chemical composition of the formed crystals. A microfluidic kidney model was developed to recapitulate the physiology of the urinary tract. We evaluated the mechanism of CaOx crystallization in the presence of bacteria in this microenvironment.

Results:

XRF analysis revealed a complex 3-dimensional stone morphology including a layered architecture, with organic and inorganic layers reminiscent of biomineralization and a uniquely-connected porosity present in each layer individually. SEM showed that the morphology of bacteria-influenced crystals significantly differs from controls; RS and EDAX confirmed CaOx composition in both groups. P. aeruginosa mediated up to 280% increase in CaOx crystal size over 10 days, with a growth rate 2.5 times that of controls. Our 3-dimensional kidney model revealed that P. aeruginosa and E. coli secretions significantly enhance CaOx crystallization, growth, and aggregation compared to controls.

Conclusion:

Our results suggest that biofilm-forming bacteria significantly promote CaOx crystal growth, propagation, and aggregation and that bacterial secretions enhance CaOx crystal formation. With our novel combined x-ray microscopy technique, locations of bacteria within stones may be identified as well as putative nucleation sites for stone formation. Collectively, these findings will give more explicit insight into the mechanistic link between bacterial biofilm formation and CaOx nephrolithiasis.

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Immune cell scRNA-seq analyses suggest a role for age-associated granzyme K-expressing T cell subset in symptomatic BPH

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Abstract

Immune cell scRNA-seq analyses suggest a role for age-associated granzyme K-expressing T cell subset in symptomatic BPH

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Background: Age-related immune dysfunction is associated with a variety of chronic conditions. Benign Prostatic Hyperplasia (BPH) is among the most common age-associated conditions in men. Prostatic immune cell infiltration is frequently observed with aging coincident with BPH nodules; however, a relationship between age-related changes in immune cells and BPH has not been defined.

Methods: scRNA-seq data of immune cells from 13 prostates from men (>50 years) were combined with previously published scRNA-seq data from three young organ donor prostates to compare young and aged prostate T cells. scRNA-seq data from the 10 small (≤40g) prostates were combined with 10 large (≥90g) prostates from aged men to compare T cell populations as prostate size increases. Velocity analysis predicted a T cell differentiation trajectory in aged prostates. Cycling and senescent BPH patient-derived fibroblasts were treated with granzyme K and senescence-associated secretory phenotype (SASP)-associated cytokines were measured by ELISA.

Results: A CD8+ Granzyme K high, Granzyme B low ($CD8^{+}GZMK^{hi}GZMB^{low}$) T cell subset, previously associated with immune aging, is increased in aged vs young prostates and positively correlated with patient international prostate symptom score (IPSS). Velocity analysis indicates altered T cell differentiation in large vs small aged prostates. Prostate stromal cells express SASP-associated genes and in vitro granzyme K treatment of cycling and senescent BPH patient-derived prostate fibroblasts variably induced SASP-associated cytokine secretion, including IL6, IL8, CCL2, CCL5, and CXCL1.

Conclusion: These data suggest a link between an age-associated $CD8^{+}$ T cell subset and symptomatic BPH. Also, granzyme K has a variable effect on patient-derived fibroblast SASP-

associated cytokine production, which may contribute to the observed variability in BPH inflammation and clinical symptoms.

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Keratin 5+ Cells are Temporally Regulated Developmental and Tissue Repair Progenitors in the Bladder Urothelium

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Abstract

Background

Bladder urothelium is a highly specialized, slow-turnover epithelium with cells organized into basal (**B**), intermediate (**I**) and superficial (**S**) layers. Biochemical and genetic labeling studies support the existence of one or more progenitor populations with the capacity to rapidly regenerate the urothelium following injury, but slow turnover, a low mitotic index, and inconsistent methodologies obscure progenitor identity. Here, we mapped the fate of bladder K5-UCs across postnatal development/maturation and following administration of cyclophosphamide (**CYC**) to measure homoeostatic and reparative progenitor capacities, respectively.

Methods

Using tamoxifen (**TMX**)-inducible $Krt5^{CreERT2}$;$R26^{tdT}$ mice, we permanently labeled K5-UCs (tdT^{K5}) and evaluated their capacity to form I and S cells during homeostasis or following CYC-induced urothelial injury. Uninjured cohort: TMX (75 mg/kg b.w.) was administered at postnatal day P1, P7, P14, P21, or P35, and mice were euthanized at P42. Injured cohort: TMX was administered at P1, P7, P14, P21, or P35, then CYC (150 mg/kg b.w.) or saline was administered at P42, and mice were euthanized 2 weeks later. Immunofluorescent microscopy was used to examine progenitor-progeny relationships during homeostasis and tissue repair. Image analysis determined tdT expression in B (Krt5+, Upk-, Fabp4-), I (Krt5+/-, Upk+/-, Fabp4-), and S (Krt5-, Upk+, Fabp4+) cells. Limiting cell RNA-seq was used for transcriptome analyses, and organoid assays were used to evaluate progenitor capacity *in vitro*.

Results

Baseline assessments (24hrs after TMX) confirmed tdT labeling was consistently efficient and highly specific at each TMX induction stage. We found that basal K5-UCs were temporally regulated progenitors, with neonatal K5-UCs having the greatest ability to form I cells. Consistent with some reports, adult K5-UCs did not restore suprabasal urothelium following injury. Transcriptome analysis (645 DEGs) identified enrichment of mitotic and cell differentiation pathways in neonatal (P7) K5-UCs. When compared to adult (P42) K5-UCs, neonatal K5-UCs exhibited a superior progenitor capacity in organoid forming assays (measured size and count).

Conclusions

By accounting for age, our findings unite what is considered a “basal cell vs. intermediate cell progenitor theory,” and support a linear model of urothelial formation and regeneration (B→I→S cell).

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Attenuation of SOX2-mediated oncogenicity in prostate cancer cells by arctigenin: A promising therapeutic approach

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Abstract

Background: Targeting the altered metabolic pathways within prostate cancer cells has emerged as a promising therapeutic approach. Addressing the intricate interaction, however, among androgen signaling, glucose utilization, and metabolic flux presented a significant challenge. Recent studies by our group and others have been instrumental in recognizing SRY (sex-determining region Y)-box 2 (SOX2) as a potential oncogene, playing a vital role not only in tumorigenesis but also in the emergence of drug-resistance phenotype and functioning as a metabolic regulator in prostate cancer. SOX2 knockout cells in culture demonstrated reduced growth rates, compromised bioenergetic profiles, and high susceptibility to traditional chemotherapeutic agents, including enzalutamide. We hypothesize that directing metabolic pathway inhibitors against SOX2-positive prostate cancers could potentially lead to the targeted eradication of the cancer cells while minimizing harm to the SOX2-low benign cells. Methods: A screening of approximately 800 compounds from TargetMol Library L5200 was conducted to assess cytotoxicity against SOX2-positive PCa cell line CWR-R1, while also prioritizing minimal toxicity towards SOX2 knockout cells (CWR-R1^{SOX2KO}). Lentivirus-mediated gene transduction technique was employed to induce SOX2 overexpression in LnCaP cells (LnCaP^{SOX2OE}). Cell viability and cellular migration were assessed by CyQuant cell proliferation kit and Incucyte Live cell imaging analysis. Mitochondrial respiration and glycolysis rates were measured by SeaHorse XF analyzer. Assessment of mitochondrial membrane potential was performed using fluorescence microscopy. Gene expression was demonstrated through qPCR and Western blotting techniques. Arctigenin-resistant cells were generated by treating the cells continuously with arctigenin for at least 30 days. Results: Through drug screening, arctigenin was identified as a potential candidate compound with the ability to selectively eliminate SOX2-positive cells, while exhibiting minimal toxicity to SOX2-negative cells. Arctigenin treatment led to significant inhibition of cell growth and migration in prostate cancer cells displaying elevated SOX2 expression. Arctigenin dissipated the mitochondrial membrane potential, decreased ATP synthesis, and reduced oxygen consumption rates in CWR-R1 and LnCaP^{SOX2OE}. Interestingly, cells that survived the arctigenin exposure (arctigenin-resistant cells) exhibited downregulation of SOX2 levels, thereby highlighting a potential SOX2-dependent mechanism of sensitivity. Moreover, arctigenin sensitizes cellular responsiveness to the conventional chemotherapeutic agent enzalutamide. The activation status of Akt remained unaltered, suggesting the involvement of Akt-independent pathways in the arctigenin-induced SOX2 downregulation and cytotoxic effects. Conclusion: Our findings suggest that arctigenin holds promise as a potential candidate for reducing the oncogenic influence of SOX2 in prostate carcinogenesis.

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Collaborating for the Advancement of Interdisciplinary Research in Benign Urology (CAIRIBU)

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Abstract

Collaborating for the Advancement of Interdisciplinary Research in Benign Urology (CAIRIBU)

BACKGROUND: There is a critical need to advance our understanding of non-cancerous genitourinary (GU) diseases. Such conditions include urinary tract infections, kidney and bladder stones, prostatic enlargement, bladder dysfunction, lower urinary tract symptoms, male reproductive disorders, and neurogenic, genetic, and developmental abnormalities of the urinary tract. In spite of their classification as benign, these diseases represent a significant economic burden and have a profound and frequently long-term impact upon the lives of those affected. Developing sensitive diagnostic tests, objective criteria for symptomatic conditions, and effective treatments are complex problems that increasingly require a transdisciplinary approach. While some investigators have successfully built and participated in successful collaborative research relationships, systematic and evidence-based approaches for developing these are few and far between. Barriers and challenges to creating successful transdisciplinary research infrastructure in the non-cancerous GU research space are not well-identified.

METHODS: The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) has prioritized transdisciplinary research. Toward this end, it created a U24 grant mechanism for an "interactions core" to coalesce a collaborative community of interdisciplinary urology researchers under the umbrella of CAIRIBU (Collaborating for the Advancement of Interdisciplinary Research in Benign Urology).

RESULTS: The CAIRIBU Interactions Core has developed strategies to promote the exchange of knowledge, cultivate a collaborative and inclusive research community, develop and deliver complementary training and resources, promote professional development of early career investigators, and provide consistent logistical support to CAIRIBU Community leaders. Importantly, the CAIRIBU Interactions Core also employs program evaluation methodologies and evidence-based frameworks to assess its effect and impact and progress toward meeting goals. In addition, the CAIRIBU Interactions Core analyzes metrics that can demonstrate the efforts of CAIRIBU Community investigators and impact on non-cancerous GU science.

CONCLUSIONS: As a coordinating mechanism for transdisciplinary interactions in non-cancerous urology research, the CAIRIBU Interactions Core promotes the research of CAIRIBU Community investigators, encourages knowledge exchange and collaboration, and gathers and analyzes metrics to assess the impact of these efforts.

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Representation of the Science, Meeting Presentations, and Abstracts at Meetings of the Society of Basic Urological Research

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Abstract

Representation of the Science, Meeting Presentations, and Abstracts at Meetings of the Society of Basic Urological Research

BACKGROUND: The Society for Basic Urological Research (SBUR) holds fall and spring meetings to share knowledge and promote discussion among investigators engaged in malignant and non-malignant urology research. Each meeting features presentations (keynote speakers + lecturers); fall meetings also include abstract presentations (posters). We characterized the individuals and science presented at SBUR meetings.

METHODS: Program books for 2015-2023 meetings were located on the SBUR website ("Past Meetings"). Meeting presentations and abstracts presented during poster sessions were separated. Each group was then split into malignant or non-malignant research. Presenters' genders, institutions, and academic credentials at the time the presentation or abstract was presented were identified. Individuals presenting >1 abstract within a meeting were counted only once. Data were compiled in Excel and analyzed to identify general trends.

RESULTS: There were 12 meetings between 2015-2023 (not including fall 2023). Except for 2015, for which meeting presentations of malignant and non-malignant research were equivalent, the percentage of malignant presentations was higher (range 55-66%, mean 60% of all presentations). For abstracts from 2016-2022 (data for the 2015 & 2023 fall meetings were not available), ≥74% reflected malignant research (range 74-84%, mean 78%). Presenters during meetings between 2015-2023 were >50% men (range 51-77%, mean 63%). For abstracts (2016-2022), there was an upward trend in women presenters – 48% (2016) to 64% (2022; mean 50% across all years). From 2015-2023, more than 2/3 of meeting presenters were PhDs (including MD/- and DVM/PhD). For abstracts (2016-2022), 55% were presented by PhD presenters (including MD/- and DVM/PhD), 14% from presenters with other academic credentials, and 31% by those with no credential (thus assumed to be trainees). From 2016-2022, among 880 abstracts presented by 828 individuals from 188 institutions, 8.5% were presented by University of Wisconsin-Madison investigators; next was University of Illinois-Chicago (4.2%), UC-Davis (4.1%), Roswell Park Cancer Institute (3.1%), UT Southwestern (3.1%), and Louisiana State University (3.0%).

CONCLUSIONS: In 11 of the 12 SBUR meetings over the past 9 years, malignant urology research was the topic of most presentations and abstracts. The higher prevalence of malignant research was most pronounced in abstracts at fall meetings. Whereas the number of women abstract presenters nearly equaled men from 2016-2022 and was actually higher in 2021 and 2022, meeting presentations delivered by men outnumbered women in all years.

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Ectopic myokines promote prostate cancer growth via angiogenesis

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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. Previously, we showed that ADT induces catabolic TGFβ family myokines in muscle. Blocking signaling of these catabolic myokines using the ligand trap ActRIIB-Fc prevents ADT-induced sarcopenia. Recently we discovered that these myokines are also expressed in tumor, and that tumor derived myokines in the circulation signal from tumor to muscle to accelerate both muscle and strength loss in the PTEN null PrCa mouse model.

Methods:

Prostate tumor volume changes in Pb-Cre4 x Ptenfl/fl mice was serially quantified during myokine blockade or anti-angiogenic therapy. Tumors were imaged using high-resolution high-frequency ultrasound (HFUS) imaging and 3D tumor images reconstructed using AMIRA. Growth of CaP8 cells and organoids from PTEN null prostate tumors treated with myokines and ActRIIB-Fc was quantitated. 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. TCGA expression analysis combined partial correlation graphs, model-based clustering and case level classification.

Results:

Myokines are secreted by tumor constitutively in Pten PrCa mice and are further induced by ADT. Tumor-sourced catabolic myokines exacerbate the sarcopenic effects of the muscle-derived ADT-induced catabolic myokines. Unexpectedly, tumor volume decreased markedly in both sham and castrated mice treated with myokine blockade therapies. A subset of PrCa patient tumors show myokine affected signaling, but myokines increase in most men during ADT. 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 null mice treated with anti-VEGFR2 mAb was arrested.

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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High-fat diet and MYC cooperation promotes lactate accumulation and tumor microenvironment remodeling in prostate cancer

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Abstract

Background: Consumption of saturated fat-rich diets and obesity are associated with prostate cancer (PCa) progression and lethality. Metabolites, such lactate, kynurenine, and arginine have recently emerged as key mediators of tumor/tumor microenvironment (TME) crosstalk, immune evasion, and resistance to therapies. However, whether perturbations in systemic metabolism support oncometabolite intra-tumoral accumulation to promote PCa progression is an open question.

Methods: Prostate metabolome, transcriptome, histopathological, and immune features were analyzed in Hi-MYC transgenic mice fed with a high-fat diet (HFD) or a control diet (CTD) for 21 weeks. ^{18}F-FDG PET was used to investigate glucose uptake in vivo. Neoangiogenesis and PCa cell migration were assessed using established assays and traction force microscopy. Gene-set enrichment analysis and bulk RNA seq-based computational models were used to uncover critical pathways and changes in TME immune cell composition in both murine and human PCa from the Physicians' Health Study and the Health Professionals Follow-up Study prospective cohorts. Finally, association of tumor glycolytic hallmarks with clinico-pathological features was investigated in independent human datasets (*i.e.*, TCGA and META855).

Results: Obesogenic HFD accelerates the development of invasive PCa through metabolic rewiring. Although c-MYC alone modulates key metabolic pathways, interaction with HFD is required to induce PCa glycolytic phenotype and accumulation of the oncometabolite lactate. These metabolic effects are associated both with the activation of transcriptional programs linked to disease

progression and therapy resistance and augmented infiltration of tumor-associated macrophages (TAM) and FOXP3+ regulatory T cells. Moreover, direct exposure to lactate stimulates neoangiogenesis and PCa cell migration. PCa from patients with high saturated fat intake displays glycolytic features, which promote M2-like TAM infiltration. Finally, high mRNA levels of lactate dehydrogenase correlate with disease recurrence and shorter survival in independent human cohorts.

Conclusions: Our work supports genetic drive and systemic metabolism as partners in crime to hijack the TME and to promote PCa progression through lactate accumulation. This sets the stage for the assessment of lactate as predictive/prognostic biomarker and supports strategies of dietary intervention and/or direct lactagenesis blockade in advanced PCa.

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Morphometrics and Gene Expression Profiling of Aged and Young Female Mice Urethra

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Abstract

Background: Urinary incontinence is common in women and typically worsens as women age, deteriorates the quality of life, and results in a high economic burden on health care. Tissue aging plays a significant role in urethral function decline; physiological changes due to aging, such as the decrease in urethral striated muscle mass and increase in connective tissue, have been known since the 1980s. However, the cellular and molecular changes leading to urethral function decline have not been previously studied. To investigate age-related muscle loss in the urethra, we evaluated the effect of aging by performing morphometric and transcriptome studies on the female mice urethras.

Methods: Urethral tissues of three young (14 weeks) and aged (94 weeks) female mice were compared. A computational image-analyzing program, VIPR (Vectorized scale-Invariant Pattern Recognition), was used to identify and quantify mouse urethral tissue components (muscle layers and connective tissues). RNA sequencing was performed to evaluate the transcriptome differences between young and aged urethra tissues. RT-qPCR was performed to validate the RNA-seq data.

Results: VIPR analysis revealed that the mid-urethra of old female mice exhibit less striated muscle, more extracellular matrix/fibrosis, and diminished elastin fibers than young female mice. Gene expression profiling analysis of the total RNA-seq of the whole urethra showed more downregulated genes in aged mice than in young mice. Immune response and muscle-related pathways were predominantly enriched, whereas keratinization, skin development, and cell differentiation pathways were significantly downregulated in aged urethral tissues compared to young urethral tissues. RT-qPCR analysis of targeted genes of interest confirms the findings of the RNA-seq data.

Conclusions: Our findings showed that the morphometric analysis of the aged female mice urethra is comparable with the elderly female human urethra and exhibits less striated muscle, more extracellular matrix/fibrosis, and diminished elastin fibers. Gene expression profiling analysis of the whole urethra demonstrated immune response and muscle-related pathways were predominantly enriched, suggesting that molecular pathways (i.e., ACVR1/FST signaling and CTGF/TGF-β signaling) leading to a decreased striated muscle mass and an increase in fibrotic extracellular matrix in the process of aging, which warrants further investigations. This work also showed that our model is viable for advancing the research in urethral dysfunction and failure.

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Prune Belly Syndrome (PBS) – evidence for a gestational gene-environment interaction

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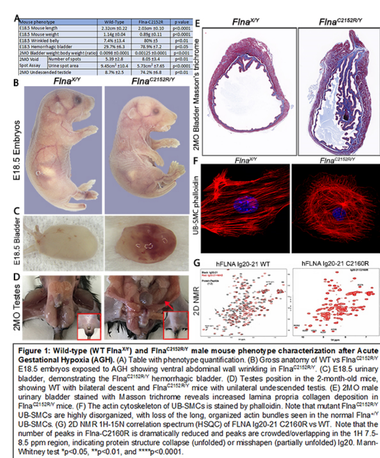
Abstract



Mouse phenotype		Wild-Type	Flna-C2152R	p value
E18.5 Mouse length		2.32cm ±0.22	2.03cm ±0.10	p<0.0001
E18.5 Mouse weight		1.14g ±0.04	0.89g ±0.11	p<0.0001
E18.5 Wrinkled belly		7.4% ±13.4	80% ±5	p<0.01
E18.5 Hemorrhagic bladder		29.7% ±6.3	78.9% ±7.2	p<0.05
2MO Bladder weight::body weight (ratio)		0.0098 ±0.0001	0.00125 ±0.0001	p<0.001
2MO Void Spot Assay	Number of spots	5.39 ±2.8	8.05 ±3.4	p<0.01
	Urine spot area	9.45cm² ±10.4	5.73cm² ±7.65	p<0.0001
2MO Undescended testicle		8.7% ±2.5	74.2% ±6.8	p<0.01

Figure 1: Wild-type (WT Flna$^{X/Y}$) and Flna$^{C2152R/Y}$ male mouse phenotype characterization after Acute Gestational Hypoxia (AGH). (A) Table with phenotype quantification. (B) Gross anatomy of WT vs Flna$^{C2152R/Y}$ E18.5 embryos exposed to AGH showing ventral abdominal wall wrinkling in Flna$^{C2152R/Y}$. (C) E18.5 urinary bladder, demonstrating the Flna$^{C2152R/Y}$ hemorrhagic bladder. (D) Testes position in the 2-month-old mice, showing WT with bilateral descent and Flna$^{C2152R/Y}$ mice with unilateral undescended testis. (E) 2MO male urinary bladder stained with Masson trichrome reveals increased lamina propria collagen deposition in Flna$^{C2152R/Y}$ mice. (F) The actin cytoskeleton of UB-SMCs is stained by phalloidin. Note that mutant Flna$^{C2152R/Y}$ UB-SMCs are highly disorganized, with loss of the long, organized actin bundles seen in the normal Flna$^{+/Y}$ UB-SMCs. (G) 2D NMR 1H-15N correlation spectrum (HSQC) of FLNA Ig20-21 C2160R vs WT. Note that the number of peaks in Flna-C2160R is dramatically reduced and peaks are crowded/overlapping in the 1H 7.5-8.5 ppm region, indicating protein structure collapse (unfolded) or misshapen (partially unfolded) Ig20. Mann-Whitney test *p<0.05, **p<0.01, and ****p<0.0001.

Background: PBS is a rare, mild to lethal human congenital GU disorder. We identified 4 humans with pathogenic filamin A (FLNA) missense mutations, including FLNA p.C2160R in the regulatory Ig20-21 repeat. The X-linked FLNA gene encodes a smooth muscle cell (SMC) mechano-sensing actin-crosslinking protein that transmits force bidirectionally between actin and integrins and binds hypoxia-inducible factor 1α to activate hypoxic gene regulation. To test mechanistic causality, we 1) created the analogous FlnaC2152R (F2152) mice using Crispr-Cas9 and assessed the effect of acute gestational hypoxia (AGH), and 2) studied the mutation's impact on ligand binding by nuclear magnetic resonance spectroscopy (NMR).

Methods: F2152 homozygous pregnant dams were exposed to AGH (8hrs of 6% O2 at embryonic day 12.5 (E12.5)) and then returned to normoxia. The male F2152 hemizygous pups were assessed at E18.5 and adult 2-months old (2MO) for gross morphology, bladder organ function (by void spot assay (VSA)) and histology. AGH F2152 urinary bladder (UB)-SMCs were cultured and phenotyped. WT Flna and F2152 Ig19-21 protein repeats were evaluated for ligand binding by NMR.

Results: When compared to WT mouse, F2152 AGH E18.5 mice manifested abdominal wall laxity and hemorrhagic, fibrotic, dilated urinary bladder, and 2MO intra-abdominal undescended testes in 80%, 78%, and 74% of mice, respectively (Fig1A-D). Histologically, the F2152 bladders showed a thicker detrusor SMC layer with increased collagen deposition (Fig1E). In F2152 AGH mice, VSA noted more urine spots with reduced spot area, indicating dysfunctional bladder (Fig1A). The F2152 dysmorphic bladder SMCs have altered actin stress fiber formation (Fig.1F), cell spreading, and focal adhesions. NMR 1H-15N correlation spectrum (HSQC) structural analysis of human FLNAC2160R revealed Ig20 protein structure collapse (unfolded) or misshapening (partially unfolded) (Fig1G).

Conclusions: Human missense mutations in the FLNA gene can cause PBS. The FLNA C2160R mutation leads to profound Ig20-21 domain dysfunction by NMR and causes PBS-like phenotype in the analogous F2152 AGH mice. Collectively, these studies of gestational gene-environment interaction provide mechanistic causality at the organ, cellular, and molecular levels.

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Investigating the role of E2F transcription factors in neuroendocrine prostate cancer.

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Abstract

Background: The progression of castration-resistant prostate cancer (CRPC), to an androgen-independent, aggressive, neuroendocrine prostate cancer (NEPC), is increasing in incidence. Few effective NEPC therapies exist, and overall survival is poor, therefore greater understanding of the molecular and epigenetic drivers of NEPC progression is required. Upregulation of *MYCN* and *EZH2*, and loss of *RB1* and *TP53* have been implicated as drivers of NEPC. We have shown that N-Myc overexpression synergizes with *RB1* loss to drive the NEPC phenotype in a novel genetically engineered mouse model (GEMM), and also confers a worse survival outcome in patients. However, the molecular underpinnings driving this synergy have yet to be elucidated.

Methods: We performed gene expression analyses and ChIP-sequencing in a prostate cancer GEMM carrying *MYCN* overexpression and *RB1* loss, and in patient-derived organoid and xenograft (PDOX) models. Transcriptional differences between models were validated using RNA-sequencing data from a CRPC and NEPC patient cohort. ChIP-sequencing data was compared to publicly available E2F1 ChIP data and to additional NEPC driver gene cistromes. E2F knockout was performed using CRISPR/Cas9 ribonucleoprotein nucleofection.

Results: Specific E2F transcription factors, *E2F-1*, *-2*, *-7* and *-8*, were significantly upregulated in N-Myc overexpressing, *Rb1* null GEMMs, and in NEPC patient samples. Single-cell RNA-sequencing revealed the enrichment of *E2f1* and *E2f2* in NE cells, but not *E2f3*, suggesting potential preferential expression, and distinct activities of activator E2Fs. Novel characterization of E2F1 and E2F2 DNA binding profiles in an NEPC PDOX model revealed a ~70% shared cistrome, which included NE gene promoters (e.g. *INSM1*, *ASCL1*). However, transcription factor motif analysis highlighted differences between the factors, which may indicate different cooperative transcription factor involvement. *E2F1* and *E2F2* knockout models have been made and will be used to investigate NEPC specific targets of E2F1 and E2F2.

Conclusions: Upregulation of specific E2F transcription factors is a potential mechanism underlying the synergy between N-Myc overexpression and RB1 loss in NEPC. E2F1 and E2F2 bind at NE gene promoters and regulate genes involved in neural-lineage related pathways, which may contribute to the development of the NE phenotype. Characterization of the roles of E2F1 and E2F2 in NEPC will provide unique insights into NEPC development, potentially identify targetable mediators of lineage plasticity, and ultimately provide rationale for future clinical strategies that will improve patient outcome.

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Alternol disturbs glycolytic metabolism by targeting GAPDH in prostate cancer

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Abstract

Background: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) catalyzes the sixth step of cellular glycolysis that is an essential for energy homeostasis. Alternol is a natural compound that induces oxidative stress-dependent apoptosis preferentially in cancer cells. We recently reported that Alternol interacts with GAPDH along with 4 Krebs cycle enzymes in prostate cancer cells. In this study, we characterized the Alternol-GAPDH interaction and the functional significance.

Methods: Prostate cancer PC-3, C4-2 and 22RV1, plus benign BPH cell line were used in the study. Alternol interaction with GAPDH was determined using CETSA assay. GAPDH activity was assessed in vitro and in cultured cells with a preassembled assay kit. Cellular glycolytic activity was evaluated using a Seahorse system. Metabolites were analyzed using a gas chromatography-mass spectrometry (GC-MS) based approach.

Results: Our data revealed that Alternol interacts with GAPDH protein on two sites, one is the NAD+ binding site on the active domain of the enzyme, postulating an inhibitory effect. As expected, Alternol directly inhibited GAPDH dehydrogenase activity in an in vitro assay with purified enzyme at a nanomole level (IC50 value 5.794 nM). Consistently, Alternol suppressed GAPDH enzymatic activity in prostate cancer cells but not in benign cells. These inhibitory effects were associated with reduced glycolytic flux in cancer cells as assessed by extracellular acidification rate (ECAR) and metabolomic analysis.

Conclusion: These results suggest that Alternol is a potent GAPDH inhibitor and that GAPDH inhibition results in disruption of glycolytic flux specifically in prostate cancer cells.

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Do members of the urobiome alter uropathogen potential

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Abstract

Urinary Tract Infections (UTIs) are a major problem globally, accounting for approximately 8 million healthcare facility visits in the US annually. The chief cause of UTI is Uropathogenic *Escherichia coli* (UPEC) accounting for ~75% of cases. Next generation sequencing and enhanced quantitative urine culture (EQUC) have allowed for the characterization of fastidious bacteria, enabling researchers to identify a community of microbial species associated with the urinary tract that has been coined the urobiome. While UPEC pathogenesis has been studied at depth, the potential interactions of UPEC with the urobiome have not yet been elucidated. Preliminary data from our lab identified the presence of *Actinotignum schaalii* in the urobiome of healthy male infants, while other studies have consistently identified *A. schaalii* in the adult urobiome. Clinical lab observations suggest that *A. schaalii* is present during UTI. Correspondingly, a handful of clinical studies have also recognized *A. schaalii* as uropathogen.

To investigate the potential role that *A. schaalii* may exhibit on UPEC pathogenesis, we developed a series of protocols that have allowed us to define the growth requirements of the bacteria. We found that growth of *A. schaalii* in liquid culture of brain heart infusion broth supplemented with a lipid mixture, or UPEC supernates is greatly enhanced. This suggests that lipids are a nutritional requirement for *A. schaalii* and that the presence of UPEC byproducts benefits *A. schaalii*. To begin evaluating the effects of *A. schaalii* on UPEC physiology and infectious potential, we next developed a co-culture scheme of *A. schaalii* and UPEC on agar plates. UPEC was spotted on a lawn of *A. schaalii* on two different types of agar: blood agar, and YESCA agar. Co-cultures were incubated at 5% CO_2, 37°C over time and imaged to assess morphological differences of UPEC in co-culture, compared to UPEC alone.

On YESCA agar plates, a visible halo was observed around UPEC as early as day 3 of growth on the *A. schaalii* lawn. On blood agar no halo was observed, however, increased roughness was seen the UPEC colony grown in co-culture. Rough colony morphology coincides with increased production of UPEC fibers and polysaccharides associated with virulence.

These preliminary data suggest that members of the urobiome such as *A. schaalii* influence UPEC physiology, and potentially pathogenesis. UPEC's ability to establish and form biofilms contribute to its virulence. To date, no study has investigated the role of *A. schaalii* virulence during infection or the potential effects on UPEC. In immediate future studies, we will define how *A. schaalii* influence UPEC pathogenesis in vivo. This work is significant, because it will be the first to determine how uropathogens respond to the presence of urinary microbiota. Our findings therefore have the potential to change current paradigms in UPEC pathogenesis.

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SOX2 mediates resistance to DNA damage via regulation of E2F1 in prostate cancer

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Abstract

Background: A leading cause of disease progression in prostate cancer patients is therapy resistance. As a result, different treatment schemes and combinations are employed to compensate as prostate cancer progresses. One mechanism that cancer cells utilize to evade treatment is through alterations in DNA damage repair pathways. Currently, there are tumor expression profiles that score homologous recombination deficiency available for patients, but this is typically only used to determine if PARP inhibitors are an appropriate treatment option. One potential biomarker we have identified, SOX2, was linked to increased Gleason Grade at diagnosis and decreased time to metastasis after biochemical recurrence. Previously, SOX2 was found to promote resistance to AR-targeted therapies and lineage plasticity in prostate cancer. Further, SOX2 has binary expression in prostate cancer making it an ideal biomarker.

Methods: The purpose of this study is to establish the role of SOX2 in mediating DNA damage. Using patient prostate cancer tumor RNA-seq datasets, we analyzed pathways and genes associated with SOX2 expression. Further, we use CWR-R1 CRISPR/Cas9 SOX2 knockout cells for RNA-sequencing and protein analysis to characterize DNA damage response. This led us to examine E2F1, a cell cycle regulating protein and SOX2 transcriptional target. We observed increased E2F1 expression in the presence of SOX2 and Enzalutamide compared to controls.

Results: Our studies show there is differential gene expression in DNA damage pathways with SOX2 expression in prostate cancer patients and prostate cancer cells. We found E2F1 is upregulated in response to increased SOX2 expression.

Conclusions: Continued investigations will focus on delineating the effect of SOX2 control on E2F1, the impact on DNA damage response from altered E2F1 expression, and the potential utility of SOX2 tumor expression as a biomarker for poor therapeutic outcomes.

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Assessing Macrophage Infiltration During the Development of Neuroendocrine Prostate Cancer

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Abstract

Background: Lineage plasticity is a proposed mechanism for the progression of treatment-induced neuroendocrine prostate cancer (NEPC). However, it is currently unknown whether this transition is mediated through a stem-like intermediate state or via direct transdifferentiation. Multiple genetically engineered mouse models of NEPC have identified Pou2f3+ populations. In particular, the population found in the $Pten^{f/f}$;$Rb1^{f/f}$;$MYCN^{+}$ (PRN) model displayed a similar but distinct chromatin accessibility profile from NEPC cells and a transcriptome enriched for stem-like gene sets, which may support the intermediate state model of lineage plasticity. The tumor microenvironment (TME) is pivotal in the development and behavior of tumor cells but its role in facilitating lineage plasticity is currently unknown. This study aims to examine the pro-tumorigenic interaction between *Pou2f3*+ cells and macrophages.

Methods: Single-cell RNA sequencing (scRNA-seq) was performed with prostate tissues from PRN and PR mice. Cells with <200 and >7500 expressed genes, <500 and >35000 transcripts, >15% mitochondrial reads, and >60% ribosomal reads were filtered out for quality control. Cell identities were determined using differentially expressed canonical cell marker genes. Cell-cell communication was analyzed using *CellChat*. Macrophage activation state was analyzed using *MacSpectrum*. Immunohistochemical (IHC) staining for AR, INSM1, and F4/80 was performed on mouse prostates and quantified using *DAB-quant*.

Results: *Il10/Il10r* signaling was identified exclusively between *Pou2f3*+ cells and macrophages in PRN mice and was not identified in PR mice. A significant increase in the relative abundance of macrophages was observed by scRNA-seq in PRN mice compared to PR. Increased macrophage infiltration was further confirmed by IHC in INSM1+ and AR- neuroendocrine (NE) foci compared to AR+ and INSM1- adenocarcinoma foci. Furthermore, there was a significant increase in the proportion of macrophages expressing M2 markers *Arg1*, *Folr2*, *Chili3*, *Mrc1*, *Ccl8* in PRN mice. Analysis of macrophage activation state revealed increased M0 and decreased M1 states in PRN mice.

Conclusions: Macrophage infiltration is increased in NE regions compared to adenocarcinoma foci which may be mediated by *Il10* production in *Pou2f3*+ cells. *Il10* has been previously implicated in the recruitment of blood derived monocytes to the TME and is known to promote M2 polarity. Thus,

increased presence of M2 macrophages may be one mechanism that supports the transition to NEPC.

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Histologic inflammation and collagen content are not positively correlated in human BPH

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Abstract

Background: Recent clinical studies have implicated prostate inflammation and fibrosis in the development of bladder outlet obstruction and lower urinary tract symptoms (LUTS). Studies utilizing rodent models, including work in our laboratory, have shown prostate fibrosis to occur as a consequence of inflammation. However, the relationship between collagen content and inflammation in human tissue samples obtained from surgical treatment of benign prostatic hypererplasia (BPH)/LUTS has not to our knowledge been previously examined.

Methods: Prostate tissue specimens from 53 patients (ages 47-88, mean 65.1) treated by open simple prostatectomy or transurethral resection of the prostate for BPH/LUTS were stained to quantitatively assess prostate inflammation and collagen content. Patients with prostate cancer present in greater than 5% of the surgical specimen were excluded. Prostate volume was determined from pelvic CT scan obtained within 2 years of surgery.

Results: Analysis of the data showed that inflammation was inversely correlated with collagen content ($r = -0.28$, $p = 0.04$). In men with prostates less than 75 cm3 inflammation increases and collagen content decreases with prostate volume ($p = 0.002$ and $p = 0.03$, respectively) while in men with prostate volume over 75 cm3 inflammation decreases and collagen content increases with prostate volume ($p = 0.30$ and $p = 0.005$, respectively).

Conclusions: Our data do not support the assumed positive association of prostate inflammation with collagen content. Coordinated analysis of scatter plots of inflammation and collagen content with prostate volume revealed a subset of prostates with volumes >50 cm3 prostate characterized by intense inflammation and low collagen content and it is this subgroup that appears most responsible for the inverse correlation of inflammation and collagen.

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A cellular atlas of normal adult male and female human lower urinary tract.

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Abstract

Background: Benign and malignant diseases of the ureter, bladder, urethra and prostate are common in aging. Fundamental questions regarding the disparate incidence and pathogenesis of lower urinary tract disease in sex and ethnicity require a deep understanding of normal. Our goal is to create a cellular atlas of normal adult male and female human lower urinary tract using a combination of single cell and spatial technologies on LUT from healthy organ donors.

Methods: Ureter, bladder, urethra and prostate (in males) was 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 was performed on FFPE and OCT sections using anchor genes derived from single cell data. Spatial transcriptomics was performed on the Nanostring GeoMx platform and analyzed with provided software.

Results: Several new cell types have been discovered in the male and female LUT, some of which appear to play central roles in disease. 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 GenitoUrinary Development Molecular Anatomy Project and Human Cell Atlas.

Conclusion: 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 common in post-menopausal women. The high incidence of tertiary lymphoid structures in healthy female vs. male bladder could help explain the 4-fold higher incidence of bladder cancer in men. The comparison of seminal vesicle to prostate could help us understand why even though both organs are androgen-regulated, 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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Deciphering a role for CD8+ T cells in *Toxoplasma gondii*-induced nodular hyperplasia of the prostate

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Abstract

Background: Inflammation is known to play a key role in the progression of benign prostatic hyperplasia (BPH), with T cells being one of the most important and expanded immune cell populations. Our lab has recently used the parasite Toxoplasma gondii to develop a novel mouse model that mimics most of the key features of human BPH, including inflammation. BPH lower urinary tract symptoms (LUTS) are associated with the formation of epithelial nodules that develop over time. These nodules arise from ring-like microglandular hyperplasia within glands that are perpetuated by a chronic inflammatory environment. Because of the strong T cell-mediated immune response that Toxoplasma gondii elicits, we hypothesize that T cells, specifically CD8+ T cells, contribute to the microglandular hyperplasia in our mouse model of prostate hyperplasia.

Methods: Ten-twelve week old CBA/j male mice were I.P. injected with either 40,000 PruDhxprt + ldh2GFP Toxoplasma gondii parasites or sterile PBS and monitored daily. Spleens and prostates were harvested at 14, 28, and 60 days p.i. Flow cytometry was performed on half of the prostates to identify T cell subsets. On the other half of the prostates, H&E histology staining and immunofluorescence were used to evaluate pathology and location of T cell subsets relative to microglandular hyperplasia.

Results: Our data show that T cells are the most expanded immune cell population. We observe that CD8+ T cells, specifically IFNg-producing CD8+ T cells, are sustained throughout Toxoplasma infection, with the most pronounced increase at 14 dpi, at the time when microglandular hyperplasia is beginning to form in this model. Additionally, IF shows that CD8+ cells associate to regions of forming hyperplasia.

Conclusion: Our work suggests a potential role for IFNg-producing CD8+ T cells in nodular formations during prostatic hyperplasia and is consistent with recent findings in human BPH. Current work is aimed at determining the dependence of nodular formations on CD8+ T cells through in vivo knockdown of these cells. Future significance of this work could be a therapeutic avenue to stall the progression of inflammation-driven BPH-LUTS.

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Batf Acts Through IL-23 Promotes Prostate Adenocarcinoma in Pten-Deficient Mice

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Abstract

Background: Inflammation is observed in almost all surgical prostate specimens; however, how inflammation promotes PCa remains unclear. CD4+ T helper (Th)17 cells have been implicated in many inflammatory and autoimmune diseases, but their role in cancer remains controversial. The activating protein 1 family member basic leucine zipper transcription factor ATF-like (Batf) controls several immune cell differentiations, especially Th17 cells, and mice deficient for Batf lack Th17 cells. However, the role of Batf-dependent Th17 cells in PCa remains to be determined. Understanding how Batf-dependent Th17 cells contribute to PCa initiation and progression is critical.

Methods: In this study, we took a genetic approach to explore the functional role of BATF-dependent Th17 cells in PCa by interbreeding Batf knockout mice with mice conditionally mutant for Pten. H&E and IHC staining to compare the histo-pathology and detect the cellular proliferation, apoptosis, and angiogenesis between Batf-/- with Pten-deficient mice (named Batf-/-) and Batf-expressing mice with Pten-deficient (Batf+). Flow cytometry compared CD4 and CD8 cell ratios and Th17 and Tregs between Batf+ and Bath-/- mice. Ex vivo culture splenocytes and prostate tissues under Th17 differentiation conditions plus IL-23 were used to determine the Batf-dependent downstream gene expression and NF-kB activation. PCa cell lines were transfected with Batf to check its downstream gene expression. Anti-IL-23p19 antibodies were used to treat Pten-deficient mice and assess treatment efficacy.

Results: Batf-/- had reduced prostate size and developed fewer invasive prostate adenocarcinoma than Batf+. Prostate tumors of Batf-/- mice exhibited reduced cellular proliferation, increased apoptosis, reduced angiogenesis, reduced inflammatory cell infiltration, and reduced activation of NF-kB signaling. Moreover, Batf-/- mice showed significantly reduced IL-23/IL-23R signaling. In the Batf-/- prostate stroma, IL-23R-positive cells reduced considerably than in Batf+ mice. Splenocytes and prostate tissues from Batf-/- mice cultured under Th17 differentiation conditions expressed reduced IL-23R than from Batf+ mice. PCa cell lines with BATF overexpression exhibited increased IL-23R expression. IL-23P19 antibody treatment of Pten-deficient mice reduced prostate tumors and reduced angiogenesis.

Conclusion: Our results suggest that Batf-dependent Th17 cells promote the initiation and progression of prostate adenocarcinoma and that a Batf-IL-23 axis is required.

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Nuclear export of m^6A-modified mRNA by METTL3-NUP93 axis promotes persistent AR signaling activation in castration-resistant prostate cancer (CRPC)

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Abstract

Background

Nuclear pore complex (NPC) is a large complex composed of approximately 30 different proteins termed nucleoporins (NUPs), which serve as the structural and functional units for macromolecules including proteins and messenger RNAs (mRNAs) shuttling between nucleus and cytoplasm. Misregulation of NPCs via abnormal expression or genetic alterations of NUP genes has been indicated to be involved in the progression of prostate cancer (PCa), but the underlying molecular mechanisms are poorly understood. Our proteomic study of NPC-associated cofactors revealed a direct interaction between the m^6A machinery and NPCs. Methylation of adenosine at nitrogen-6 position (m^6A) is the most abundant internal modification of mRNAs in eukaryotes. While functions of several m6A regulators in PCa have been recently recognized, their crosstalk with NPCs in PCa evolution has never been investigated.

Methods

We performed next generation sequencing (NGS) analyses including m^6A-MeRIP-seq, RNA-seq, and ChIP-seq to evaluate m^6A-mediated nuclear RNA export and gene regulation. We utilized ELISA analysis to examine cellular cholesterol and dihydrotestosterone (DHT) level. We also established *in vivo* mouse xenograft and patient-derived xenograft (PDX) models and confirmed our findings. Finally, we performed bioinformatic analyses of several prostate patient cohorts to evaluate the clinical relevance of our results.

Results

Here, we demonstrated that the m^6A methyltransferase METTL3 directly interacts with nucleoporin NUP93 in multiple PCa cells, which seems to be more robust in castration-resistant prostate cancer (CRPC) cell lines. The METTL3-NUP93 interaction is required for enhanced nuclear export of m6A-modified mRNAs and oncogenic transformation. METTL3-NUP93 axis promotes nuclear export of a large subset of m^6A-modified mRNAs including cholesterol biosynthesis enzymes, increases cellular cholesterol and DHT level, and triggers the activation of persistent AR signaling in CRPC cells.

Conclusion

Our current findings demonstrated that disruption of this m^6A-METTL3-NUP93 mediated-mRNA transport axis may represent a promising therapeutic strategy for CRPC, the lethal form of the disease.

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Cell Signaling Pathway for the Downregulation of AR in Stromal Cells

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Abstract

Background: Prostate cancer is one of the leading causes of death in Men worldwide. It is one of the top three leading causes of death in men and is specially found in older men. Cancer cells are in constant crosstalk with their surrounding environment. This environment involves the fibroblasts, immune cells, and the extracellular matrix and these can be an important factor in determining disease outcomes. In the prostate gland, cancer cells are dependent on the androgen receptor (AR) for their growth, which is contradictory to stromal fibroblasts where loss of AR increases with aggressiveness of the cancer. Our goal was to determine the mechanism behind the loss of this AR in stromal fibroblasts to identify signaling pathways which we could validate across multiple cell lines and patient fibroblasts. We previously identified TNFα and TGFβ as two cytokines produced by cancer cells, which were capable of suppressing AR expression in fibroblasts.

Methods: The immortalized fibroblast cell line BHPrS1, primary patient fibroblasts, and their immortalized derivatives were screened for signaling events downstream of TNFα/TGFβ using immunoblotting and immunofluorescence.

Results: Treatment of BHPrS1 cells will TNFα for 2 hours, caused increase nuclear localization and phosphorylation of NF-κB. While p38α was predominately nuclear localized and already phosphorylated in untreated cells, there was a slight increase in p38α phosphorylation following TNFα treatment. Treatment of BHPrS1 cells and a primary patient fibroblast isolate with TNFα and TGFβ for 24 hours both caused loss of AR expression as measured by immunoblotting. However, only TNFα caused an increase in NF-κB phosphorylation. An increase in p38α phosphorylation over basal levels was not observed with either TNFα or TGFβ

Conclusion: Treatment of prostate stromal fibroblasts with TNFα triggers the stimulation of NF-κB signaling and reduces AR expression. While, TGFβ reduced AR expression, it was not associated with an increase in NF-κB or p38α activation, suggesting another mechanism for the reduction in AR. Future experiments would involve testing these signaling molecules in patient samples using IHC to see how TGFβ reduces AR in prostate stromal fibroblasts. This information will help us understand how AR reduction in the prostate stroma creates more aggressive cancer cells.

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The Epigenetic Role of Nuclear Receptor Co-Repressor 2 (NCOR2) in Neuroendocrine Prostate Cancer Progression

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Abstract

Background: In prostate cancer (CaP), most patients develop androgen-driven luminal-like adenocarcinoma. Those patients who present with, or progress to, advanced metastatic disease are treated with androgen deprivation therapy (ADT). While this approach is initially highly effective, nearly all patients recur with castration-resistant prostate cancer (CRPC) and a subset transform into the aggressive, androgen-independent Neuroendocrine Prostate Cancer (NEPC). NEPC exhibits heterogeneous morphology and limited treatment options, resulting in poor survival. This transition to NEPC may involve clonal divergence from a CRPC precursor, facilitated by aberrant epigenetic reprogramming.

Methods: This study investigates the role of Nuclear Receptor Co-Repressor 2 (NCOR2) in NEPC development. Our prior research has demonstrated that reduced NCOR2 expression is associated with shorter time to biochemical recurrence, acquisition of neuronal-like characteristics, and epigenetic changes promoting NEPC progression. NCOR2 is well-known to recruit histone deacetylases, establishing a repressive chromatin state. We hypothesized that NCOR2 serves as an epigenetic barrier at lineage-specific enhancer regions, constraining the activation of developmental programs thereby inhibiting trans-differentiation to NEPC. Loss of NCOR2 would enhance lineage plasticity under ADT pressure by eliminating the epigenetic barriers at lineage-specific enhancer regions.

Results: Utilizing 10x Genomics Multiome (GEX+ATAC) single cell-seq, we identified gene expression and genome accessibility changes that drive NCOR2-dependent transdifferentiation to NEPC occurring as early as seven days following castration in the CWR22 prostate cancer xenograft mouse model. Using CUT&RUN-seq for a marker of active enhancers, H3K27ac, we found that NCOR2 knockdown triggers earlier activation of neuronal-related super enhancers compared to control CRPC cells under androgen withdrawal.

Conclusions: This study emphasizes the therapeutic potential of targeting NCOR2-dependent epigenetic plasticity as a complimentary strategy with the androgen receptor signaling axis. Understanding the pivotal role of NCOR2 in NEPC progression may open new avenues for the treatment of this aggressive prostate cancer subtype.

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Lipid Dysregulation in Benign Prostatic Hyperplasia: Unveiling Regional Relationships

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Abstract

Background:

Benign prostatic hyperplasia (BPH) is a common condition affecting older men, characterized by the non-malignant growth of the prostate. This can lead to a host of deteriorating symptoms collectively known as lower urinary tract symptoms (LUTS). Immunological processes are implicated in BPH, though their pathological significance remains undetermined. Using a mouse model of BPH, we previously identified the migration of macrophages into the lumen. These luminal macrophages accumulated lipid droplets and transformed into foam cells indicating a dysfunction in lipid metabolism. However, lipid accumulation within the context of BPH remains unexplored. Our current research is focused on comprehensively understanding and characterizing how lipids are distributed in different prostate regions affected by BPH.

Methods:

Wholemount frozen sections of donor and BPH prostates were procured at the University of Texas Southwestern Medical Center. Tissues were stained with Oil Red O (ORO) and regions of glandular nodules, internodular regions in BPH and normal transition zone in donor tissues were imaged at 40x magnification. Tissues were also subjected to immunohistochemistry (IHC) using an antibody against CD68. Tissue samples were analyzed using a Mantra II. Pathological Workstation and InForm software. Mann-Whitney test was used for statistical analysis.

Results:

Lipid content was significantly higher in the BPH glandular nodules compared to the transition zone of donor tissues (5.5-fold, $p<0.001$). Epithelial cells also contained more lipids than stromal cells. In the internodular region, lipid deposition varied due to the presence of both epithelial and stromal components. Notably, elevated intraluminal lipid accumulation was found almost exclusively in BPH prostates. Additionally, luminal macrophages were significantly elevated in BPH and co-localized with lipid positivity, indicating their phenotype as foam cells.

Conclusion:

This study provides the first comprehensive analysis of intraprostatic lipid accumulation in BPH. Increased lipids within glandular nodules suggests the involvement of lipid dysregulation in BPH progression and the presence of foam cells links disrupted lipid balance with immune responses. Further, lipid accumulation specifically in the lumen may contribute to macrophage infiltration and foam cell differentiation. These findings enhance our knowledge of the involvement of lipids in BPH, potentially opening avenues for targeted therapies to manage its progression.

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CHD7 suppresses androgen signaling in neuroendocrine prostate cancer

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Abstract

Background: The progression from castration-resistant prostate cancer (CRPC) to neuroendocrine (NE) prostate cancer (NEPC) is driven by several molecular events, including the acquisition of epigenetic changes. In a cohort of advanced prostate cancer patients, we found that the chromatin modifier CHD7 was overexpressed in NEPC patients. Moreover, using a genetically engineered mouse model (GEMM) of prostate cancer we revealed that CHD7 is hypomethylated and overexpressed in NE foci compared to adenocarcinoma. While these findings suggest that CHD7 may be linked with NEPC, the direct mechanism by which CHD7 is involved in disease progression remains unknown.

Methods: We used single-cell based approaches, such as scRNA-seq and scATAC-seq, in conjunction with bulk RNA-seq and RRBS, to fully characterize the development of NEPC in a novel GEMM driven by N-Myc in the context of *Pten* and *Rb1* loss. We modeled androgen deprivation in LNCaP cells and assessed transcriptomic changes by RNA-seq. We utilized CRISPR-mediated gene editing to delete *CHD7* in human NEPC patient-derived models. In these newly created models, we determined transcriptional changes by RNA-seq and assessed epigenetic changes to chromatin accessibility and enhancer activation by ATAC-seq and H3K27ac ChIP-seq.

Results: We performed scRNA-seq and scATAC-seq on GEMM prostates collected at 6 and 8 weeks of age which revealed that increased *Chd7* expression correlated with the expression of NE markers. Analysis of the methylome demonstrated concordant hypomethylation and overexpression of *CHD7* in both human and mouse models of NEPC. Using an *in vitro* model of long-term androgen withdrawal in LNCaP cells, we showed that chronic androgen deprivation synergizes with known genetic drivers of NEPC to promote the expression of *CHD7* and NE markers while reducing responsiveness to androgen stimulation. Genomic deletion of *CHD7* in human NEPC samples led to the increased expression of AR target genes and genes containing ARE motifs. These changes induced by CHD7-loss also correlated with increased chromatin accessibility around ARE motifs and the increased deposition of H3K27ac at the enhancers of AR target genes.

Conclusions: We have shown that CHD7 is hypomethylated and upregulated during the progression to NEPC. Using NEPC patient-derived models we have revealed that CHD7 normally functions to suppress androgen signaling and may help to maintain NE differentiation. Future studies will determine the potential to target CHD7 therapeutically to prevent or reverse the transition to NEPC.

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Gαi2 plays a vital role in Rac1 dependent activation of Wave2 axis and essential for cell migration in prostate cancer cells.

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Abstract

Gαi2 plays a vital role in Rac1 dependent activation of Wave2 axis and essential for cell migration in prostate cancer cells.

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and

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Background: Gαi2 is a subunit of the heterotrimeric G protein complex, classified into Gs, Gi/o, Gq, G12 in the form of activated Gα-GTP, and Gβγ subunits that are required for signal transduction in response to activation of G-protein coupled receptors (GPCRs). Previously we have shown that Gαi2 protein plays an essential role in cell migration and invasion in response to ligands acting via both GPCR and protein tyrosine kinase receptors (PTKR). Cell migration and motility involves dynamic and spatially regulated changes to the cytoskeleton and cell adhesion, activating numerous proteins, including Rho GTPases. Some of the most widely studied proteins belong to Rho family of GTPases including RhoA, Rac1 and Cdc42 whose functions are essential in migrating cells. Previous studies have shown that Gαi2 plays a role in the regulation of cell motility and invasion independently or downstream of Rac1 activation. In this study, we investigated the possible effects of Gαi2 at the level of Rac1-dependent F-actin polymerization and branching, specifically at the level of activation of Wave2 and Arp 2/3 proteins.

Methods: We generated PC3 stable cell lines overexpressing constitutively active Rac1 and were transfected with control siRNA or Gαi2 siRNA to knockdown endogenous Gαi2 expression. We also generated PC3 with transient overexpression of constitutively active Gαi2 (Gαi2-Q205L) in PC3 cells. Rhodamine-phalloidin staining was used to detect F-actin filaments and DAPI staining was used to detect nuclei for morphological analyses.

Results: The western blot analysis showed Rac1 dependent activation of Wave2 and Arp 2/3 are impaired in the absence of Gαi2. Furthermore, overexpression of Gαi2-Q205L in PC3 cells significantly increased compared to cell transfected with control plasmids. In the parallel experiments we used a specific Gαi2 inhibitor, which blocked Giα2-Q205L induced cell migration in PC3 cells. Expression of constitutively active Gαi2 in PC3 cells also caused significantly morphological changes and formation of lamellipodia at the leading edge of migrating cells.

Conclusion: We conclude that Gαi2 plays a crucial role in the activation of proteins downstream of Rac1. These proteins are important for cell migration in prostate cancer cells.

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Investigating the Role of FOXA2 During the Transition to Neuroendocrine Prostate Cancer

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Abstract

Background: Acquired resistance to androgen receptor (AR)-targeted therapies and the progression to metastatic-castrate resistant prostate cancer (CRPC) remains a significant clinical problem. Mechanisms of acquired resistance include lineage plasticity, by which CRPC tumors can become AR-negative and acquire neuroendocrine features (neuroendocrine prostate cancer [NEPC]). Clinical prognoses for patients with NEPC are very poor and we lack a comprehensive understanding of the molecular mechanisms underlying lineage plasticity and NEPC progression. Single cell analyses from genetically engineered mouse models and patient tumor data show that FOXA2, a key lineage-determining pioneer transcription factor (TF), is significantly upregulated in subsets of CRPC and NEPC patient tumors. However, little is known about the role of FOXA2 in regulating lineage plasticity and progression from CRPC to NEPC. Elucidation of this mechanism is essential to identify novel biomarkers and potential therapeutic targets for NEPC treatment.

Methods: In this study, we first examined the role of FOXA2 in suppressing androgen signaling and promoting progression towards an NEPC phenotype in vitro. We then conducted ATAC-seq and FOXA2 ChIP-seq in patient-derived NEPC models to characterize how FOXA2 alters the chromatin accessibility landscape and to identify potential FOXA2 interactors. We further validated these identified FOXA2-interactors using biochemical approaches.

Results: We found that FOXA2 overexpression suppressed androgen signaling and promoted progression to a NEPC phenotype under short- and long-term androgen deprivation conditions. Further, FOXA2 redirected the chromatin accessibility landscape to be consistent with an NEPC gene expression program, including increased chromatin accessibility for key NEPC TFs. FOXA2 ChIP-seq showed FOXA2 to be bound at known NEPC driver genes and epigenetic modifiers. Lastly, we discovered that FOXA2 physically interacts with key NEPC TFs and epigenetic regulators, suggesting that these FOXA2 physical interactions are required for NEPC progression.

Conclusions: Overall, our data from murine and human indicate that FOXA2 functions to suppress androgen signaling and function as a pioneer TF to redirect chromatin accessibility towards an NEPC molecular program. Further, we characterized the FOXA2 cistrome in patient-derived NEPC models and found that FOXA2 physically interacts with critical NEPC TFs and epigenetic regulators. These findings provide novel mechanistic insights underlying how FOXA2 regulates lineage plasticity and NEPC progression.

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Leveraging polyamine metabolic stress to enhance sensitivity to epigenetic therapy for prostate cancer.

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Abstract

Leveraging polyamine metabolic stress to enhance sensitivity to epigenetic therapy for prostate cancer.

Background: Advanced stage prostate cancer (CaP) is currently treated with androgen deprivation therapy. Despite initial response, most men recur with castrate resistant prostate cancer. Deregulation of epigenetic processes is a contributing factor to resistance, which creates a strong rationale for development of therapeutic strategies targeting epigenetic mechanisms. However, due to the relative non-specificity of epigenetic drugs in terms of both target cells and affected genomic positions, they have demonstrated limited efficacy and often are toxic. Strategies that would sensitize the target cells may both increase efficacy as well as reduce systemic toxicity, thereby broadening the therapeutic window of epigenetic therapy.

Methods: The flux through the polyamine catabolic pathway creates potential metabolic dependencies that can be exploited. The degree to which this catabolic pathway is activated is unique to prostate. This flux strains the homeostatic control of methyl donor pools (S-adenosyl methionine (SAM)) and acetyl donor pools (acetyl-CoA). Proper regulation of the acetyl-CoA and SAM pools is important to epigenetic regulation, as they are the donor molecules for histone acetyltransferases and both histone and DNA methyltransferases. We hypothesized that pharmacologically induced flux through polyamine metabolism and connected one carbon metabolism would disrupt SAM and acetyl-CoA pool homeostasis and make CaP more sensitive to epigenetic therapy that targets SAM and acetyl-CoA utilization.

Results: Using cell line models, *ex vivo* prostatectomy specimens, and a patient derived xenograft model, we found that activation of polyamine catabolism combined with inhibition of the methionine salvage pathway significantly disrupted acetyl-CoA pools and SAM pools. In a mouse xenograft model, the effects on metabolites were primarily limited to the xenograft tissue and normal prostate, with limited numbers of metabolites affected in other normal tissues. In cell lines, these effects were propagated to the epigenome with losses of H3K27ac, less open chromatin, and loss of DNA methylation. Moreover, both cell line and *ex vivo* models became more sensitive to relatively low dose Decitabine and JQ1.

Conclusions: The unique polyamine metabolism in prostate can be exploited to sensitize CaP cells to epigenetic therapy. This may hold promise for improving the therapeutic window for epigenetic therapies in prostate cancer patients, potentially resulting in better efficacy with lower toxicity.

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Identifying tumor immune cell metabolic interactions in prostate cancer of African American men

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Abstract

Background

Unequal access to health services, discrimination and other socioeconomic factors contribute to health disparities. The mechanisms by which these social pressures impact biological processes remain to be determined.

Methods:

We mined a retrospective race-matched Genomic Resource Intelligent Discovery (GRIDTM) database, NCT02609269 and prospective VANDAAM study (NCT02723734) to explore how oxidative stress and inflammatory signaling promote progression of prostate cancer (PCa) in African American men (AAM).

Results:

We found that the mitochondrial electron transport chain (ETC) complex I, III related genes are the most differentially pathways associated with oxidative damage expressed in AAM. Interestingly, AAM cell lines exhibited differential sensitivity to complex I & III inhibition. The correlational discordance of ETC complex I & III expression scores and immune-related genes suggest that complex I & III contributes to immunosuppressive microenvironment in PCa from AAM. Further analyses and ex vivo culture demonstrate enrichment of inflammatory cytokines in PCa from AAM. We then identified Aminopeptidase N (ANPEP) as the most race dependent differential expression in clinical cohorts. High expression of ANPEP enhanced production of immunosuppressive cytokines with a possible role in tuning adaptations to cellular stress in PCa of AAM.

Conclusions:

We identified race dependent differences in mitochondrial ETC which may reflect adaptations to chronic stress. Unraveling the roles of mitochondria in promoting immune suppression will have significant implications to understand how the intersection between social pressures and biological responses is impacting oncologic processes.

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Molecular analysis of bladder cancer cells with conditional FASN knockdown: Implications for IFNα gene therapy.

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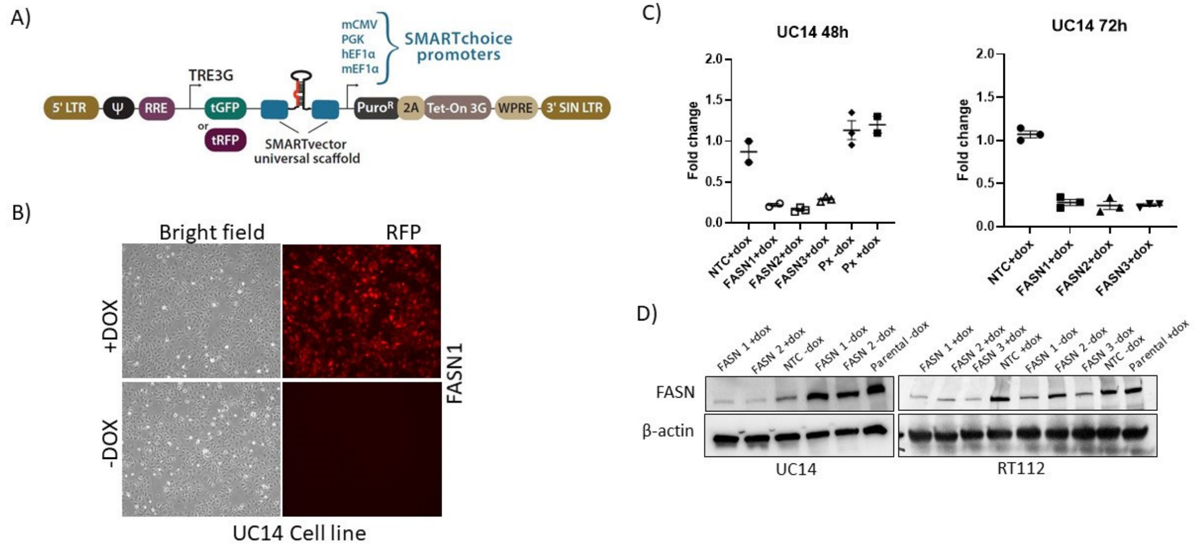
Abstract

Background: Interferon α (IFNα) gene therapy has been approved by the FDA for patients with BCG unresponsive bladder cancer. Using cell lines, preclinical models, and clinical samples we identified changes in the tumor metabolic pathway involved in fatty acid biosynthesis as one of the key pathways altered in bladder tumors treated with IFNα. Fatty acid synthase (FASN) is a key enzyme in this biosynthetic pathway and was downregulated in both murine models and in human bladder tumors. To define the role of FASN in IFNα gene therapy, we generated conditional knockout cells for FASN in two bladder cancer cell lines: UC14 and RT112. We molecularly characterized these cells to better understand mechanisms that drive acquired resistance to IFNα gene therapy.

Methods: Human bladder cancer cell lines were cultured in MEM supplemented with 10% FBS and transduced with three lentiviral particles expressing doxycycline-inducible shRNA for FASN. Knockdown cells were selected with puromycin and exposed to doxycycline to induce FASN knockdown. RNA was isolated using RNAqueous Total RNA Isolation Kit (ThermoFisher) and RT-qPCR was used to confirm knockdown with Agpath One Step RT-PCR Kit (ThermoFisher) using Taqman probes. Proteins were extracted using 1X RIPA buffer supplemented with Complete mini protease inhibitor cocktail and Western blot was used to confirm knockdown at the protein level. RNA quality was assessed using TapeStation and RNAseq analysis was carried out using Ion Proton Sequencer according to the manufacturer's instructions.

Results: Human bladder cancer cell lines UC14 and RT112 were successfully transduced with shRNA vectors and knockdown was induced by doxycycline treatment. Knockdown of FASN was confirmed at mRNA and protein levels. RNAseq analysis was used to identify genes/pathways enriched in knockout cells. Principal component analysis separated the cells based on treatment. Heatmap identified differentially regulated genes between the knockdown and control cells. Ingenuity pathway analysis identified several pathways including positive enrichment for interferon signaling in the knockdown cells. Downregulated pathways included natural killer cell signaling in UC14 and Oxidative phosphorylation and EIF2 signaling in RT112 cells.

Conclusion: In conclusion, we successfully established and characterized doxycycline inducible cell lines for RT112 and UC14 and identified genes/pathways that are altered after FASN knockdown. We also identified upregulation of interferon response in these cells after FASN knockdown that may contribute to acquired resistance to IFNα gene therapy. In the future, we will directly test this *in vitro* and *in vivo* by exposing FASN knockdown cells to human Ad-IFNα gene therapy vectors. Our studies will be impactful in understanding mechanisms of acquired resistance to IFNα gene therapy.



Generation of FASN knockdown in bladder cancer cell lines. A) SMART vector for generation of knockdown. B) Cells showing RFP expression after doxycycline treatment in UC14 cell line. C) Western blot analysis confirming knockdown in UC14 at 48h and 72h. D) Confirmation of knockdown by western blot analysis in UC14 and RT112.

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Targeting AR-WDR77 interactions for prostate cancer therapy

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Abstract

Background: Androgen receptor (AR) drives prostate cancer (CaP) even after androgen deprivation therapy (ADT) failed. Inhibiting AR's transcription factor function to overcome acquired resistance to ADT is an attractive therapeutic avenue. However, which AR-coregulator interaction should be targeted is not clear, AR-coregulator complex higher order structures and stoichiometry are unknown, and how manipulating AR-coregulator interactions impacts the AR cistrome is not understood. We explore the feasibility of targeting interactions between AR and WD repeat 77 (WDR77, non-catalytic component of the methylosome complex), which are enriched in ADT-resistant CaP and mediate CaP cell survival.

Methods: CUT&RUN assays defined AR and WDR77 cistromes. Cell viability, cell proliferation and CaP xenograft studies studied the effect of WDR77 on CaP growth. CoIP studies, Biacore and BioLayer lnterferometry (BLI) assays defined AR-WDR77 interactions and effects of peptides and small molecules targeting AR-WDR77 interactions on AR's protein complexation.

Results: Significant overlap was found between AR and WDR77 cistromes and the AR cistrome was reduced considerably after WDR77 loss, suggesting that WDR77 controls AR complex formation at target genes. WDR77 overexpression induced CaP cell proliferation before and after ADT. WDR77 silencing decreased cell proliferation, delayed CaP progression and prolonged survival of ADT-naïve and -resistant CaP xenografts. Cell-free co-IPs and BLI showed that WDR77 directly interacts with AR and isolated a central WDR77 domain critical for AR binding and complex formation. CoIP and BLI assays using peptides spanning the central WDR77 domain narrowed down the AR-WDR77 interaction site to a 21 aa region. Overexpression of the central WDR77 domain and 21 aa region altered AR-WDR77 interactions and AR complex composition in in vitro and in vivo CoIPs. A rationally designed WDR77 inhibitor similarly interfered with AR-WDR77 interactions and AR transcription complex formation.

Conclusion: Our studies confirm the importance of WDR77 for ADT-resistant CaP growth, AR complex formation and AR cistrome. Disrupting AR-WDR77 interplay is feasible and may be a novel CaP treatment strategy.

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Shift in Estrogen Receptor Alpha and Beta expression and location during *T.gondii*-induced prostatic hyperplasia

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Abstract

Background: *T.gondii* is an obligate intracellular parasite capable of infecting all nucleated cells in warm-blooded animals. We found that systemic *T. gondii* infection can colonize the prostate and lead to void pattern disruption and nodular hyperplasia in mice. In addition, we found that men who have tested seropositive for *T.gondii* IgG were more likely to have more severe BPH (Benign Prostatic Hyperplasia) pathology than seronegative patients. However, we have yet to understand the mechanism behind this phenomenon. Hormone treatment is known to establish nodule formation and urinary dysfunction, which lead us to hypothesize that *T.gondii* can contribute to the manipulation of the steroid hormone pathway, contributing to the development of BPH pathology and glandular nodule formation. Herein, we focus on estrogen and the estrogen receptors, due to recent studies showing the ratio of estrogen to testosterone plays a key role in the development of BPH pathology.

Methods: We analyzed the pro-hyperplastic Estrogen receptor alpha (ERα) and anti-hyperplastic Estrogen Receptor Beta (ERβ) presence in mouse prostate tissue using Immunofluorescence and immunoblotting to determine the amount and localization of the receptors adjacent to *T. gondii* induced inflammation, hyperplasia and nodule formation. Finally, we used single cell RNASeq to assess cell-type dependent gene activity related to estrogen receptors between infected and uninfected mice.

Results: Immunofluorescence indicates that Ki67, an indicator of cell proliferation, is increased in the epithelial cells of *T.gondii*-infected animals relative to control prostates. Increased proliferation is concurrent with increased nuclear ER-alpha presence. In addition, immunoblotting indicates that ERα is increased in T.gondii infected mice compared to uninfected mice at 60 d.p.i. Finally, our preliminary RNAseq data shows an impact on ER-regulated gene expression.

Conclusions: Our results indicate that *T.gondii* infection impacts the steroid hormone pathway and induces the abundance and location of the estrogen receptors. Our future studies include knockout analysis of ERα and ERβ receptors in both infected and uninfected animals, as well as further analysis of gene expression seen in the RNASeq analysis.

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Using Dynamic MRI to Determine Metrics of Bladder Shape, Wall Tension and Stretch During Voiding

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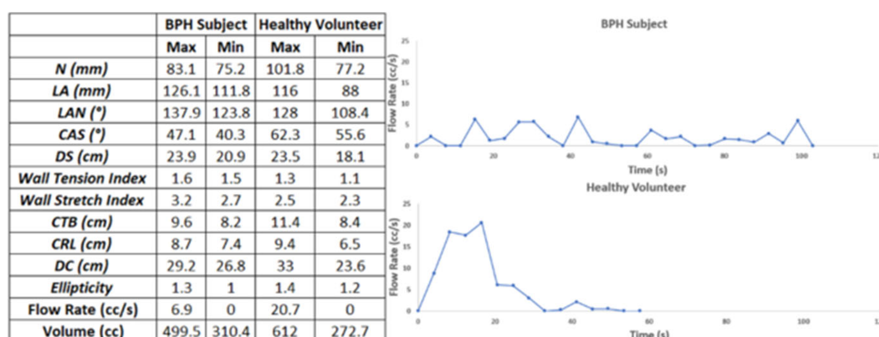
Abstract

Background: Voiding physiology is most studied with video-urodynamics. Its limitations include lack of high resolution and quantitative imaging data to study bladder anatomy while voiding. We describe the use of dynamic MRI to determine metrics of bladder shape during voiding in a subject with benign prostatic hyperplasia and lower urinary tract symptoms (BPH/LUTS) and a healthy volunteer.

Methods: MRI was performed using a clinical 3T scanner according to an IRB-approved protocol, acquiring bladder images every 3.7 s during voiding supine in the scanner. 3D anatomic models were created and analyzed with MIMICS (Materialise, Leuven, Belgium). We determined distances from the top of the trigone to the pubic symphysis (N) and the longest axis (LA) and the angle between them (LAN). Curvature angle (CA) and curvature distance (DS) were measured in the sagittal plane and curvature (DC), top-bottom (CTB) and right-left (CRL) distance were measured in the coronal plane. All of these metrics were measured at each time point of the voiding event.

Figure 1: A) Sagittal and B) coronal views from the healthy volunteer showing bladder shape metrics measured during voiding.

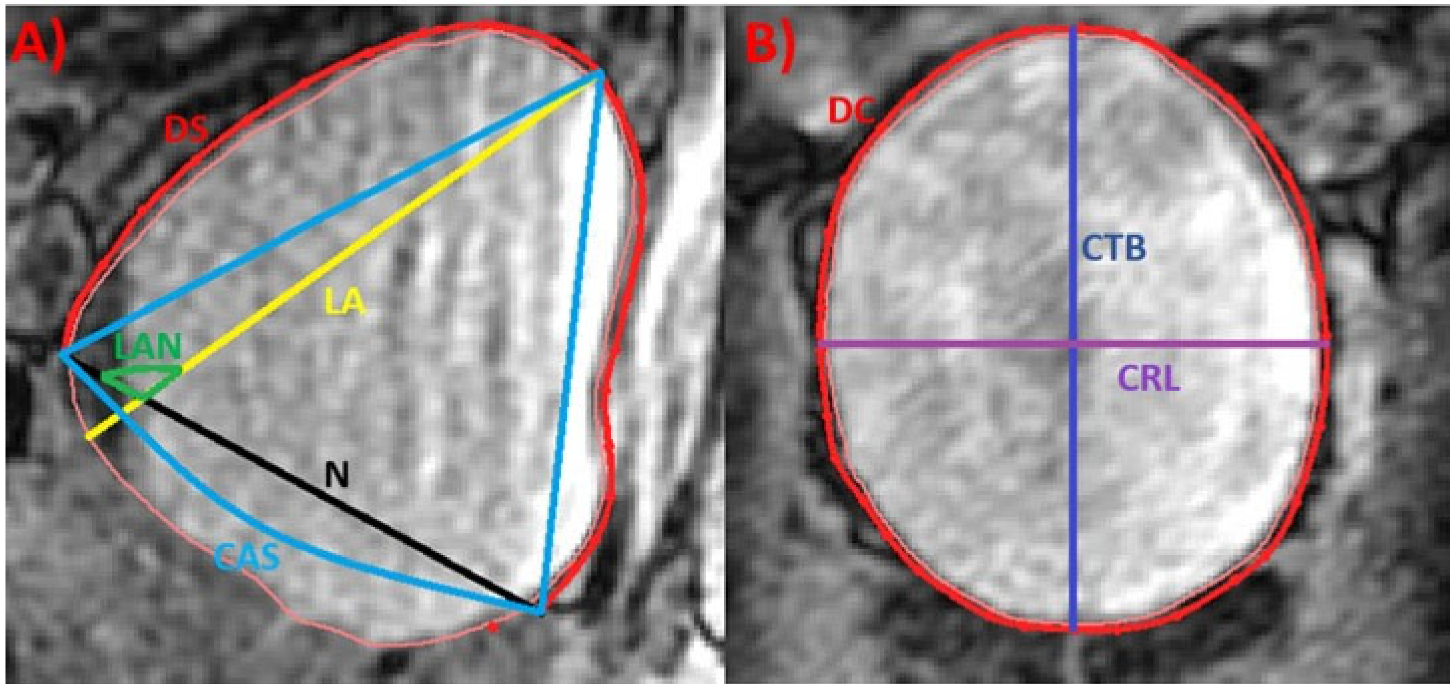
Results: From the metrics displayed in Figure 1, dynamic indices of wall tension (LA/N), wall stretch (DS/N) and ellipticity (CTB/CRL) were calculated. The table in Figure 2 reports the maximum and minimum values of these metrics during the void. The calculated urine flow curve is shown adjacent. The healthy volunteer exhibited a larger range of motion across all metrics, lower wall tension and wall stretch indices. Linear correlation coefficients (R2) between these metrics and flow rate were found to be higher in the healthy volunteer.



	BPH Subject		Healthy Volunteer	
	Max	Min	Max	Min
N (mm)	83.1	75.2	101.8	77.2
LA (mm)	126.1	111.8	116	88
LAN (°)	137.9	123.8	128	108.4
CAS (°)	47.1	40.3	62.3	55.6
DS (cm)	23.9	20.9	23.5	18.1
Wall Tension Index	1.6	1.5	1.3	1.1
Wall Stretch Index	3.2	2.7	2.5	2.3
CTB (cm)	9.6	8.2	11.4	8.4
CRL (cm)	8.7	7.4	9.4	6.5
DC (cm)	29.2	26.8	33	23.6
Ellipticity	1.3	1	1.4	1.2
Flow Rate (cc/s)	6.9	0	20.7	0
Volume (cc)	499.5	310.4	612	272.7

Figure 2: (Left) Maximum and minimum values of calculated metrics throughout the void. (Right) Voiding curves for both subjects.

Conclusion: Conclusion: These two studies exemplify the potential of dynamic MRI to provide dyanmic anatomical detail, quantitative measurements and calculated metrics of wall tension and stretch to investigate the biomechanics of normal voiding and voiding in patients with LUTS.



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Identification and Characterization of Novel Pathogenic Genes in Prostate Cancer

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Abstract

Background: Prostate cancer (PCa), one of the most frequently diagnosed cancers, is a global issue with an estimated 192,500 men living with metastatic prostate cancer in the US alone. While there are some methods available to diagnose and treat PCa, such as prostate specific androgen test and chemical castration, PCa remains the second leading cause of cancer-related deaths in men. PCa is a highly complex disease, and its development can often be attributed to mutations in particular genes. Thus, efforts to discover and characterize novel pathogenic genes and their protein products could lead to a better understanding of the disease as well as the development of diagnostic markers and possible drug targets.

Methods: A collection of 503 single nucleotide substitution datasets obtained from patients with PCa were downloaded as variant call format files from The Cancer Genome Atlas public resources. After data preprocessing, an in-silico screen of the variants was conducted using a computational workflow that integrated frequency analyses, functional effect prediction, and protein-protein interaction networks to score the protein-coding genes involved for their propensity for association with PCa. This screening identified eight potentially pathogenic genes that have not been previously characterized in PCa. Utilizing CRISPR/Cas 9 genome editing, we have developed knockout (KO) cell pools of four of the genes of interest in a 22Rv1 cell line. Classic experimental measures of cancer cell growth including proliferation, migration, and apoptosis assays will be performed to assess cancer cell growth and invasiveness for each gene.

Results: The eight genes identified in the in-silico screen are: CSMD3, TRRAP, CHD4, VWF, EPHB1, HERC2, MCM3, and NKX3-1. While most of these genes have been implicated in various endocrine cancers, they have not been previously described in PCa. We have successfully created KO cell pools for CSMD3, VWF, EPHB1, and NKX3-1. The remaining four genes (TRRAP, CHD4, HERC2, and MCM3) proved essential to cell survival and therefore we will rely on overexpression of these genes to assess cancer cell behavior.

Conclusions: Through the studies described above, we will characterize these novel genes and gain an understanding of their role in PCa. This will initially be done in a PCa cell model and at length we aim to perform murine studies to evaluate tumor growth and behavior in vivo. We expect these studies will lead to the identification of potential targets and pathways for diagnostic markers and drug targets for the treatment which will improve prognosis for PCa patients.

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IL-1 Signaling and Inflammatory Nodular Hyperplasia in the Mouse Prostate

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Abstract

Background: Approximately 50% of men will develop Benign Prostatic Hyperplasia (BPH) by the age of 50 which leads to difficulty urinating, nocturia, and severe conditions such as urinary retention. A hallmark feature of BPH is the formation of microglandular structures composed of epithelial and stromal cells, forming large nodules. These microglandular structures are correlated to severe patient symptoms. As the prevalence and size of nodules increase, they contribute to prostate enlargement which can restrict the urethra. Current treatment options for BPH only temporarily treat the symptoms. It is well known that chronic inflammation is strongly associated with BPH. The cytokine IL-1 has been shown to be involved in prostatic hyperplasia and has been shown to be the most upregulated in inflammation models. Previous studies have shown that IL-1 plays a key role during prostate development, and it has been suggested that developmental pathways are reactivated during BPH by IL-1 signaling. The common parasite Toxoplasma (T.) gondii infects the mouse prostate which leads to the formation of microglandular hyperplasia. We hypothesize that T. gondii infection upregulates IL-1 which leads to prostatic microglandular hyperplasia.

Methods: We infected CBA/J mice with T. gondii and harvested the prostate 28 days post infection (DPI). Prostates were harvested, formalin fixed, paraffin embedded, and assessed via Hematoxylin and Eosin staining. IL-1 levels at 28 DPI were measured with both BioPlex and ELISA. IL-1 signaling was assessed by comparing the amount of phospho-IRAK1 to IRAK1 via western blot. Additionally, single cell RNA sequencing was also conducted.

Results: Infected animals showed microglandular nodules when examined histologically. The levels of IL-1 more than doubled in the infected animals at 28DPI. This increase in IL-1 also lead to a significant increase in downstream IL-1 signaling. Finally, single cell RNA sequencing results also indicate IL-1 dependent signaling to be occurring in the T. gondii infected, inflamed mouse prostate.

Conclusions: T. gondii infection results in an inflammatory phenotype with IL-1 being a key player as shown by RNA, protein, and downstream signaling. Future studies include determining the degree of IL-1 signaling throughout the course of the infection as well as whether IL-1 signaling is necessary for the formation of mouse prostate microglandular hyperplasia. This work will help elucidate the role of IL-1 within the course of T. gondii infection and the pathologies associated with it.

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ATPase copper transporting beta (ATP7B) contributes to docetaxel resistance in human metastatic prostate cancer

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Abstract

Background

Docetaxel has been the standard first-line chemotherapy for metastatic prostate cancer (mPCa) since 2004, but resistance to docetaxel treatment is common. The molecular mechanisms of docetaxel resistance remain largely unknown and could be amenable to interventions that mitigate resistance.

Method

A fluorescence probe was developed to image cellular levels of copper in living docetaxel-resistant and parental PCa DU145 and 22Rv1 cells. Cell viability and colony formation assays were performed to evaluate the combination effects of docetaxel and disulfiram (DSF), an FDA-approved drug for the treatment of alcoholism, in combination with copper. Knockdown of ATP7B was achieved by silencing RNA (siRNA) transfection and protein alterations after treatments were detected by Western blotting. The cellular thermal shift assay was used to determine the potential engagements of DSF and copper with ATP7B. The combined anti-tumor effects of docetaxel and DSF/copper were determined in PCa patient-derived organoids and xenograft models of docetaxel-resistant PCa cell lines. The prognostic effect of ATP7B deletion on mPCa patients undergoing chemotherapy was further evaluated in a cBioPortal database (SU2C/PCF dream team, PNAS 2019).

Results

We have discovered that several docetaxel-resistant mPCa cell lines exhibit less uptake of cellular copper and express markedly higher levels of ATP7B protein without significant changes of other copper transporters, such as ATPase copper transporting alpha (ATP7A) and high-affinity copper uptake protein 1 (CTR1). Knock-down of ATP7B by siRNAs sensitized docetaxel-resistant mPCa cells to the growth inhibitory and apoptotic effects of docetaxel. Importantly, deletions of ATP7B existed in about 9.9% of mPCa tissues and predicted significantly better survival of patients after their first chemotherapy than those with wild-type ATP7B ($P = 0.0006$). In addition, DSF and copper acted synergistically with docetaxel and enhanced the in vivo antitumor effects of docetaxel in xenograft models of docetaxel-resistant PCa cells. Our analyses also revealed that DSF and copper engaged with ATP7B to decrease protein levels of COMM domain-containing protein 1, S-phase kinase-associated protein 2, and clusterin, and markedly increase protein expression of cyclin-dependent kinase inhibitor 1.

Conclusions

Our results suggest that ATP7B is a clinically relevant target, and that DSF/copper can be repurposed for improving the efficacy of docetaxel in the treatment of mPCa.

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Inflammation impacts androgen receptor signaling in basal prostate stem cells through interleukin 1 receptor antagonist and promotes basal stem cell proliferation and transdifferentiation

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Abstract

Majority of patients with benign prostate hyperplasia (BPH) exhibit chronic prostate inflammation and the extent of inflammation correlates with the severity of symptoms. How inflammation drives or contributes to BPH and the underlying mechanisms are not clearly understood. We have established a unique mouse model (Prostate Ovalbumin Expressing Transgenic 3 (POET3)) that mimics the autoimmune prostatitis in human to study the role of inflammation in prostate hyperplasia. After the injection of ovalbumin peptide-specific T cells, POET3 prostates exhibited an influx of inflammatory cells and an increase in pro-inflammatory cytokines that led to epithelial and stromal hyperplasia. We have previously demonstrated with POET3 model that inflammation expands the basal prostate stem cell (bPSC) population and promotes bPSC differentiation in organoid cultures. In this study, we investigated the mechanisms underlying the impact of inflammation on bPSC. We found that AR activity was enhanced in inflamed bPSC and was essential for bPSC differentiation in organoid cultures. Most importantly, we identified, for the first time, interleukin 1 receptor antagonist (IL-1RA) as a key regulator of AR. IL-1RA was one of the top genes upregulated with inflammation and inhibition of IL-1RA abrogated the enhanced AR nuclear accumulation and activity in organoids derived from inflamed bPSC. The mirroring effects of IL-1RA recombinant protein and IL-1a neutralizing antibody suggest that IL-1RA may function by antagonizing IL-1a. Furthermore, we established a lineage tracing model to follow bPSC during inflammation and under castrated conditions. We found that inflammation induced bPSC proliferation and differentiation into luminal cells even under castrated conditions, indicating that AR activation driven by inflammation in bPSC is sufficient for their proliferation and differentiation under androgen-deprived conditions. However, proliferation of the differentiated bPSC in the luminal layer significantly diminished with castration, suggesting inflammation may have little impact on AR in stromal cells, as stromal cells deprived of AR activity after castration could no longer provide paracrine growth factors essential for luminal proliferation. Taken together, we have discovered novel mechanisms through which inflammation may modulate AR signaling in bPSC and induce bPSC luminal differentiation that contributes to prostate hyperplasia.

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Sensitivity to PARP1 inhibition in prostate cancer is enhanced by depletion of the androgen receptor-regulated protease FAM111A.

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Abstract

Background: Prostate carcinoma (PCa) remains the most diagnosed cancer in men in the United States. Age, positive family history and ancestry are prominent risk factors for PCa development. Two independent studies identified SNPs within the FAM111A protease gene that predispose to PCa or to an aggressive form of PCa arguing FAM111A may have a role in this malignancy. FAM111A harbors a trypsin-like domain and can autoproteolyse, but target proteins remain unidentified. FAM111A also contains a PCNA binding domain and ssDNA binding region.

Methods: In vitro and in vivo studies, and analyses of publicly available databases were used to uncover the importance of FAM111A in PCa.

Results: AR represses FAM111A in castration sensitive and resistant cells, and FAM111A and AR transcripts are reciprocally regulated in metastatic lesions. FAM111A levels are significantly lower in matched castration resistant then sensitive LuCaP cells, and lower in patient derived metastatic lesions than in primary tumors. An AR binding site within the FAM111A gene has been identified and confirmed. Moreover, FAM111A depletion reduces AR target gene PSA and TMPRSS2 transcript levels, suggesting a FAM111A-AR co-regulatory loop. FAM111A protein localizes to the nucleus and predominantly to nucleoli in castration sensitive cells, while nucleoli localization greatly decreases with castration resistant in the LNCaP-C4-2 model. Lastly, depletion of FAM111A in castration sensitive and resistant cells enhances the efficacy of PARP1 inhibitors olaparib and niraparib.

Conclusion: Our studies argue that FAM111A has a role in double-stranded DNA repair. Thus, tumors with reduced FAM111A levels may be more sensitive to PARP1 inhibition.

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Amino acid transport inhibition resensitizes enzalutamide-resistant prostate cancer cells to enzalutamide

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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 have been proposed to contribute to the development of resistance to enzalutamide. These include androgen receptor variants, alternative signaling mechanisms, metabolic pathways, androgen receptor by-pass mechanisms etc. Our previous studies showed 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 involved by metabolomics indicated that amino acid metabolism and amino acid transporters may play an important role in the acquisition of resistance to enzalutamide. Hence, we tested whether co-targeting amino acid transport resensitizes enzalutamide-resistant PCa cells to enzalutamide.

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. We treated xenografts of enzalutamide-resistant PCa cells in mice with amino acid transport inhibitors singly or in combination with enzalutamide and assessed tumor growth.

Results: We found that expression levels of several amino acid transporters are increased in PCa tissues. We also found that amino acid transporter expression is significantly upregulated in enzalutamide-resistant PCa cells. Treatment with amino acid transport inhibitors JPH203 and V9302 inhibited enzalutamide-resistant PCa cell survival preferentially. Co-treatment with amino acid transport inhibitors and enzalutamide resensitized enzalutamide-resistant PCa cells to enzalutamide.

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

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EXOGENOUS NITRIC OXIDE AMELIORATES THE LINEAGE REPROGRAMING IN NEUROENDOCRINE PROSTATE CANCER

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Abstract

INTRODUCTION AND OBJECTIVE:

The androgen receptor (AR) is the principal driver of prostate cancer development and resistance. Most drug therapies revolve around re-activation of this AR-signaling axis. However, a subset of prostate tumours lose dependence on the AR pathway and co-opt alternative lineage programs to bypass therapeutic pressure and sustain tumour growth. Clinically, this lineage reprogramming has been associated with loss of luminal epithelial identity and the ensuing transition from a typical prostate adenocarcinoma to an aggressive neuroendocrine prostate cancer (NEPC). Previous studies in our lab has found the efficacy of S-nitrosoglutathione (GSNO) in reducing the secondary tumors in castration-resistant prostate cancer; which hypothesizes the lineage reprogramming potential of GSNO in reducing the overall tumor load. In the current study, we evaluated the mechanistic aspect behind this reduced tumor burden in NEPC tumors.

METHODS:

For the in-vivo study, castrated SCID mice were orthotopically grafted with H660 cells to generate NEPC murine models. Once the tumors became palpable, the animals were divided into two groups. Group 1 received vehicle (Control) and group 2 received GSNO treatment at the dose of 10mg/kg/day intraperitoneally (ip), three times a week until sacrifice. The experiment was terminared on day 30. Tumors were harvested, and the ALDH population in CTL and GSNO-treated tumors was regrafted orthotropically onto the prostate in another set of castrated SCID mice that were not exposed to any treatment. Tumor growth and metastases were checked in either experiment using IVIS imaging. At the end of the experiment, tumors, blood, lungs, liver, brain, bones were isolated and studied.

RESULTS:

In-vivo results highlighted that treatment with GSNO reduced the overall tumor burden by showing a significant decrease ($p<0.05$) in the tumor volume (plotted as MFI) as compared to the untreated control. The overall tumor weight were also reduced. The IVIS imaging showed the overall metastases in lungs, bones, liver and brain was reduced in the GSNO treated mice. Also, the percentage of ALDH+ cells which represent the stem cell population were significantly reduced in the GSNO treated tumors. Importantly, the mice that received ALDH+ cells showed a reduction in overall metastasis in lungs. Mechanistically, we highlighted for the first time a critical molecular interaction

between MYC and DOT1L in the neuroendocrine cancer stem cells which was inhibited by the NO treatment.

CONCLUSION:

This study shows that increased nitric oxide effectively re-regulate the lineage reprogramming of neuroendocrine prostate cancer stem cells by inhibiting the MYC-DOT1L axis in NEPC stem cells.

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Using Synthetic Histology Images for Training AI Models: A Novel Approach to Prostate Cancer Diagnosis

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Abstract

Background : Prostate cancer (PCa) presents significant challenges for timely diagnosis and prognosis, leading to high mortality rates and increased disease risk and treatment costs. Recent advancements in machine learning and digital imagery offer promising potential for the development of automated and objective assessment pipelines that can reduce human capital and resource costs. However, the reliance of AI models on large amounts of clinical data for training presents a significant challenge, as this data is often biased, lacking diversity, and not readily available. To address this limitation, this study employed generative adversarial network (GAN) models to produce high-quality synthetic images of different PCa grades (radical prostatectomy (RP)), which were customized to account for the granularity associated with each Gleason grade and required a significantly smaller training data sample size.

Methods : A deep convolutional GAN was used to create synthetically generated images. The generated images were subjected to multiple rounds of benchmarking and quality control assessment by board-certified pathologists before being used to train an AI model (EfficientNet) for grading digital histology images of adenocarcinoma specimens (RP sections) obtained from the cancer genome atlas (TCGA).

Results : Remarkably, the AI model trained with synthetic images outperformed the model trained with original digital histology images. Validation was performed using the AI model trained with synthetic data to grade digital histologies from an in-house active surveillance trial (for RP) and needle biopsy data from Radbound University Medical Center, Karolinska Institute. This study demonstrates the potential of customized GAN models to generate a large cohort of synthetic data that can train AI models to effectively grade PCa specimens, with a small sample size of training data.

Conclusions : This approach could potentially eliminate the need for extensive clinical data for training any AI model in the domain of digital imagery, leading to cost and time-effective diagnosis and prognosis.

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Combined Phenotypic and Kinesin Targeting Overcomes Therapeutic Resistance in Advanced Prostate Cancer

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Abstract

Background: Therapeutic resistance remains the main contributor to lethality in patients with metastatic prostate cancer. Currently, taxane chemotherapy (1st line docetaxel and 2nd line cabazitaxel) is the only treatment strategies to confer a survival benefit in patients with metastatic castration resistant prostate cancer (mCRPC). Our lab has demonstrated that cabazitaxel induces phenotypic reprograming via the interconversion of epithelial-mesenchymal transition (EMT) to mesenchymal-epithelial transition (MET).

Methods: This study investigates the molecular mechanisms underlying phenotypic changes in therapeutically resistant C4-2B cells. Transcriptomic profiling defined gene signatures of the differential response to taxanes underpinning therapeutic resistance. We subsequently assessed the protein expression in PDX models from patients with lethal mCRPC after treatment with radiation, ADT, antiandrogens, and chemotherapy including cabazitaxel. Tumors were profiled for EMT, and expression and cellular localization of mitotic spindle protein HSET. The combined effect of kinesin inhibitors (ispinesib and CW069), with cabazitaxel on cell death and phenotypic landscape was determined in models of advanced prostate cancer.

Results: RNAseq analysis revealed unique gene signatures underlying the differential response of resistant and sensitive C4-2B cells to cabazitaxel. Pathway association analysis revealed that cabazitaxel treatment of sensitive cells exhibit genes involved in DNA damage, while genes involved in protein regulation and EMT/stemness are found in C4-2B taxane resistant cells. Tumors (from PDX models from patients with advanced prostate cancer that received treatment with cabazitaxel following ADT showed elevated E-cadherin, decreased vimentin and HSET, and phenotypic re-differentiation. Combination of cabazitaxel with kinesin inhibitors resulted in significantly higher efficacy than either drug given as monotherapy at (a) inducing tumor cell death and (b) inducing phenotypic reprograming toward MET in both sensitive and taxane resistant prostate cancer cells.

Conclusions: This study provides new insights into the effect of phenotypic reprograming of prostate tumors and targeting mitotic kinesins to confer therapeutic vulnerability. Our findings are of translational significance in identifying novel treatment combination/sequencing strategies (cabazitaxel and kinesin inhibition), as an avenue towards improved therapeutic response, to overcome lethal prostate cancer.

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Hyaluronic Acid levels correlate with IPSS in BPH, and an oral HA inhibitor has efficacy in a mouse model of inflammation-induced LUTS.

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Abstract

Hyaluronic Acid levels correlate with IPSS in BPH, and an oral HA inhibitor has efficacy in a mouse model of inflammation-induced LUTS.

Background: Benign Prostatic Hyperplasia (BPH) is a pathologic condition in aging men associated with lower urinary tract symptoms (LUTS). Urine storage and voiding pathologies recorded as IPSS (International Prostate Symptom Score) drive LUTS' treatment. Hyperplasia and fibrosis in the transition zone (TZ) of the prostate are implicated in BPH-related symptoms, with chronic inflammation as a contributor. HA is causally linked to inflammatory and fibrotic diseases. We tested the clinical significance of HA in BPH and the efficacy of 4-methylumbelliferone (4-MU) in a mouse model of prostate inflammation and hyperplasia. 4-MU is an oral small molecule HA-synthesis inhibitor used to treat liver ailments with a high safety profile for prolonged use.

Methods: We examined HA and HA-family (HA-synthases, CD44, Col1A1) levels in BPH tissues in two patient cohorts: patients with large (L; ³ 100 g) and small (S; ≤ 30 g) volume prostates with either high (Hi; ~ 26±6) or low (Lo; ~ 5±1.6) IPSS. Thus, creating 4 categories by volume and IPSS: L-Hi, L-Lo, S-Hi, S-Lo. A mouse model was created by intraurethral injection of LPS in C57BL/6J male mice and treated with oral 4-MU. Histology, HA-test, IHC/ICC, qPCR, void spot assay (VSA), and LC-MS analyzed the HA/HA family expression and 4-MU's efficacy. Multi-parametric tests were used to assess the statistical significance.

Results: HA, HA-family, CD44-EGFR complex and activated EGFR (pEGFR) were high in BPH tissues from L-Hi and S-Hi groups, with no relation to volume. In L-Hi and S-Hi specimens, infiltration of immune cells was 5-fold higher, and the cells were embedded in a dense HA matrix. A signature consisting of HA-family and HA-induced cytokines classified the BPH specimens by IPSS (Hi vs Lo) but not by volume. In epithelial and fibroblast co-cultures from BPH tissues of patients with Hi-IPSS, HA-family, and EGFR- mitogenic signaling levels increased ≥ 5-fold but were inhibited by 4-MU. HA-family levels were high in the mouse sub-renal growth of TZ prostatic epithelial cells. Prostate tissues from the LPS-mouse model showed high HA-family and immune infiltration, and the mice showed fewer primary and total voids. 4-MU oral gavage eliminated these manifestations at a human equivalent dose with little tissue or serum toxicity. 4-MU and its active metabolites accumulate in the prostate.

Conclusions: HA and HA-family levels correlate with IPSS. The LPS-induced inflammatory prostate model can test novel oral agents, such as 4-MU, for treating BPH-induced LUTS.

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Estrogen-dependent and independent metabolic maladaptations contributing to BPH

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Abstract

Background: While androgens and estrogens are likely to impact the development and progression of benign prostatic hyperplasia (BPH), the role of metabolic maladaptation driven by steroid receptors or other signaling pathways in disease remains unresolved.

Methods: The impact of ERß on prostatic mitochondrial function was assessed in aged global ERß knockout mice. Mechanistic studies to assess the impact of mitochondrial dysfunction utilized the BHPrS1 benign human prostate stromal cell line. Glucose-deprivation forces BHPrS1 cells to rely predominantly on oxidative phosphorylation (OXPHOS) for ATP production and maintaining metabolic homeostasis, sensitizing them to disruptions in OXPHOS. We tested the impact of glucose deprivation on cell growth, fibrosis-related gene expression and mitochondrial respiration via cell growth assays, qPCR and Oroboros analyses. Expression of anoikis resistance markers was assayed using qPCR and immunofluorescent staining.

Results: Aged but not young ERß knockout mice displayed reduced activity of mitochondrial electron transport chain (ETC) complex I and II. Under glucose-deprivation conditions, BHPrS1 proliferation was significantly but reversibly inhibited and fibrosis-related gene expression was increased. Glucose deprivation sensitized cells to mitochondrial stress induced by rotenone, further reducing proliferation as well as loss of cellular adhesion. Anoikis resistance markers (ZC3H4 and TrkB) were up-regulated.

Conclusions: ERß may play a protective effect in aging prostate to maintain mitochondrial ETC activity and metabolic homeostasis. Metabolic maladaptations resulting from reduced glycolytic ATP production may promote fibrosis in prostate stromal cells. Prostate stromal cells appear to be sensitive to complex I inhibition and may adapt to mitochondrial dysfunction through promotion of anoikis resistance.

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SCAP IS A PROMISING PHARMACOLOGICAL TARGET FOR TREATING LIPOGENESIS-DRIVEN PROSTATE CANCER

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Abstract

Background:
Prostate cancer (PC) is the most common and second most lethal cancer in men. Despite therapeutic advances in androgen receptor (AR)-targeting agents, progression to lethal, drug-resistant, castration-resistant PC (CRPC) remains a major clinical problem. Thus, there is an unmet need for novel treatment approaches and novel, druggable therapeutic targets in CRPC. Profiling of PC patient samples shows increases in two master regulators of lipogenesis-sterol regulatory binding proteins 1 and 2 (SREBP1 and SREBP2) and their transcriptional targets. Overexpression of SREBP1 and SREBP2 and their target genes has been associated with tumor aggressiveness, poor clinical outcomes, and drug resistance in PC. The activation and nuclear translocation of SREBPs is closely regulated by SREBP cleavage-activating protein (SCAP). We propose targeting SCAP will simultaneously block the activation and subsequent activity of SREBP1 and SREBP2, representing a promising therapeutic strategy for CRPC.

Methods:
In this study, we utilized genetic and pharmacological approaches to characterize inhibition of SCAP/SREBP signaling. Changes in gene expression were characterized by global RNA sequencing. Cell proliferation and viability were assessed using MTT and flow cytometry-based assays. We also characterized cell migration and invasion behaviors under SCAP inhibitor conditions. We observed reduction in AR signaling and de novo lipogenesis as well as induction of apoptosis. Changes in lipids and metabolites were profiled using mass spectrometry. All experiments were conducted and confirmed using multiple PC cell lines of diverse origin. Additionally, we evaluated the anticancer activity and safety profile of our novel SCAPi using CDX mouse models.

Results:
We found that both genetic and pharmacological inhibition of SCAP yielded similar gene signatures, particularly the perturbation of cell cycle and lipogenesis genes. We identified a novel SCAPi that is orally bioavailable and blocks proteolytic cleavage of precursor SREBPs into their mature transcription factor forms. We determined SCAPi inhibits de novo lipogenesis signaling and dramatically inhibits both AR- and SREBP-target genes in multiple PC cell lines. Through flow cytometry (Annexin V) and western blots (cleaved PARP and caspase-3) we found SCAPi induces apoptosis in multiple PC cell lines. Pilot studies in tumor-bearing mice show SCAPi significantly increases survival without inducing animal weight loss.

Conclusions:
There is a critical need for novel targets in PC, and dysregulated lipogenesis is an untargeted oncogenic pathway. Our work furthers the field's understanding of hijacked lipogenesis in PC and paves the way for a first-in-class inhibitor of lipogenesis to treat lethal PC.

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De novo lipid synthesis in the pathogenesis of benign prostatic hyperplasia.

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Abstract

INTRODUCTION:

Benign prostatic hyperplasia (BPH) is the most prevalent urological condition in older men, affecting ~ 50% of men above the age of 50. As the prostate gland enlarges around the urethra, urine flow is obstructed, driving a collection of deteriorating symptoms known as Lower Urinary Tract Symptoms (LUTS). BPH is known to be driven by an age-related steroid hormone imbalance and inflammation. Steroid hormone imbalance can be reproduced in mice via testosterone and estradiol treatment (T+E2). In this model, we previously identified foam cell differentiation, a lipid-laden macrophage phenotype, specifically in the lumen. Foam cells indicate lipid dysregulation, but the source of excess lipids in the BPH prostate is unknown. Therefore, in this study, we assessed the gene expression pattern of genes of the de novo lipid synthesis pathway in the T+E2 model.

METHODS:

Male C57BL/6J mice were implanted with pellets containing 25 mg (T) and 2.5 mg (E2), and their ventral prostates were collected two weeks later. Cells were then dissociated with cold protease and loaded on Chromium Next GEM (7000 cells/sample). Each sample was sequenced on NextSeq2000 at 100 million reads/sample. In situ hybridization (ISH) was performed to confirm scRNA-seq results using probes for FASN. The effect of E2 (0.1-100 nM) in vitro was tested on BPH-1 cells.

RESULTS:

The scRNA-seq analysis of T+E2 ventral prostates identified two luminal, a basal, a proliferating and a progenitor epithelial cell cluster. Expressional analysis indicated an increase in the majority of genes of the de novo lipid synthesis pathway (i.e., Fasn, Scd-1, Acly, and Acat1/2) in most epithelial clusters. Increased Fasn expression was confirmed via ISH ($p < 0.01$). Expression of genes of the de novo lipid synthesis pathway was also explored in BPH-1 cells in response to E2.

CONCLUSIONS:

Our findings indicate a potential role for lipid dysregulation and an increase in de novo lipid synthesis in the pathogenesis of BPH regulated by steroid hormone imbalance. Our future studies aim to

determine whether E2-treated epithelial cells can directly stimulate foam cell formation in co-culture models.

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Prostate Cancer Cell Response to TGFβ Determines Therapeutic Vulnerability

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Abstract

Background: Anoikis ("homelessness") is an apoptotic process which occurs when cells lose their connection to the extracellular matrix. Resistance to anoikis confers cell survival, invasion and metastatic characteristics, facilitating tumor progression. The novel quinazoline-based α1-adrenoceptor antagonist (DZ-50) is a therapeutic for prostate cancer (PCa) via loss of epithelial mesenchymal transition (EMT) phenotypic effectors and tight junctions that are associated with induction of anoikis and anti-angiogenic properties. This study investigates the functional intersection between EMT and anoikis in PCa cells.

Methods: To test the hypothesis that *DZ-50 induces anoikis vulnerability in PCa cells by interjecting with TGFβ-associated EMT*, we used the following human PCa cell lines as models: LNCaP TβRII (androgen-sensitive; TGFβ responsive); VCaP (androgen-sensitive; TGFβ responsive, taxane resistant); and C42B-TaxR (TGFβ unresponsive, taxane resistant). Cell death of PCa cells in response to treatment with DZ-50, and/or TGFβ, and/or cabazitaxel, was assessed using the MTT assay. Expression of signaling effectors of anoikis, EMT and actin cytoskeleton was analyzed by Western blotting and protein expression was correlated with cell survival, migration and invasion.

Results: Our results demonstrate that (1) In LNCaP TβRII cells, DZ-50 treatment led to anoikis induction and reprogramming of EMT to mesenchymal-epithelial transition (MET). (2) In contrast, DZ-50 failed to induce anoikis in VCaP cells. Exposure to TGFβ however led to significant loss of cell survival in these cells. (3) Therapeutically resistant cells C42B-TaxR, showed increased sensitivity to cabazitaxel after co-treatment with anoikis inducing agent DZ-50.

Conclusions: This study provides new insights into the response of PCa cells to anoikis-inducing agents and/or EMT inducer TGFβ. We found that DZ-50 led to significant anoikis induction in LNCaP TβRII cells after TGFβ-associated phenotypic changes, while in VCaP cells DZ-50 failed to induce anoikis, but TGFβ led to apoptosis bypassing the phenotypic changes. We provide novel evidence of differential response to TGFβ: EMT or apoptosis in PCa cells. The dynamic landscape of the prostate tumor microenvironment can be sensitized to anoikis by DZ-50 by "priming" tumor cells to phenotypic reversion (EMT–MET). Ongoing studies focus on transcriptomic analysis of the differential response to TGFβ and the *in-vivo* anoikis-driven effect in pre-clinical models of advanced PCa.

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Exercise rescues aging-associated androgen receptor decline in mice

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Abstract

BACKGROUND: As men age, the serum level of testosterone (T) decreases, while the level of estrogen (E_2) remains stable. Studies suggest that the imbalance in androgen receptor (AR) and estrogen receptor (ER) signaling may result in the reactivation of prostatic growth and further lead to benign prostatic hyperplasia (BPH). Interestingly, it has been reported that exercise reduces the risk of BPH in men but the mechanisms are not fully understood. Since men and mice show similar changes in circulating T and E_2 as they age, the study of AR-ER signaling and exercise in mice may show insight into disease development and progression in humans. Our previous study found that aging (24-month-old) but not young (2-month-old) mice showed lower urinary tract dysfunction (LUTD). Here, we hypothesize that exercise alleviates LUTD in mice by restoring AR-ER signaling balance. To test our hypothesis, we evaluated the expression of nuclear AR during aging and studied the effects of exercise on AR expression and LUTD in the aging male mouse prostate.

METHODS: We examined the ventral prostate (VP) from young (2-month-old) and aging mice (24-month-old) C57Bl/6 mice with/without 4-week exercise. The aging mice with exercise were singly housed, and running wheels were freely accessible. Each wheel was individually monitored throughout the duration of the study. LUTD was assessed using void spot assays and analyzed with the Void Whizzard software. Nuclear AR expression was determined via immunohistochemistry. The percentage of AR positivity was measured by the Inform software.

RESULTS: Aging mice developed LUTD when compared to young mice. In VP, assessment of the same mice showed a significant decrease in nuclear AR ($p < 0.01$) among the aging (28.17% positivity, n=4) compared to the young mice (91.73% positivity, n=5). After 4 weeks of exercise, the aging mice (n=7) showed a significantly increased level of nuclear AR in VP (78.49% positivity, $p<0.05$) and alleviated LUTD, compared with the no exercise controls (n=4).

CONCLUSIONS: Prostatic AR decreases with age in mice, and exercise rescues this decline in aging mice VP. The changes in ER signaling during aging and before and after exercise will be examined in the future.

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Neuronal PD-1 is a target for *Staphylococcus epidermidis*-derived Lipoteichoic Acid to mediate anti-nociceptive activity in Chronic Pain

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Abstract

Neuronal PD-1 is a target for Staphylococcus epidermidis-derived Lipoteichoic Acid to mediate anti-nociceptive activity in Chronic Pain

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Introduction-- Chronic pelvic pain syndrome (CPPS), the most common form of prostatitis, is a complex multi-symptom syndrome with unknown etiology and limited effective treatments. A novel lipoteichoic acid derived from a commensal strain of S. epidermidis (SELTA), was previously demonstrated to reduce tactile allodynia in Experimental Autoimmune Prostatitis (EAP), a murine model of CPPS. Our previous studies demonstrated that modulation of immune homeostasis in the prostate was an important contributor to the amelioration of allodynia in the murine model of CP/CPPS. In this study, a sensory-specific PD-1 conditional knock-out mouse model was established to verify our hypothesis of neuronal PD-1-mediated SELTA analgesic effect. We focused on the role of SELTA on neuronal pathways by examining peripheral nociceptive neurons at lumbar-sacral levels following exposure to SELTA.

Methods—EAP was induced by subcutaneous injection of rat prostate antigen and adjuvant, as previously described (1). Pain responses were assessed by tactile allodynia using Von Frey filament behavioral testing every seven days. Animals were sacrificed on day 28 following injection, and prostates were collected. For ex vivo experiments, the DRGs (dorsal root ganglion) were collected at the level of L4-S2 from B6 mice and co-cultured for 24 hours with SELTA treatment. Intracellular live calcium imaging was performed on adult DRG neuron cultures using the fura-2 ratiometric dye. Images were taken on multi-color confocal microscopy and analyzed by NIH Image J. Statistical analyses were performed using Microsoft Excel and GraphPad Prism software.

Results— Following SELTA instillation, PD-1 immunoreactivity of EAP-induced prostate tissue on day 28 was increased and localized to bIII-tubulin-labeled nerve fibers in the stroma and nerve endings surrounding epithelial cells. Elevated PD-1 immunoreactivity was similarly observed in L4-S2 DRG by SELTA treatment ex vivo. Dye-labeled PD-1 RNA probe showed SELTA-induced increased PD-1 mRNA expression in the prostate and DRG. Functionally, SELTA inhibited ATP-induced Ca^{2+} influx on cultured DRG neurons. Both a TLR2 antagonist as well as neutralizing antibodies to PD-1 reversed the inhibitory effect of SELTA in neurons. Moreover, inhibitory cytoplasmic residues of PD-1 and downstream signaling adapters were shown to be specifically phosphorylated in cultured DRG neurons following SELTA treatment. Finally, abrogation of PD-1 in the PD-1 CKO mice removed the ability of SELTA to mediate pain relief in the induced-EAP model of chronic pelvic pain.

Conclusions—Taken together, our data demonstrated that SELTA is a novel exogenous inducer of PD-1 signaling in neurons that mediates anti-nociception. These results further suggest that SELTA possesses neuro-immunomodulatory activity by virtue of its actions on PD-1 expressed in immune and neuronal cells

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Elucidating the mechanism of MEIS1/HOXB13-mediated tumor suppressive phenotype in 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 HOXB13 mutants were a generously shared from Dr. Sandor Spisak, Ph.D. and Matthew Freedman, M.D.. An in vivo study utilizing SCID mice were used to xenograph cell types mentioned.

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, consistent with a HOXB13-MEIS1 driven phenotype.[VGD1] G84E tumors grew slower 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 need 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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Development and characterization of a novel sensory neuron-specific PD-1 conditional knockout mouse

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Abstract

Development and characterization of a novel sensory neuron-specific PD-1 conditional knockout mouse

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Introduction—Chronic pelvic pain syndrome (CPPS), the most common form of prostatitis, is a complex multi-symptom syndrome with unknown etiology and limited effective treatments. A novel lipoteichoic acid derived from a commensal strain of S. epidermidis (SELTA), was previously demonstrated to reduce tactile allodynia in Experimental Autoimmune Prostatitis (EAP), a murine model of CPPS. Our previous studies demonstrated that modulation of immune homeostasis in the prostate was an important contributor to the amelioration of allodynia in the murine model of CP/CPPS. However, our new data further indicated that neuronal PD-1 is likely a crucial player in the development and modulation of chronic pain and contributes to SELTA-associated analgesic mechanisms. To better understand the role of neuronal PD-1 in chronic pain, we established a sensory neuron-specific PD-1 knockout mouse model. The development, behavioral phenotype, and PD-1 expression in the peripheral and central nervous systems of this CKO mouse were characterized.

Methods—The sensory neuron-specific PD-1 knockout mouse model was established by crossing advillin promoter-driven Cre male and floxed PD-1 female mice (f/f). By crossing back with f/f mice, both floxed PD-1-Cre and floxed PD-1 lines were obtained. Animals were housed in a 12-hour light/dark cycle environment with free access to water and food. For the characterization of CKO mice, we used 8-10 weeks-old male animals and recorded the following features: 1. General development such as potential differences in animal hair, skin development, and body weight between PD-1 CKO and f/f mice. 2. For PD-1 expression, the prostate, dorsal root ganglion (DRG), spinal cord, thalamus, hippocampus, cortex, spleen, intestine, and lymph nodes were evaluated by FISH, immunoblot, and immunohistochemistry. 3. The effect of PD-1 loss was evaluated in several behavioral analyses. Images were taken on multi-color confocal microscopy and analyzed by NIH Image J. Statistical analyses were performed using Microsoft Excel and GraphPad Prism software.

Results— 1. PD-1 CKO mice developed normally and showed no differences in body weight or other physiologic parameters with control f/f mice. 2. Sensory neurons of DRG at L2 to S1 level and trigeminal ganglion showed reduced PD-1 immunoreactivity, particularly small diameter sensory neurons that represent 65-80% of all neurons. FISH analysis of PD-1 CKO mice with dye-labeled PD-1 RNA probes showed reduced PD-1 mRNA expressions in small-diameter DRG neurons and afferents in the prostate. A similar change in PD-1 expression was also observed in neurons located in the

dorsal horn of the spinal cord but no difference was observed in the motor neurons. No change in PD-1 expression was observed in the thalamus, hippocampus, and cortex in PD-1 CKO mice. Immune cells in lymph nodes and spleen showed no change in PD-1 expression. 3. To assess the influence of PD-1 knockout on the cognitive tasks, we completed several behavioral analyses including Y maze, novel object recognition, and dark & lightbox tests. No difference was found.

Conclusions—Our data demonstrates the creation of a new transgenic mouse strain with sensory neuron-specific depletion of PD-1. This novel PD-1 CKO mice shall allow further understanding of the role of PD-1 in chronic pain conditions. The PD-1 CKO mice shall contribute to the investigation of neuron-immune cell crosstalk and its impact on the development and maintenance of chronic pain.

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Translational Studies to Advance anti-ROR1 Cancer Stem Cell Targeting Therapy for Metastatic Prostate Cancer.

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Abstract

BACKGROUND-- The heterogeneity of metastatic castration resistant prostate cancer (mCRPC) makes it challenging to treat and resistance to therapies usually arises. Wnt5A and its non-canonical Wnt receptor, ROR1, have emerged as a promising new signaling pathway target. Wnt5A is a significant marker of poor prognosis in circulating tumor cells (CTCs) of mCRPC patients. ROR1 is expressed in lethal types of mCRPC. A therapeutic ROR1 antibody, Zilovertamab, has been clinically tested in CLL and metastatic breast cancer and shown to be safe. We investigated Zilovertamab-based therapies for metastatic prostate cancer.

HYPOTHESIS -- Wnt5A/ROR1 signaling may mediate a cancer stem cell program which makes cells resistant to therapies which target the cell cycle and proliferation. Blocking ROR1 may reveal vulnerabilities which sensitize cancer cells to chemotherapies like docetaxel.

METHODS-- ROR1 expression was determined using RNASeq, qRT-PCR, FACS and Western blotting. ROR1 signaling was blocked using the anti-ROR1 therapeutic antibody, Zilovertamab, or CRISPRCas9 ROR1 knock out. Cell growth was measured using an Incucyte real time imaging system. Cell cycle analysis was performed in live cells in 2D cultures and 3D organoids using the Fucci2BL bicistronic Fluorescent, Ubiquitination-based Cell Cycle Indicator reporter system. PDX PCSD13 tumor growth was measured via calipers and IVIS. RNASeq was performed on tumors.

RESULTS-- We showed that ROR1 was expressed at high levels in 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. Cells expressing the FUCCI live cell cycle tracker showed docetaxel led to G2 arrest and ROR1 signaling inhibition increased efficacy of docetaxel. Treatment of PCSD13 PDX in vivo with Zilovertamab plus docetaxel synergistically increased tumor growth inhibition in vivo.

CONCLUSIONS-- Cancer stem cells represent the fundamental precursors from which all diverse tumor subpopulations evolve. Thus, 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 our PDX and PCs cell line models to

Zilovertamab plus docetaxel. We are now conducting a phase 1b clinical trial with zilovertamab plus docetaxel in patients with metastatic CRPC (CirmD, NCT05156905, PI R Mckay).

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Efficacy of an FGF2 mimetic peptide (FGF-PT) as a mitigator of radiotherapy-induced damage

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Abstract

Background: Radiation doses high enough to cure abdominal tumors, including prostate cancer, often damage surrounding tissue, leading to multiple organ system injury. Although there are several FDA-approved radiation mitigators for those at risk of bone marrow failure, none are available for GI acute syndrome (GI-ARS). A peptide FGF2 mimetic (FGF-PT) was designed to mitigate radiation damage in multiple tissues, including the gastrointestinal tract and bone marrow.

Methods: Male NIH Swiss mice and Wistar rats underwent radiation with or without bone marrow sparing ($LD_{70-80/30}$) in a dual source cesium or xray irradiator, respectively. After 24 hr, animals, were injected with FGF-PT (10 or 20 mg/kg) or saline vehicle alone daily for 1 or 3 days. For rats, blood was collected weekly for counts of blood cell populations (CBC). At Day 30, intestinal crypt cell proliferation and maturation markers were assessed using immunofluorescence (IF) confocal microscopy. Mitochondrial biogenesis and cytokine expression were assessed using realtime PCR and multiplex ELISA assays.

Results: Administration of FGF-PT provided a survival advantage in both mice and rats, of about 1-2 days, after radiation. Mice treated with FGF-PT had reduced GI bleeding, improved stool formation, and shorter duration of diarrhea. Irradiated mice receiving FGF-PT had better preservation of proliferating crypt cells, mitochondrial function, and metabolite transporter expression compared to vehicle controls, improving survival at 30 days. FGF-PT administration to rats produced similar results. When G-CSF was given as supportive care for bone marrow damage, the addition of FGF-PT provided additional benefit. Circulating pro-inflammatory cytokines were reduced in both rodent models.

Conclusions: FGF-PT produced survival benefits in irradiated rodent models by reducing pro-inflammatory cytokines, preserving intestinal villi function, and improving bone marrow recovery. These results show that the synthetic FGF-PT peptide has potential as a safe and effective mitigation agent for radiation-induced GI-ARS.

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Matrix stiffness mechano-sensitizes transformed prostate epithelial cells towards a luminal phenotype.

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Abstract

Background: 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 mechanoresponsive 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, wherein patient-derived prostate epithelial cells (PrECs) upon induction with FGF-7, form a suprabasal layer and differentiate into luminal epithelial cells (K8/18, TMPRSS2). Given the mechanoresponsive properties of Notch proteins, we explored the role of mechanical stimuli in PrEC cell differentiation.

Methods: We cultured normal and transformed PrECs on soft (0.5kPa) and stiff (35kPa) matrix poly-acrylamide hydrogels. After cells reached confluency, we induced differentiation for 1, 3, 5, and 7 days with KGF/FGF-7 supplement (20ng/ml) on alternating days. 24 hours prior to assay termination, a bolus of androgens (10nM R1881) was provided for facilitate transcription of Androgen Receptor (AR) regulated genes. Cells were collected fixed in 4% PFA and immunostained for differentiation markers (CK5/14, 8/18, TP63, TMPRSS2) and for cytoskeleton (Paxillin and Phalloidin).

Results: Our data show that in normal PrECs, NOTCH3 expression increased on soft relative to stiff matrix after 3 days of culture concurrent with increased expression of luminal epithelial markers (K8/18, TMPRSS2). However, in cells transformed with mutation in ERG, overexpressing c-MYC, and knockdown of PTEN (EMP) this phenotype is dysregulated, where increased NOTCH3 expression and differentiation markers increase in stiff vs soft. EMP cells exhibit increased mechano-sensitivity as as measured with increased protrusions and spreading.

Conclusion: Our results highlight the dysregulation in mechano-sensitivity in transformed epithelial cells. Transformed tumor cells are arrested in epithelial differentiation process and shift towards more mechano-sensitive phenotype.

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BARRIERS TO INCREASING FUNDING FOR NON-CANCEROUS GENITOURINARY RESEARCH

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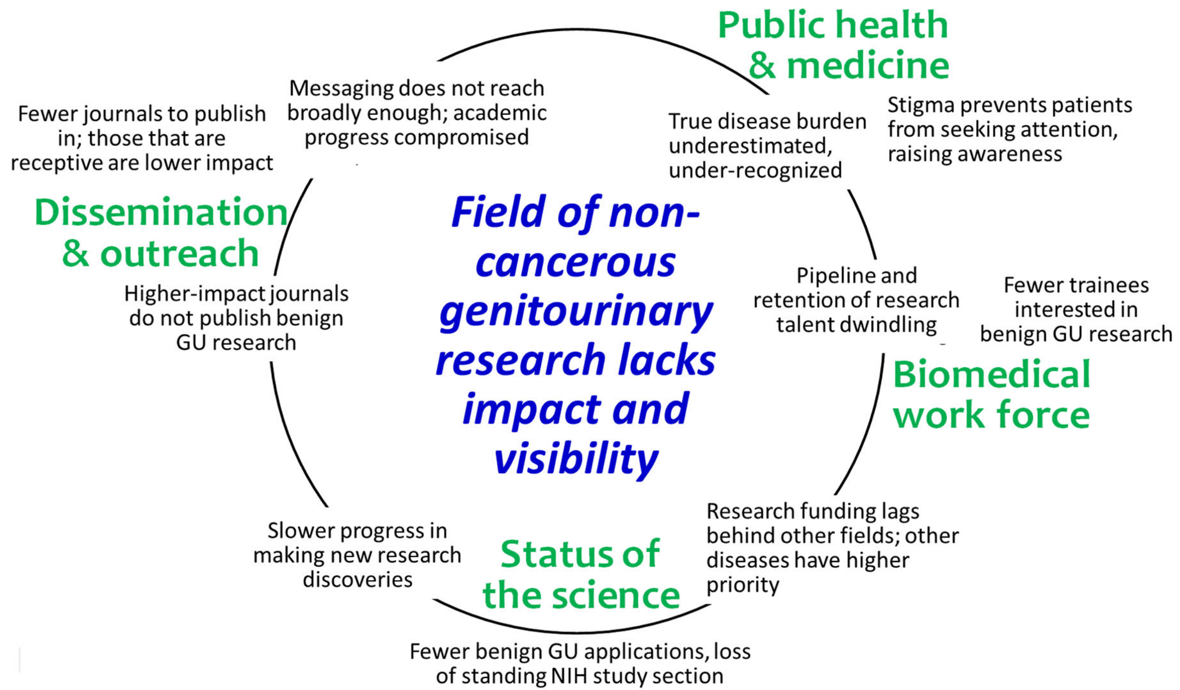
Abstract

BACKGROUND: Non-cancerous genitourinary conditions (NCGUCs) affect a growing number of people, especially in an aging population, and confer high burdens on a healthcare system already experiencing shortages of urologists and urogynecolosits. Conditions such as urinary tract infections, functional and control problems of the bladder, prostate enlargement and lower urinary tract symptoms, urinary tract stones, male infertility, chronic pelvic pain, and genital dysfunction are increasingly common. The burden of NCGUCs is high, albeit underreported, and includes medical charges for diagnostics, procedures, and prescriptions, and out-of-pocket expenses for specially-adapted wearable items (e.g., pads and pants), analgesics, and equipment (e.g., catheters). Knowledge gaps related to NCGUCs are significant and require more research funding to drive progress. But barriers exist (Figure). Our objective was to identify whether lower visibility of NCGU research in urology/GU journals is a factor.

METHODS: Using common bibliometric processes, we reviewed higher-ranked non-oncology urology/GU journals publishing between 2017-2021 for impact factor, editorial board composition, and articles.

RESULTS: We identified 25 of the highest-impact journals that publish NCGU research; nearly half were North American, 40% European, and 12% Asian. We identified general urology journals (n=13) and those focused on andrology (5), neurourology (2), urogynecology (2), endocrinology (1), prostate (1), and kidney (1). Among nearly 20,000 articles, NCGU research was published less frequently in journals with higher impact factors; the prevalence of editorial board members whose research was primarily related to malignant genitourinary (MGC) conditions was correlated.

CONCLUSIONS: The burden of NCGUCs in society is already high and is growing. Research funding has not kept pace, and this is likely due to multiple reasons. We identified lower prevalence of NCGU research published in higher impact urology journals, which may be a contributor to and/or consequence of research funding that has not kept pace with societal impact.


Public health & medicine
True disease burden underestimated, under-recognized
Stigma prevents patients from seeking attention, raising awareness
Fewer journals to publish in; those that are receptive are lower impact
Messaging does not reach broadly enough; academic progress compromised
Dissemination & outreach
Higher-impact journals do not publish benign GU research
Field of non-cancerous genitourinary research lacks impact and visibility
Pipeline and retention of research talent dwindling
Fewer trainees interested in benign GU research
Biomedical work force
Research funding lags behind other fields; other diseases have higher priority
Slower progress in making new research discoveries
Status of the science
Fewer benign GU applications, loss of standing NIH study section

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CRISPR/Cas9 Extracellular Vesicle Delivery System to Knockout ACSL4 in Prostate Cancer Cells

CRISPR/Cas9 Extracellular Vesicle Delivery System to Knockout ACSL4 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). Treatment of ADT can result in androgen-receptor (AR) pathway independent tumor growth that is resistant to AR signaling inhibitors (ARSIs). PCa cells in an AR stripped environment upregulate Acyl-CoA Synthetase Long Chain Family Member 4 (ACSL4) due to its dysregulation of fatty acid metabolism. Our previous study has shown that ACSL4 is a potential therapeutic target for AR-independent PCa tumors. CRISPR/Cas9 machinery provides a method to produce a genomic knockout of ACSL4 in PCa cells. However, delivery of CRISPR/Cas9 machinery requires an effective delivery vehicle. We have demonstrated that extracellular vesicles (EVs) are natural biological vesicles for drug delivery that can efficiently package CRISPR/Cas9 through a modification of N-myristoylation in the Cas9 protein.

Methods: Lentiviral vector expressing myristoylated Cas9 (mCas9) was created by fusion of the octapeptide derived from the N-terminus of Src kinase into the N-terminus of Cas9 gene. Additionally, three individual candidate sgRNAs for targeting eGFP or ACSL4 were bicistronically cloned into the lentiviral vector. The product was confirmed via Sanger sequencing. An mCas9/sgRNA-ACSL4 lentivirus was produced and used to infect PC3 cells. Infected cells were collected and analyzed via Western blotting. Proof-of-Concept EVs were produced by co-transfection of mCas9/sgRNA-GFP and VSVG in 293T cells. Conditioned media was collected for sequential ultracentrifugation. EVs-producing cells and EVs were analyzed via Western blotting.

Results: The mCas9/sgRNA-GFP EVs expressed expected EVs markers and myristoylated Cas9 protein. The EVs encapsulating mCas9/sgRNA-eGFP knocked out GFP gene in recipient cells. Additionally, sgRNA-eGFP was substituted with sgRNA-ACSL4 to produce the mCas9/sgRNA-ACSL4 construct. Sequencing revealed that sgRNA-ACSL4 was successfully cloned into the mCas9 lentiviral vector. PC-3 cells infected with mCas9/sgRNA-ACSL4 lentivirus showed an ACSL4 knockout via Western blot analysis. Future studies will include the encapsulation of mCas9/sgRNA-ACSL4 in EVs to target a variety of prostate cancer cells.

Conclusion: N-myristoylated Cas9/sgRNA ribonucleoprotein complex targeting ACSL4 produced an effective knockout. This result displays mCas9/sgRNA-ACSL4 as a successful construct that provides promise for future EVs-based gene therapy.