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Molecular mechanisms of urological diseases and treatment resistance: meeting report of the 2021 society for Basic Urologic Research (SBUR) annual meeting

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The Society for Basic Urologic Research (SBUR) 2021 Annual Meeting convened online on November 4-7, 2021, via a digital meeting platform. At the very start of the meeting, Dr. Michael Freeman from Cedars-Sinai Medical Center gave a tribute to Dr. Leland W. K. Chung, who passed away this year. Dr. Chung was an internationally renowned urological cancer researcher, mentor, and educator, and a lifetime member and past president of the SBUR. The meeting was officially kicked off with the keynote Leland W.K. Chung lecture delivered by Dr. Padmanee Sharma from MD Anderson Cancer Center, followed by six plenary sessions focused on: 1) Emerging Technologies and Models for Urological Research; 2) Endocrine and Genetic Regulations in Urological Biology; 3) Epigenetic Regulations of Urological Development and Diseases; 4) Infection, Inflammation, and Immune Response in Urological Biology; 5) Biomarkers, Environmental Factors, and Health Disparities in Urological Diseases; and 6) Urological Stem Cells, Lineage Plasticity, and Treatment Resistance. Dr. Martin Gleave from the Vancouver Prostate Centre delivered this year's American Urological Association (AUA) lecture. There were 302 registrants from 5 countries that participated in the meeting.

The keynote Leland W.K. Chung lecturer Dr. Padmanee Sharma was introduced by Dr. Gail Prins from the University of Illinois at Chicago. Dr. Sharma discussed the tight regulation of immune responses wherein the "ON" signals drive the "OFF" signals. She shared the seminal discovery by Dr. James P Allison to prolong T cell anti-tumor responses by blocking the CTLA-4 inhibitory pathway, which laid the groundwork for multiple clinical trials in a wide range of cancers and a new field called Immune Checkpoint Therapies (ICT). However, Dr. Sharma recognized that, despite the promise of ICT, the patient response rate is only around 30-40%. There are many research questions that remain

to be addressed to increase patient response and reduce the toxicity of the treatment. Dr. Sharma implemented pre-surgical or tissue-based trials in earlier disease settings to obtain appropriate samples from patients for mechanistic and biomarker studies. The first of these was a neoadjuvant (pre-surgical) clinical trial with anti-CTLA-4 in patients with localized bladder cancer, which for the first time showed that bladder cancer could be responsive to ICT. Analyses of pre- and post-treatment tissues identified ICOS (Inducible T-Cell Co-Stimulator), a member of the CD28/CTLA-4 family, being significantly up-regulated following anti-CTLA-4 treatment. Further, only the responder patients showed increased $ICOS^+$ CD4 T cells post-treatment, suggesting that ICOS and its ligand ICOSL may be necessary for effective anti-tumor immune response in the setting of anti-CTLA-4 therapy. To take the hypothesis back to the laboratory, Dr. Sharma used transgenic mice to show that ICOS or ICOSL knockout (KO) mice had impaired tumor rejection and survival compared to the wild-type mice following CTLA-4 blockade. Mechanistically, this is due to ICOS-induced signaling via PI3-kinase to promote T-bet expression, which is critical for Th1 responses and tumor rejection.

Consequently, concurrent stimulation of ICOS improved the anti-tumor responses and survival of bladder tumor-bearing mice treated with anti-CTLA-4. Next, Dr. Sharma presented her group's work on prostate cancer (PCa) as a "cold" tumor with poor T-cell infiltrations. Analyses of clinical specimens from a neoadjuvant study of PCa treated with anti-CTLA-4 revealed compensatory immune inhibitory pathways, such as up-regulation of PD1, PD-L1, and VISTA in CD8+, CD68+, as well as tumor cells. This work led to a clinical trial of anti-CTLA-4 and anti-PD-1 combination in patients with metastatic castration-resistant prostate cancer (CRPC). In addition, Dr. Sharma reported less frequent clinical responses in patients with bone metastases than soft-tissue metastases. Analyses of clinical samples revealed that Th1 cells are up-regulated in soft-tissue metastases, but not bone metastases that express higher levels of TGFβ. Indeed, a combination of ICT with anti-TGFβ treatment increased Th1 cells, reduced tumor growth in bone, and improved survival in murine models of bone tumors. Lastly, Dr. Sharma discussed other critical pathways for consideration as resistant mechanisms and highlighted the epigenetic regulator EZH2, the enzymatic subunit of the polycomb repressive complex 2 (PRC2). While CD28 and TCR (T cell Receptor) signaling are both essential in driving T cell responses, they increase the expression of EZH2. EZH2 catalyzes H3K27 trimethylation (H3K27me3), leading to gene repression and stabilization of the FoxP3 transcription factor that mediates immune suppression via Treg cells and thus tumor resistance to ICT. Accordingly, EZH2 inhibitors impact the phenotype and function of Tregs and improve anti-tumor responses of ICT in animal models. This work laid the foundation for a new clinical trial of EZH2 inhibitors in combination with anti-CTLA-4 in patients of metastatic PCa, bladder cancer, and renal cell carcinoma, including patients who have failed prior anti-PD-L1 therapy.

The first plenary session discussed Emerging Technologies and Models for Urological Research. Drs. Natasha Kyprianou from the Icahn School of Medicine at Mount Sinai and Allen Gao from the University of California, Davis, were the discussion leaders for this plenary session. Dr. Cory Abate-Shen from Columbia University discussed the benefits and challenges of genetically-engineered mouse models (GEMMs) and the uses of Cre recombinase to activate or inactivate genes in specific tissues, of inducible promoters for temporal recombination, and of reporters for lineage tracing. Dr. Abate-Shen presented a catalog of Nkx3.1-Cre-driven GEMMs that represent all stages of PCa and are available at the Jackson Laboratory. Lastly, Dr. Abate-Shen presented her recent work using new technologies to generate refined GEMMs that model bladder cancer for novel translational findings. Dr. Christina Jamieson from the University of California, San Diego, discussed patient-derived xenograft (PDX) and organoids (PDO) as invaluable resources for studying PCa bone metastasis. Dr. Jamieson presented PDX models with different growth under distinct microenvironments, i.e., soft tissue versus bone, and varying responses to anti-androgen therapies, identifying a PDX model of bone-metastatic PCa that is castration-resistant in the bone-niche. Dr. Stanley Qi from Stanford University discussed their research to expand genome engineering tools beyond genome editing, including CRISPRi/a for programmable gene regulation, the fusion of dCas9 to different enzymatic domains of epi-

genetic regulators to either methylate or demethylate DNA or histones for epigenomic regulation, and the creation of heterochromatin and long-range DNA interactions using CRISPR-EChO. Dr. Qi further presented novel CRISPR tools for *in vivo* use, which utilized structure-guided engineering to enhance the activities of dCasMINI and improve its delivery.

The second plenary session focused on Endocrine and Genetic Regulations in Urological Biology. Drs. Zhou Wang from the University of Pittsburgh Medical Center and Scott Dehm from the University of Minnesota (Meritorious Achievement Award Recipient) were the discussion leaders for this plenary session. This session was kicked off by Dr. Zhou Wang, who presented on the role of E-cadherin loss and luminal epithelial permeability in glandular benign prostatic hyperplasia (BPH). Dr. Wang found that E-cadherin was downregulated at sites of inflammation in BPH patient samples and used an inducible prostatic luminal epithelial-specific E-cadherin KO mouse model, as well as cell line models, to demonstrate how E-cadherin loss weakens epithelial cell-cell junctions and induces prostatic inflammation and bladder overactivity. Next, Dr. Mehdi Mollapour from Suny Upstate Medical University discussed a novel role of the tumor suppressor Folliculin (FLCN) in inhibiting the Warburg effect by blocking Lactate Dehydrogenase A (LDHA) activity in kidney cancer. Dr. Mollapour also presented his lab's efforts to develop FLCN peptides for cancer treatment, which showed promising efficacy to inhibit LDHA activity *ex vivo* and induce apoptosis in cancer cells. Dr. Yan Dong from Tulane University discussed the roles of Androgen Receptor (AR) splice variant AR-V7 in PCa and showed that AR-V7 could drive gene expression and DNA damage repair, independently of full-length AR, suggesting the potential of targeting AR-V7 for PCa therapy. Dr. Dong further showed that circular AR transcripts are increased during CRPC and serve as a prospective non-invasive marker for AR variant expression to predict resistance to AR inhibitors. Dr. Joshi Alumkal from the University of Michigan discussed the role of AR pathway loss in PCa lineage plasticity and therapy resistance upon enzalutamide treatment. Work from his laboratory revealed that enzalutamide treatment activates lineage plasticity programs in PCa cells with low AR activity and increases the activity of E2F Transcription Factor 1 (E2F1), which can be blocked by bromodomain and extraterminal (BET) inhibition.

The third plenary session focused on Epigenetic Regulations of Urological Development and Diseases. Drs. Amina Zoubeidi from the Vancouver Prostate Centre, Jelani Zarif from Johns Hopkins University, and Leigh Ellis from Cedars-Sinai Medical Center served as discussion leaders for this plenary session. Dr. Haojie Huang from Mayo Clinic discussed the roles of polycomb group protein EZH2 in catalyzing H3K27me3 and how this epigenetic function is modulated by EZH2 phosphorylation at various residues by CDK1/2 and AKT. Dr. Huang presented recent work from his laboratory demonstrating that ZMYND8 (Zinc Finger MYND-Type Containing 8) is overexpressed in kidney cancer and preferentially binds phosphorylated EZH2 (at T487) under hypoxia conditions, switching EZH2 binding from PRC2 to FOXM1 to induce MMP genes and promote kidney cancer migration and invasion. Dr. Irfan Asangani from the University of Pennsylvania (Young Investigator Award Recipient) presented research in his laboratory focusing on the regulation of AR signaling in PCa. Dr. Asangani discussed the expression and function of NSD2, a histone methyltransferase that catalyzes H3K36 dimethylation in relation to the AR pathway and PCa progression. Dr. Praveen Thumbikat from Northwestern University reported dysregulated immune responses in chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS). Dr. Thumbikat discussed how early-life events, infection, and environment drive epigenetic changes such as ITGAL hypomethylation and IL-10 hypermethylation in the immune compartment of CP/CPPS patients, resulting in immune regulatory deficits and chronic inflammation. Dr. Kexin Xu from the University of Texas Health San Antonio presented her research on methylation of adenine bases at the nitrogen-6 position (m6A) in PCa. The m(6)A methyltransferase METTL3 was shown to induce m6A on the mRNA of HMBOX1 (Homeobox Containing 1), which leads to mRNA degradation. The loss of HMBOX1 expression results in telomere shortening and p53 inactivation, causing genomic instability and PCa progression. Dr. Pavlos Msaouel from MD Anderson Cancer Center presented his group's discovery that SMARCB1 loss increased c-Myc-induced DNA replication stress in renal medullary carcinoma (RMC), conferring tumor vulnerability to drugs target-

ing replication stress. Dr. Msaouel reported a distinct immune profile in RMC, characterized by significant up-regulation of the cGAS-STING pathway, and discussed ongoing tissue-rich prospective clinical trials to determine optimal immunotherapies to target stress-induced vulnerabilities. Immediately following this session, Dr. Carolyn Best, Director of Research at the AUA, provided an update on the education, diversity, advocacy, and funding programs at the AUA.

The fourth plenary session discussed Infection, Inflammation, and Immune Response in Urological Biology. Drs. Shafiq Khan from Clark Atlanta University, Zongbing You from Tulane University, Douglas Strand from UT Southwestern Medical Center, and Travis Jerde from Indiana University were discussion leaders for this plenary session. Dr. Sarki Abdulkadir from Northwestern University presented novel therapeutic approaches using microbes or MYC inhibitors (MYCi) to turn immunologically "cold" prostate tumors "hot" and sensitize prostate tumors to ICT. Dr. Abdulkadir demonstrated that UroPathogenic Escherichia Coli (UPEC) strain CP1 and MYCi treatments reprogram the tumor immune microenvironment and promote tumor-infiltrating lymphocytes to enhance immunotherapy response in PCa. Next, Dr. Karen Sfanos from Johns Hopkins University showed that bacterial infections and inflammation might contribute to oncogenic TMPRSS2:ERG gene fusions that occur in proliferative inflammatory atrophy (PIA), a putative risk factor lesion for PCa. Analysis of prostatectomy specimens with bacterial infections revealed high rates of ERG+ PIA lesions, some of which were transitioning to early invasive cancer, supporting TMPRSS2:ERG fusion as an early event in PCa development. Dr. Xin Lu (Young Investigator Award Recipient) from the University of Notre Dame presented promising immunotherapeutic approaches for treating penile cancer and PCa, including PMN-MDSC (polymorphonuclear myeloid-derived suppressor cells) targeted approaches in combination with ICT. Using a GEMM of penile cancer with deletion of tumor suppressors *Smad4* and *Apc* in the mouse penis (SA model), Dr. Lu found that MDSCs are increased in the tumors of SA mice, MDSC-targeted therapy synergizes with ICT to inhibit tumor growth, and PARP inhibitors also show promising anti-tumor activity. Next, Dr. A. Lenore Ackerman from the University of California, Los Angeles, presented on the urinary microbiome, showing that fungi are part of the urinary microbiota and are associated with urinary tract disorders and that the effect of fungi on urinary tract function is determined by strain-dependent virulence factors. Dr. Ackerman proposed a "three-hit hypothesis" suggesting that Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) development requires the convergence of three factors: genetic susceptibility in the host, a disease-causing microbial strain, and an environmental stimulus. Dr. Maria Hadjifrangiskou from Vanderbilt University discussed UPEC functions during a Urinary Tract Infection (UTI), showing that UPEC uses aerobic respiration in the intracellular niche and shifts urothelial metabolism toward glycolysis. The intracellular infection induces hypoxia and HIF1 activity in a cytochrome bd-dependent manner to drive this shift towards anaerobic metabolism. Dr. David DeGraff from Penn State University presented the relationship between pioneer transcription factor FOXA1 and bladder cancer tumor heterogeneity and the role of FOXA1 in regulating interferon-stimulated genes. Dr. DeGraff showed that squamous tumors exhibit FOXA1 loss, increased mutational burden, and PD-L1 expression. Mechanistically, Dr. DeGraff found that FOXA1 loss induces global increases in H3K27ac to promote the expression of interferon-stimulated genes, including PD-L1. Dr. Petros Grivas from the University of Washington discussed immunotherapy advances in the treatment of urothelial carcinoma and provided important updates on several clinical trials. Some highlights from Dr. Grivas' presentation include: adjuvant nivolumab has been FDA-approved in high-risk urothelial carcinoma; atezolizumab has been approved as a first-line option for cisplatin-ineligible patients, and pembrolizumab has been approved as a first-line option for platinum-unfit patients. The final lecture of this session was the AUA lecture delivered by Dr. Martin Gleave from the Vancouver Prostate Centre. Dr. Martin Gleave presented his lab's work on understanding and targeting treatment resistance and adaptive stress pathways in advanced PCa. Dr. Gleave discussed the importance of molecular subclassification of advanced PCa and meaningful prognostic and predictive biomarkers, which is complicated by several limitations in genomics and precision oncology. While genomic alterations are key factors driving therapy resistance,

non-genomic factors also play important roles. Additionally, obtaining CRPC bone metastasis tissues is difficult and is further complicated by inter-tumor heterogeneity. A promising alternative to obtaining CRPC tissue samples is to use liquid biopsies. Dr. Gleave presented his group's work using plasma ctDNA (circulating tumor DNA) to examine the PCa genome and monitor treatment-induced genomic alterations in metastatic CRPC. Interestingly, Dr. Gleave found that elevated levels of ctDNA correlated with poor response to AR inhibitors. In addition, ctDNA exhibited similar mutation profiles and gene copy numbers to metastatic CRPC tissue biopsies. Analyses of patient ctDNA also revealed frequent alterations in AR, underscoring the need for novel agents targeting AR reactivation, such as AR chaperone inhibitors and AR degraders. Dr. Gleave then discussed other common alterations, including DNA-repair deficiency and PI3K alterations that can be targeted respectively by PARP inhibitors and ipatasertib in combination with AR pathway inhibitors. Next, Dr. Gleave discussed the value of PET imaging in identifying oligomets and highlighted the potential of PSMA (prostate-specific membrane antigen)-targeted radioligand therapy. Finally, Dr. Gleave discussed his work investigating stress responses to identify novel drug targets for combination with AR pathway inhibitors. Notably, Dr. Gleave and collaborators used cryo-electron microscopy (cryo-EM) and computer-augmented drug design to identify a lead compound (ivermectin) that inhibits Hsp27 (Heat shock protein 27), a stress adaptor protein that regulates survival signaling in CRPC.

The fifth plenary session discussed Biomarkers, Environmental Factors, and Health Disparities in Urological Diseases. Drs. Hari Koul from Louisiana State University Health Sciences Center and Shuk Mei Ho from the University of Cincinnati were discussion leaders for this plenary session. Dr. Larisa Nonn from the University of Illinois at Chicago presented her work exploring how Vitamin D deficiency and Megalin hormone salvaging activity contribute to PCa disparities in African American men. Dr. Nonn identified that low vitamin D in African American men leads to increased androgen import into the prostate by Megalin, which is highly expressed in PCa metastasis. Dr. Kosj Yamoah from Moffitt Cancer Center discussed his work studying PCa disparities and emphasized the need for more basic, clinical, and epidemiological studies in diverse populations. Dr. Yamoah showed that African American PCa patients exhibit enriched immune and inflammatory pathways, lower DNA damage repair, and higher radiosensitivity than European American men, highlighting the need for further studies in diverse PCa populations for a better understanding of factors that may influence diagnosis and therapeutic response. Following these presentations, a panel discussion was held on the topic of Health Disparities in Urological Diseases, moderated by Dr. Adam B. Murphy from Northwestern University. The panelists included Drs. Jennifer Anger from Cedars Sinai, Rosalyn Adam from Harvard University (Distinguished Service Award Recipient), Clayton Yates from Tuskegee University, and patient advocate Mrs. Jan Manarite from Cancer ABCs. Dr. Jennifer Anger discussed sex and gender disparities in urologic diseases and the need for basic science research on pain responses, hormonal therapy, puberty blockade, and animal models for cis and transgender individuals. Dr. Rosalyn Adam discussed racial, ethnic, and socioeconomic disparities and differences in urological diseases, highlighting the need for better research, education, advocacy, inclusion, and funding. Dr. Clayton Yates discussed PCa disparities in African American men and further described how PCa in African American men exhibits increased inflammatory signature, expression of immune checkpoints, and responsiveness to Vitamin D treatments. Patient Advocate Mrs. Jan Manarite provided critical perspectives to the conversation, emphasizing the importance of asking good questions and shared decision-making for patients.

The sixth plenary session focused on Urological Stem Cells, Lineage Plasticity, and Treatment Resistance. Drs. Jiaoti Huang from Duke University, Leah Cook from the University of Nebraska, Tim Ratliff from Purdue University, and SBUR President Dr. Susan Kasper from the University of Cincinnati were discussion leaders for this plenary session. Dr. Wei-Qiang Gao (SBUR 2021 International Speaker) from Shanghai Jiao Tong University presented on single-cell RNA seq (scRNA-seq) analysis of novel mouse models of acute and chronic bladder injury induced by cyclophosphamide (CPP), which provided important insights into the composition of the murine bladder mucosa layer after CPP, lineage relationships during urotheli-

al cell regeneration, and the niche associated with urothelial regeneration. Dr. Gao also discussed scRNA-seq analyses of human bladder cancer and characterized the intra-tumoral heterogeneity and invasive subpopulations in bladder cancer. Dr. Jung Wook Park from Duke University presented a human prostate organoid transformation assay, which involves isolating cell populations of interest (e.g., basal, luminal, neuroendocrine) from benign prostatectomy samples followed by lentiviral transduction with oncogenic drivers, short-term organoid culture, and subcutaneous injection into mice. This system was used to explore how cell origin determines cancer cell phenotype and profile temporal transcriptional changes during small cell PCa development. In addition, Dr. Park discussed how this organoid transformation assay can be applied to the study of other cancers, such as small cell bladder cancer and small cell lung cancer, and found that ion channel blockers sensitize small cell bladder cancer to chemotherapy. Dr. Dolores J. Lamb from Cornell University discussed promising techniques for artificial gamete generation and autologous transplantation for men with gonadotoxic drug exposure or infertility due to genetic causes. Notably, Dr. Lamb spoke on the potential of using cryopreserved spermatogonial stem cells from pre-pubertal boys to restore spermatogenesis after gonadotoxic drug treatment. Next, Dr. Yuanyuan Zhang from Wake Forest University presented his lab's work characterizing urine-derived stem cells (USCs) for tissue repair and engineering for various urological applications. Dr. Zhang successfully used USCs to develop 3D renal organoids and improve renal function in a rat model of chronic kidney disease. Dr. Hansen He from the University of Toronto discussed cell-free DNA methylome profiling using over 200 plasma samples of localized and metastatic PCa patients and found global hypermethylation in metastatic PCa. The methylome data distinguished localized and metastatic PCa and predicted genetic variations (e.g., copy number gain/loss) with high accuracy. Dr. Ping Mu from UT Southwestern integrated RNA-seq and ATAC-seq data and performed a CRISPR-based functional screen to identify transcription factors driving Enzalutamide resistance upon the loss of chromatin remodeling protein CHD1 in PCa. Dr. Mu showed that CHD1 loss promotes lineage plasticity and drives tumor heterogeneity, which leads to heterogeneous mechanisms of resistance and populations of resistant cells caused by different genes upon Enzalutamide treatment. Finally, Dr. Isla Garraway from the University of California, Los Angeles, discussed how aggressive PCa and metastases co-opt stem-cell features, with particular focus on the role of keratin 13 (KRT13). Analyzing human prostate tissue samples, Dr. Garraway successfully isolated and characterized a prostate stem-like population marked by KRT13 expression. Dr. Garraway found that KRT13 expression in primary PCa is, in fact, a poor prognostic factor and drives bone colonization, perhaps by regulating cell stiffness.

Lastly, eight trainees were selected from 131 submitted abstracts to receive Virtual Travel Awards and give oral presentations within these plenary sessions. In support of SBUR's goal to train the next generation of urologic researchers, a mission supported by the NCI and NIDDK, Drs. Daniel Frigo and Tanya Stoyanova 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 two parts: Part 1 was a 1.5-hour career panel discussion and Part 2 was a 1-hour fellowship/grant writing session. The turnout for the Trainee Symposium was over 90 trainees, surpassing attendance of any prior SBUR Trainee Symposium, in-person or virtual. The career panel discussion included six panelists that spanned a range of academic and non-academic occupations. The panelists included Drs. Cimona Hinton (Clark Atlanta University), Cindy Lebron (Seagen), James Brooks (Stanford University), Sarah Amend (Johns Hopkins School of Medicine), Meghan Rice (Revolution Medicines), and Matthew Smith (Genentech). During the career panel session, questions pertaining to careers in Academia and Industry were addressed. These included a comparison of career paths in Academia vs. Industry, required skill sets and expertise for each of these career paths, potential to switch between these two career tracks, pre-requisites for tenure-track positions in academia, challenges associated with starting an independent research group, insights into funding opportunities, challenges in obtaining tenure in academia, comparing scientist positions in established companies versus start-ups, and the importance of mentorship. During the transition from the career panel discussion to the fellowship/grant writing session, Dr. Larisa

Nonn gave a short presentation on the IRACDA postdoctoral fellowship program, the details of which can be found on the following webpage: https://iracda.uic.edu/program/. The fellowship/grant writing portion of the symposium was led by Dr. Travis Jerde (Indiana University School of Medicine) and included short presentations and discussions with Drs. Amina Zoubeidi (Vancouver Prostate Centre), Leigh Ellis (Cedar-Sinai Medical Center), and Jill Fehrenbacher (Indiana University). Different types of fellowship mechanisms were discussed, as were common pitfalls and potential strategies for successful grant writing.

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Hepcidin upregulation correlates with immune-suppressive tumor environment and unfavorite survival outcomes in renal clear cell carcinoma patients

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Abstract

Background: Current therapies for renal cell carcinoma (RCC) focus on tyrosine kinase inhibitors and immune checkpoint inhibitors. Unfortunately, clinically reliable biomarkers for treatment selection are rare, and we aimed to discover novel biomarkers for immune precision medicine. The iron-regulating peptide hormone hepcidin was reportedly increased in RCC patient sera and tissues. However, its potential implication as a prognostic and immunotherapy biomarker for RCC management remains exclusive.

Methods: Multiple RNAseq and cDNA microarray datasets were utilized to analyze hepcidin expression profiles connected with patient pathological parameters and survival outcomes. Single-sample gene set enrichment analysis (ssGSEA) was conducted to explore the correlation of hepcidin expression with immune infiltration cell subtypes. Two single-cell RNA-seq (scRNA-seq) datasets were used to analyze hepcidin expression at the single-cell level on the tumor microenvironment (TME).

Results: Hepcidin expression was drastically upregulated in both clear cell RCC and papillary RCC tissues. However, hepcidin upregulation correlated with disease progression and survival outcome only in ccRCC but not in pRCC tissues. Hepcidin upregulation was parallel with a significant hypomethylation at its promoter region and a drastic downregulation of the SOSTDC1 gene (BMP protein antagonist) in ccRCC tissues. Spearman correlation analysis found a strong correlation of hepcidin upregulation with inflammatory factors, immune responsive cytokines, and immune checkpoint receptors. Meanwhile, hepcidin upregulation was positively associated with immune suppressive infiltrations (macrophages and Treg cells). Anti-PD-L1 immunotherapy largely reduced hepcidin expression in anti-cancer immune cells in ccRCC tissues. The negative impact of hepcidin upregulation on patient survival outcomes was diminished in immune infiltration enriched with anti-cancer cell types.

Conclusion: Hepcidin upregulation was strongly associated with disease progression, had a negative impact on patient survival, and was associated with a suppressive immune microenvironment in ccRCC tissues. This study provides a novel biomarker, hepcidin upregulation, as an indicator for immune precision medicine.

2

A novel of 9 immune-related gene prognostic index for clear cell renal cell carcinoma

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Abstract

Purpose: In order to screen out the immune checkpoint inhibitor (ICI) benefit subgroup patients in renal cell carcinoma (RCC), and the immune characteristics in ICI benefit patients.

Experimental Design: We download The Cancer Genome Atlas kidney renal clear cell carcinoma dataset (n=611). Immune genes were downloaded from Immunology Database and Analysis Portal. Immune-related gene prognostic index (IGPI) was constructed by bioinformatics and Cox regression modeling. Furthermore, the clinical phenotypes, immune cell characteristics, and ICI therapy benefit in high and low IGPI risk score groups were all analyzed.

Results:

A total of 702 differentially expressed immune genes were identified, PDIA2, CCR10, IRF9, VAV3, SAA1, SEMA3G, AMH, GDF7, ANGPTL3 (n=9) genes were related to prognosis. IGPI for clear cell RCC was constructed on the basis of 9 genes. IGPI high risk score patients were correlated with high *SETD2* and *BAP1* mutation; high infiltration Treg cells; and more aggressive phenotypes. In IGPI test and train groups, the survival rate between IGPI high risk score and IGPI low risk score patients were all significant ($p<0.001$, $p<0.001$, respectively), 1-year AUCs (area under the curve) were 0.770 and 0.848 in test and train groups, respectively. IGPI high risk score patients had better clinical benefit from CTLA4 and PD-1 inhibitor therapy.

Conclusions: IGPI, immune gene based prognostic model, provided a biomarker for ccRCC patient ICI therapy.

3

The PRC2-dependent epigenetic remodeling exacerbates inflammation and severity of urinary tract infections

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Abstract

The PRC2-dependent epigenetic remodeling exacerbates inflammation and severity of urinary tract infections

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Background: Urinary tract infection (UTI) is a pervasive health problem worldwide. Standard treatment for UTIs often lead to antibiotic resistance due to its recurrent nature. Patients with a history of UTIs suffer increased risk of recurrent infections, yet the underlying mechanisms remain unclear. In this study, we aim to determine whether UTI induces epigenetic remodeling of bladder urothelial cells and, if so, whether targeting epigenetic remodeling decreases severity of UTIs.

Methods: Mice are infected with clinically relevant uropathogenic *E. coli* UTI89 to model acute, chronic and severe UTIs. Function of PRC2 in bladder urothelial cells was studied by conditionally knockout *Eed* gene using a urothelium-specific Cre driver. In addition, *in vitro* organoid model was also used to model urothelial cell-specific response to bacterial infection. Effect of PRC2-specific small molecular inhibitors were also used to examine their therapeutic potential to improve outcomes of chronic and severe UTIs.

Results: Here, we show that bladder infections induce expression of *Ezh2* in bladder urothelial cells. *Ezh2* is the methyltransferase of polycomb repressor complex 2 (PRC2), a potent epigenetic regulator. Urothelium-specific inactivation of PRC2 results in a reduced urine bacterial burden, muted inflammatory response, and decreased activity of the *NF-kB* signaling pathway. PRC2 inactivation also

enhances urothelial damage repair by attenuating basal cell hyperplasia and increasing urothelial differentiation. Treatment with Ezh2-specific small molecule inhibitors improves outcomes in mice with chronic and severe bladder infections.

Conclusions: These findings collectively suggest that the PRC2-dependent epigenetic reprograming controls the amplitude of inflammation and corresponding severity of UTIs and, further suggest that Ezh2 inhibitors may be a viable non-antibiotic strategy to manage chronic and severe UTIs.

4

Androgen Production, Uptakes and Conversion (APUC) genes converge as a signaling pathway and are target genes in metastatic castration resistant prostate cancer.

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Abstract

Background.

The androgen receptor (AR) signaling pathway regulates progression of prostate cancer (PC). Metastatic castration resistant prostate cancers (mCRPC) patients generally receive AR targeted therapies (ART) or androgen-deprivation therapies (ADT) with initial response, however resistance is inevitably observed. Prior studies have shown activity and upregulation of APUC genes based on genomic analyses of patient germlines. Some APUC genes, such as the conversion gene, *HSD3B1*, predict response to 2nd generation androgen targeted therapies in the recent CHAARTED trial. The overall role of APUC genes, each with unique actionable enzymatic activity, in mCRPC progression and patient outcomes remains unclear.

Methods.

In this study, we interrogated genomic and clinical features of APUC genes based on whole exome sequencing (WES) and whole transcriptome sequencing (WTS) data from mCRPC. We focused on 12 “tissue-specific” APUC (tsAPUC) genes that were generally not detected in normal prostate tissue. We utilized current informatics and behavior profiling algorithms to study associated genes and to identify clinical, genomic, and functional characteristics of tsAPUC genes.

Results.

The aggregate of tsAPUC genes were recurrently amplified or overexpressed (gains) across 22 prostate cancer studies. In mCRPC, tsAPUC gene gains were observed in up to 40% of patients. Of the 12 tsAPUC genes, mCRPC patients co-upregulated up to 8 tsAPUC genes in one tumor. Gains in tsAPUC genes also predicted a patient population with reduced sensitivity to AR signaling inhibitors. In orthogonal *in vitro* experiments, we found overexpression of tsAPUC genes yielded increase proliferation when PC cell lines were treated with ADT and the ART, enzalutamide. To elucidate function of tsAPUC genes, we scored AR or neuroendocrine PC (NEPC) activities using gene signatures but found no association with these pathways and tsAPUC genes. We next deployed an integrating WTS profiling algorithm developed to study gene-gene interactions of tsAPUC genes with each of the 20K genes detected in mCRPC. All 12 tsAPUC exhibited highly convergent signaling activity, with notable increases in oncogenic KRAS signaling.

Conclusions.

Our observations demonstrate that genomic detection of the aggregate of tsAPUC genes identifies one mechanism of ART and ADT resistance in subset of mCRPC tumors. Our findings in conjunction with prior studies demonstrating survival differences amongst patients with tsAPUC perturbations should guide future pre-clinical studies in which actionable precision therapeutics can be developed upon stratifying

mCRPC patients based on gains in these APUC genes.

5

MOLECULAR CHARACTERIZATION TO DELINEATE THE CLONAL EVOLUTION OF PRIMARY PROSTATE CANCER WITH SYNCHRONOUS LYMPH NODE

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Abstract

Background: Primary prostate cancers (PCa) harbor multiple spatially distinct tumors with significant inter- and intra-tumoral molecular heterogeneity. This genomic diversity gives rise to many competing subclones that may drive the biological trajectory of localized PCa. Previous large scale sequencing efforts have focused on the evolutionary process of metastatic PCa, revealing a potential clonal progression to castration resistance. However, the clonal origin of lymph node (LN) metastases in primary disease is still unknown. Here, we performed multiregional targeted DNA/RNA next generation sequencing (NGS) of primary PCa with synchronous LN metastases to better define the cancer foci or region capable of metastasis.

Methods: Patients who underwent radical prostatectomy and LN dissection that revealed node positive disease were identified for this IRB-approved study. All pathology slides were re-reviewed by a dedicated genitourinary pathologist. Punch biopsies were performed from pre-identified multiple regions of cancer on formalin fixed paraffin embedded (FFPE) prostate and LN specimens from which DNA/RNA samples were co-isolated. High depth, targeted, multiplexed, polymerase chain reaction (PCR)-based DNA NGS was performed to characterize the genomic profile of each tumor region using two targeted DNA NGS panels: a custom PCa NGS panel (135 genes, 3,127 amplicons) and the comprehensive cancer panel (CCP; 409 genes and 15,992 amplicons). Targeted RNA NGS sequencing was performed to evaluate gene fusion status of each sample. We determined and compared somatic DNA mutations, copy number alterations (CNA), and gene fusion status between primary and LN disease. Phylogenetic analysis was performed to determine the likely clonal source of LN metastasis.

Results: We analyzed 88 primary tumor (1°) and 23 LN metastases samples from 14 patients. After quality control, 11 patients (69 and 18 primary and LN tumor samples, respectively) had sufficient quality data for analyses. Seven patients had evidence of extraprostatic extension (EPE), with phylogenetic analysis supporting this as the source of the LN metastasis in 4 cases. In patient #1, while all 1° regions showed concordant *TP53* and *TPR* non-synonymous mutations and broad CNAs with two LN metastasis foci, only two of the 1° regions showed concordant high-level CNAs with both LN metastasis foci. In two patients with pT2N1 disease, sub-clonal seeding and clonal evolution was observed with metastases arising from a GG5 area. Seven patients had cribriform pattern in both LN and dominant 1° foci. One patient showed *CDK12* mutation within the 1° and LN foci. *FOXA1* was mutated in two

patients within 1° and LN foci.

Conclusions: Using targeted genomic and transcriptomic NGS to assess primary PCa and synchronous LN metastases, we demonstrate that LN metastases is idiosyncratic, likely related to a combination of histopathologic and genomic factors, including presence of high-grade disease, EPE, cell morphology such as cribriform pattern, and shared driver mutations such as CDK12 and FOXA1. These findings highlight the need to develop robust prognostic biomarkers for identification of novel therapeutic targets for LN metastasis.

6

Defining the roles of genetic factors in the cellular reprogramming of androgen receptor-active prostate cancer to neuroendocrine prostate cancer

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Abstract

Background: Neuroendocrine (NE) prostate cancer occurs in 10-15% of advanced prostate cancers and arises primarily through NE transdifferentiation (NEtD) as a mechanism of resistance to androgen receptor (AR) signaling inhibitors. NEtD of prostate cancer involves cellular reprogramming from an AR^+/NE^- (ARPC) to an AR^-/NE^+ (NEPC) state. While multiple genetic events have been associated with this process, tractable models to define their functional effects on the AR and NE programs have been lacking.

Methods: Cell lines were subjected to a cell reprogramming assay in which they were transduced with lentiviruses expressing combinations of dominant-negative TP53 R175H, short hairpin RNA targeting Rb1 (shRB1), N-Myc, Bcl-2, ASCL1, SRRM4, NR0B2, KRAS G12V or an empty vector at a multiplicity-of-infection of 4 for each factor. 72 hours after transduction, cells were replated in neural stem cell media absent of androgens. Media was changed every 72-96 hours. Cells were collected 14 days after transduction for phenotypic analysis by immunoblotting, immunocytochemistry (IHC), or by RNA-seq. Cells were also engrafted in NSG mice to assess tumor initiation and phenotype based on morphology and IHC.

Results: Introduction of the lentiviral pool into the C4-2B and LNCaP cell lines in the cell reprogramming assay led to a loss of AR protein markers and gain of NE protein markers which was also confirmed using validated AR and NE transcriptional signatures. Leave-one-out analyses nominated ASCL1 as the single factor in the pool capable of silencing the AR program and inducing the NE program. Factor reconstitution studies revealed variable effects of the genetic insults on cell fitness with ASCL1 causing a significant detriment. Partial least squares regression analysis of transcriptome data also highlighted the relative importance of ASCL1 compared to SRRM4 on the phenotypic conversion from ARPC to NEPC. NEUROD1 could replace ASCL1 in driving NEtD but other putative drivers could not. Additional cell line models such as MDA PCa 2b and PC-9 (EGFR-mutant non-small cell lung cancer) were also amenable to NEtD using this approach.

Conclusions: We established an *in vitro* methodology to rigorously investigate the functional contributions of genetic perturbations to the cellular reprogramming of ARPC to NEPC. Our findings suggest that the neural transcription factors ASCL1 and NEUROD1 act as master lineage factors by coordinately modulating the AR and NE programs during NEtD. We also find that optimization of cell fitness is an important factor associated with NEtD of prostate cancer.

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ERG Participates in Two Distinct Interactions with Co-Activator EWS

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Abstract

Background: Prostate cancer is responsible for the second most cancer-related deaths in American men. A hallmark of this disease is the gene fusion between the promoter of an androgen-driven gene and an ETS transcription factor. The most common fusion, accounting for 50% of cases, is *TMPRSS2-ERG*, which results in expression of ERG and subsequently increased migration and tumor growth. However, ERG function is dependent on an interaction with the co-activator, EWS. An understanding of this interaction can reveal new therapeutic targets to inhibit ERG in prostate cancer.

Methods: Affinity pulldowns, direct interaction assays, and co-IPs were used to determine protein-protein interactions. Affinity pulldowns rely on a purified His-tagged protein bound to His-beads to pull down protein from cell lysates. Direct interaction assays utilize a purified His-tagged protein and a GST-tagged purified protein. IP-mass spectrometry was performed on HA-tagged ctEWS in duplicate alongside ctEWS-negative controls. Hits were cross referenced with previous ERG IP-mass spectrometry data to determine possible interacting partners. Knockdown cell lines are being generated with lentiviral transduction of an shRNA.

Results: ERG directly interacts with the N-terminus of EWS, and this interaction is dependent on ERG's proline 436. However, ERG fails to interact with YS37 EWS, in which its ability to phase separate is abolished. An interaction between ERG and the C-terminus of EWS (ctEWS) occurs in cell extracts but not with purified protein, indicating this interaction is indirect. This ERG-ctEWS interaction is independent of ERG's proline 436, but may be dependent on RNA. To determine additional factors that facilitate this indirect interaction, IP-mass spectrometry of ctEWS was performed. One hit has been confirmed to interact with ERG and ctEWS. Ongoing experiments aim to determine the nature of a multisubunit complex that includes ERG and EWS, and test the importance of this complex in prostate tumorigenesis.

Conclusions: Taken together, these data have led to the hypothesis that ERG is part of a higher order, possibly phase-separated, structure that is required for activation of ERG transcriptional targets. Continued work to define this complex can provide new therapeutic targets or approaches to targeting ERG in prostate cancer.

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CREB5 reprograms nuclear interactions to promote resistance to androgen receptor targeting therapies

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Abstract

Background.

Metastatic castration resistant prostate cancers (mCRPC) are treated with therapies that antagonize the androgen receptor (AR). Almost all patients develop resistance to AR-targeted therapies (ART). Our previous work identified CREB5 as an amplified and overexpressed target gene in human mCRPC that promoted resistance to all clinically-approved ART including enzalutamide, apalutamide and darolutamide. The mechanisms by which CREB5 promotes progression of mCRPC or other cancers remains elusive.

Methods.

We interrogated how cells overexpressing CREB5 promoted extensive reprogramming of nuclear protein-protein interactions in response to the ART agent enzalutamide. We integrated ChIP-seq, rapid immunoprecipitation and mass spectroscopy of endogenous proteins (RIME), and genome-scale gene perturbation screens. To establish clinical relevance of the findings, we compared CREB5 binding patterns to AR and FOXA1 binding sites in mCRPC tissue. In addition, we utilized the patterns of CREB5 nuclear protein interactions and mCRPC patient transcriptomes to predict how CREB5 promotes reprogramming and ART resistance.

Results.

In promoting proliferation in ART, CREB5 exhibited a strong degree of interaction with known AR transcription machinery including FOXA1, HOXB13 and GRHL2 as well as novel prostate cancer regulators TBX3 and NFIC. The robust degree of CREB5-FOXA1 interaction was exhibited at both transcription regulatory elements and bound nuclear proteins. The factors TBX3 and NFIC interacted with CREB5 but required an intact CREB5 B/L-Zip domain. TBX3 and NFIC, two nuclear factors that are amplified or overexpressed in mCRPC, were lineage-specific prostate cancer vulnerabilities including ones that were ART resistant, and bound to FOXA1 transcription regulatory elements. Informatics modeling of the CREB5 activity through protein interactions and mCRPC transcription patterns indicated CREB5 is associated with pathways found in patients resistant to enzalutamide including TGFb and Wnt signaling and epithelial to mesenchymal transitions.

Conclusions.

Our study indicates that the dynamic binding properties of CREB5 mediates assembly of essential factors to AR and FOXA1 to promote ART resistant transcriptional processes. In addition, NFIC and TBX3 are lineage-specific regulators of prostate cancer. This knowledge informs of developmental targets to antagonize ART transcription factors.

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Spatial transcriptomics reveals a transition from a prostate luminal to club-like cell state in 5-alpha reductase inhibitor treated BPH patients

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Abstract

Background: Understanding how prostate luminal cells adapt and survive in a low androgen environment is central to the clinical treatment of benign and malignant disease. 5-alpha reductase inhibitors (5ARIs) block the conversion of testosterone to dihydrotestosterone (DHT) and are used to treat Benign Prostatic Hyperplasia (BPH). 5ARI treatment reduces tissue DHT levels and causes involution of prostate glands. However, the histologic response to 5ARIs is heterogeneous and lower urinary tract symptoms in treated BPH patients often persist or worsen, leading to surgical intervention. We examined transcriptional changes accompanying 5ARI treatment to determine the cellular mechanisms of treatment resistance.

Methods: Mass spectrometry was performed to measure 5ARI drug and hormone levels in prostate tissue from BPH patients. Prostates from untreated and 5ARI treated men were selected for Visium spatial transcriptomics. Immunostaining and in situ hybridization were used to confirm gene expression changes.

Results: We observed that patients with detectable levels of 5ARI drug display reduced DHT and increased testosterone. In 5ARI treated patients, regions of normal prostate glandular architecture can often be seen adjacent to regions with smaller atrophied glands, defined as histologically-resistant and -responsive, respectively. Spatial transcriptomics performed on histologically-resistant vs. -responsive regions revealed a urethral club-like molecular signature in small atrophied glands. Based on the degree of club-like gene expression, distinct prostate luminal cell states could be identified that represented a transition from a secretory luminal to a club-like state. Prostate luminal to club-like transition was accompanied by a decrease in gland size, reduced luminal infoldings, increased activation of the NF-κB signaling pathway and reduced androgen receptor mediated signaling. Atrophied regions displayed increased phosphorylation of the NF-κB subunit p65 and expression of the NF-κB target gene BCL-2.

Conclusions: We conclude that 5ARI treatment results in small atrophied glands lined by cells with a club-like gene signature. Our results indicate that prostate luminal cells gradually acquire this club-like gene signature in response to 5ARI treatment. Further research is needed to determine whether inhibition of androgen signaling or inflammation associated with 5ARI treatment is responsible for this adaptation.

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Landing and homing in the bone niche, a milestone associated with metabolic rewiring of prostate tumor cells triggered by bone precursors.

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Abstract

BACKGROUND: Communication between prostate tumor cells and the microenvironment is determinant for tumor progression. Alterations in energy metabolism emerged as one of the hallmarks of cancer. In this work, we aimed at identifying key metabolic genes that fuel prostate cancer (PCa) bone metastasis.

METHODS: We used a transwell co-culture system where PCa cells (PC3) and bone progenitor cells (MC3T3 or Raw264.7) shared the medium but were not in direct physical contact. We then assessed by RNA-seq analysis the transcriptomic profile of PC3 cells modulated by soluble factors released from bone precursors. Metabolic deregulated genes were identified using the *Reactome* database and specific metabolic pathways alterations were identified by GSEA. Further, we evaluated the clinical relevance of the selected metabolic signature performing an integrative bioinformatics analysis using publicly available datasets.

RESULTS: a significant dysregulation of metabolic associated genes in PC3 cells co-cultured with osteoblasts/osteoclasts precursors was detected; particularly a strong activation of the lipidic metabolism ($P<0.05$) was observed. Activation of metabolic regulatory pathways including PPAR and PI3K-Akt was detected in tumoral cells ($P<0.05$). We selected the altered metabolic genes of our *in vitro* model for an unsupervised clustering analysis. Using transcriptomic data from primary tumors and bone metastasis patients samples from the Nelson Lab, Fred Huchinson CRC dataset (GSE74685), we noted that those genes could precisely cluster PCa patients in two clearly defined groups: primary PCa ($n=14$) and bone metastasis ($n=20$). These findings highlight that the early transcriptional metabolic alterations triggered in our co-culture model were able to discriminate primary tumors from bone metastatic clinical samples. Further, we studied the independence of these metabolic genes through overall survival (OS) analyses using data from the SU2C-PCF Dream Team project. We found that *VDR*, *PPARA*, *SLC16A1* and *GPX1* (lipid associated genes) expression levels could be independent risk-predictors of death (HR: 4.96, 2.85, 3.93 and 3.67, respectively; $P<0.05$), and that the combined expression of these four genes correlates with a worst outcome in metastatic patients (HR: 2.65, $P<0.05$).

CONCLUSIONS: a novel lipid associated metabolic signature present at the onset of PCa metastasis appears to be critical for survival in PCa patients, pointing out to new attractive druggable targets for the disease.

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Does Treatment in Academic Centers Decrease Racial and Insurance-Based Disparities in Patients with Muscle-Invasive Bladder Cancer?

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Abstract

Background: Racial and insurance-based disparities in muscle-invasive bladder cancer (MIBC) have been well documented. However, little is known whether these disparities are affected by the type of treatment facility. We aimed to investigate the impact of treatment in academic facilities on disparities in patients with MIBC.

Methods: We queried the National Cancer Database (NCDB) for patients with muscle-invasive non-metastatic bladder cancer (cT2-T4/M0) between 2004 and 2015. Patients were stratified according to treatment facility type (community cancer program, comprehensive cancer program, academic research program and integrated network). Sociodemographic, clinical and treatment pattern variables were assessed among different groups. Kaplan-Meier survival curves and Cox proportional hazards model were fitted to evaluate overall survival (OS) as a function of race, insurance status and facility type after adjusting for confounding variables.

Results: A total of 73,780 patients with stage cT2-T4/M0 MIBC were identified. Of whom 27,227(37%) were treated at academic centers (AC) and 46,553(63%) were treated at non-academic centers (Non-AC). Overall, Medicare patients had lower overall survival (OS) when compared to the privately insured (HR1.73, CI1.69–1.7, p<0.001), and African Americans (AA) compared to whites (HR1.16, CI1.12–1.20, p<0.001). Notably, patients treated at Non-AC had lower survival compared to AC (HR1.25, CI1.23–1.28, p<0.001). When stratified by facility type, in Non-AC group, overall survival was significantly diminished for AA compared to whites (HR1.18, CI1.10–1.27, p<0.001). Similarly, uninsured and Medicaid patients had worse OS compared to privately insured patients (HR1.23, CI1.09–1.39, p=0.001 and 1.38, 1.25–1.52, p<0.001; respectively). Adjusted subgroup analysis of patients treated at AC revealed persistence of racial disparities. AA showed persistently worse survival compared to whites (HR1.22, CI1.13–1.32, p<0.001). Likewise, insurance-based disparities persisted with uninsured and Medicaid patients showing worse OS compared to private insurance (HR1.2, CI1.04–1.38, p=0.014 and 1.23, 1.11–1.36, p<0.001, respectively).

Conclusion: Treatment at academic research facilities did not mitigate racial and insurance-based disparities. AA race and uninsured/Medicaid status were consistently associated with worse overall survival even in AC.

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TLK1 phosphorylation of MK5 promotes prostate cancer cell migration and pathologic features of aggressiveness

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Abstract

TLK1 phosphorylation of MK5 promotes prostate cancer cell migration and pathologic features of aggressiveness

Background: Metastatic dissemination of prostate cancer (PCa) accounts for majority of PCa related deaths. However, the exact mechanism of PCa cell spread is still unknown. We uncovered a novel interaction between two unrelated promotility factors, tousled-like kinase 1 (TLK1) and MAPK-activated protein kinase 5 (MK5), which initiates a signaling cascade promoting metastasis. In PCa, TLK1-MK5 signaling might be crucial as androgen deprivation therapy (ADT) leads to increased expression of both TLK1 and MK5 in metastatic patients. Moreover, TCGA analysis also revealed higher expression of both TLK1 and MK5 in metastatic tumors. Therefore, we hypothesize that **TLK1-MK5 axis promotes PCa cell migration and invasion through actin reorganization as well as focal adhesion proteins modification and disruption of this interaction may help to keep the tumor localized.**

Methods and Results: We conducted scratch wound repair and chemotactic migration assays to determine if TLK1 and MK5 can regulate motility. Both genetic depletion and pharmacologic inhibition of TLK1 and MK5 resulted in reduced migration of both non-malignant MEF and PCa cells without affecting cellular proliferation rate. We confirmed TLK1 binding of MK5 by co-immunoprecipitation, His- and GST-pull down assays and established that TLK1 phosphorylates MK5 on three residues (S160, S354, S386) that results in the catalytic activation of MK5. While immunoblotting detected substantial amount of pMK5 S354 and TLK1 in all major PCa cell lines, anti-androgen treatment increases pMK5 S354 level in a dose-dependent manner and pharmacologic inhibition of TLK1 reduces pMK5 S354 level in LNCaP cells, which suggest TLK1 as an authentic pMK5 S354 kinase. IHC analysis of TRAMP mice and human TMA also revealed higher pMK5 S354 level in metastatic lesions compared to the low-risk ones. In addition, pMK5 S354 was found to colocalize with neuroendocrine marker chromogranin A, together of which may suggest pMK5 as a potential biomarker of PCa aggressiveness. The motility reduction of PCa cells upon MK5 inhibition is a consequence of impaired actin filamentation as we observed in LNCaP cells. Our immunoblotting analysis demonstrated that MK5 inhibition results in decreased FAK Y861, Paxillin Y118, and HSP27 S78/82 phosphorylation, which leads to reduced actin polymerization and motility.

Conclusion: Our data support that TLK1-MK5 axis is functionally involved in driving PCa cell motility and clinical aggressiveness, hence, disruption of this axis may inhibit the metastasis of PCa.

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Novel AKR1C3 inhibitor synergizes enzalutamide treatment in advanced prostate cancer

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Abstract

BACKGROUND: AKR1C3, also named HSD17B5, is one of the most important genes involved in androgen metabolism and elevated expression of this enzyme is associated with prostate cancer progression and drug resistance. Multiple inhibitors have been developed to target AKR1C3 activation in xenograft tumor models, including Indomethacin, GTx-560 and ASP9521. However, none of the inhibitors have shown promising results in clinical settings so far. Thus, there is a great unmet need to develop superior AKR1C3 inhibitor for further clinical testing. In this study, we will investigate a novel AKR1C3 inhibitor and test its function *in vitro* and *in vivo*.

METHODS: The AKR1C3 inhibitor was modified from celecoxib. Computational simulation of inhibitor and AKR1C3 active pocket interaction was performed by AutoDock vina and PyMOL. The steroid profile including androgens from cells and xenograft tumors were analyzed by Liquid Chromatography-Mass Spectrometry (LC-MS). Gene expression was determined by real-time PCR and western blot. The effects of the AKR1C3 inhibitor on AKR1C3 activity and enzalutamide sensitivity were characterized in enzalutamide-resistant prostate cancer models *in vitro* and *in vivo*.

RESULTS: The novel AKR1C3 inhibitor (PB) displayed superior potential to inhibit AKR1C3 activity and suppress enzalutamide resistant prostate cancer cell growth. Through AutoDock computational simulation, PB bound with the active pocket of AKR1C3 and the binding affinity was -9.4kcal/mol, superior to celecoxib and Indomethacin (-7.4kcal/mol). At the same dose, PB significantly suppressed enzalutamide resistant C4-2B MDVR cell growth compared to celecoxib, Indomethacin and enzalutamide. Notably, PB showed better enzalutamide combination effects than Indomethacin in cell growth, colony formation and AR/AR-V7 signaling inhibition. PB also improved enzalutamide treatment and decreased intratumoral testosterone level *in vivo* by using the relapsed VCaP xenograft tumor model.

CONCLUSIONS: We have developed a novel AKR1C3 inhibitor and showed synergistically effects in combination with enzalutamide treatment in vitro and in vivo.

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Prostate-specific overexpression of the colony-stimulating factor 1 (CSF1) inducesprostatic intraepithelial neoplasia dependent of epithelial-Gp130.

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Abstract

Background: Inflammatory signaling plays a critical role in initiation and progression of various types of cancer including the prostate cancer. Among immune cells, macrophages are often increased in human benign prostatic hyperplasia and prostate cancer, and they are the major sources of many inflammatory cytokines such as Il1α/β etc. in the mouse prostate. Macrophage colony-stimulating factor (M-CSF/CSF1) plays a critical role in macrophage recruitment and survival as well as polarization of the cancer-related anti-inflammatory M2 phenotype of macrophage. In this study, we hypothesize ectopic expression of Csf1 in mouse prostates should recruit macrophages and other types of immune cells, leading to an increased level of various cytokines and chemokines within the prostate.

Methods: To investigate how increased macrophages affects prostate homeostasis, we develop and analyze a *Pb-Csf1* mouse model with prostate-specific overexpression of *Csf1*. And to demonstrate the key mechanisms, RNA-seq analysis is performed, and *Pb-Csf1* mouse is bred with *Il1r* knockout mouse and *Gp130*-floxed mouse, respectively. Furthermore, to determine whether p53-mediated senescence restricts the initiation and progression of PIN lesions in the *Pb-Csf1* model, *Pb-Csf1;Pb-Cre;P53*$^{fl/fl}$ mice are analyzed. Lastly, to investigate whether inflammation affects the Ar binding at a genome scale in *Pb-Csf1* model, AR ChIP-seq analysis is performed.

Results: Csf1 overexpression promotes immune cell infiltration into the prostate, modulates the macrophage polarity in a lobe-specific manner, and induces senescence and low-grade prostatic intraepithelial neoplasia (PIN). The *Pb-Csf1* prostate luminal cells exhibit increased stem cell features and epithelial-to-mesenchymal transition. *P53* knockout alleviates senescence but fails to progress PIN lesions. Ablating epithelial *Gp130* but not *Il1r1* substantially blocks PIN lesion formation. The androgen receptor (AR) is downregulated in *Pb-Csf1* mice. ChIP-Seq analysis reveals altered AR binding in 2482 genes although there is no significant widespread change in global AR transcriptional activity.

Conclusions: Our study shows that macrophage infiltration causes PIN formation dependent of epithelial *Gp130* but is incapable of transforming prostate cells even in the absence of senescence.

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TBX2 drives neuroendocrine prostate cancer through exosome-mediated repression of miR-200c-3p

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Abstract

Background: Deciphering the mechanisms that drive the transdifferentiation to neuroendocrine prostate cancer (NEPC) is crucial towards identifying novel therapeutic strategies against this lethal and aggressive subtype of advanced prostate cancer (PCa). Further, the role played by exosomal microRNAs (miRs) in driving NEPC signaling, and in the propagation of the NEPC phenotype remains largely elusive.

Methods: The unbiased differential miR expression profile of human PCa cells genetically modulated for TBX2 expression identified miR-200c-3p. The experiments included gain and loss of function studies, chromatin immunoprecipitation, and immunohistochemical profiling in a xenograft mouse model of PCa metastasis.

Results: Our findings using *in vitro* and *in vivo* approaches have unraveled the TBX2/miR-200c-3p/SOX2/MYCN signaling axis in NEPC transdifferentiation. Mechanistically, we found that: *1)* TBX2 binds to the promoter and represses the expression of miR-200c-3p, a miR reported to be lost in CRPC, and *2)* the repression of miR-200c-3p results in the upregulation of its targets SOX2 and MYCN – two established drivers of the NEPC phenotype. In addition, rescue of mir-200c-3p in the context of TBX2 blockade has shown that miR-200c-3p is the critical inter-mediary effector in TBX2 regulation of SOX2 and MYCN. Further, our studies reveal that in addition to the intra-cellular mode, the TBX2/miR-200c-3p/SOX2/N-MYC signaling axis can promote NEPC transdifferentiation via exosome-mediated inter-cellular mechanism, an increasingly recognized and key mode of propagation of the NEPC phenotype.

Conclusions: Taken together, our study unearths the role of TBX2 in driving SOX2/MYCN mediated NEPC through exosome-mediated transfer of miR-200c-3p.

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Systematic Review of Recruitment Bias in U.S. Phase 2 and 3 Randomized Clinical Trials for Non-Neoplastic Genitourinary Disease of Adult Males: 2008-2019

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Abstract

Background: To promote health equity in the field of urology, clinical trials should strive for unbiased representation.

Methods: A systematic review of randomized phase 2 and 3 clinical trials recruiting U.S. male adults for treatment of non-neoplastic genitourinary disease and initiated between 2008 and 2019, was performed using four databases: Medline, Embase, Central, and the U.S. Clinical Trial registry. The Cochrane Handbook of Systematic Reviews of Interventions and PRISMA guidelines were used to identify trials. Meta-analyses were performed to obtain summary proportions and 95% confidence intervals (CI) of ethnicity and race groups. Summary proportions were compared with the corresponding Census proportions based on the 2010 U.S. Census.

Results: We identified 36 trials (total participants, n=6141) related to the treatment of non-neoplastic genitourinary disease in males (e.g. benign prostatic hyperplasia, erectile dysfunction, Peyronie's disease, secondary hypogonadism). Among them, 28% reported ethnicity (10 trials) and 44% reported race of the participants (16 trials). The summary proportions of American Indians (0.46%, 95% CI 0.19-0.83), Asians (0.91%, 95% CI 0.06-2.34), and multiracial participants (0.17%, 95% CI 0.02-0.49) were lower than Census proportions (1.10%, p=0.002; 5.01%, p<0.001; 1.56%, p<0.001). The summary proportion of Native Hawaiians and Pacific Islanders (NHPI) (0.72%, 95% CI 0.40-1.29) was higher than Census proportion (0.20%, p<0.001). The summary proportions of Hispanics (18.7%, 95% CI 10.1-29.3), blacks (15.9%, 95% CI 10.6-22.1), and whites (80.0%, 95% CI 73.1-86.1) were not significantly different from Census proportions (14.2%; 12.3%; 79.8%).

Conclusions: These results highlight disparities in clinical trial recruitment for treatment of non-neoplastic genitourinary diseases of males. More than half of the trials reported neither ethnicity nor race. The results suggest that American Indians, Asians, and multiracial participants were underrepresented; NHPI were overrepresented. Overall, trials for treatment of non-neoplastic genitourinary diseases of males may not reflect the demographics of the populations sought to be served.

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FGF7 peptide (FGF7p) mimetic mitigates urothelial injury from cyclophosphamide or radiation

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Abstract

Background: Hemorrhagic cystitis from chemical or radiation bladder injury can cause life-threatening bleeding and/or urothelial cancer. While fibroblast growth factor 7 (FGF7) blocks urothelial apoptosis induced by cyclophosphamide (CPP) or radiation, limitations include inability to use via direct bladder infusion and high cost due to the large size of the protein (18.8 kDa). We previously identified a small peptide that equaled or exceeded the mitigation effect of full length FGF2 on acute radiation syndrome. Benefits of developing a similar FGF7 biomimetic peptide include lower likelihood of inflammation, longer shelf life, higher purity, and lower cost than the full-length protein.

Methods: Based on the 3-dimensional structure of FGF2 peptide, a corresponding FGF7 peptide (FGF7p) consisting of 19 amino acids was identified and synthesized. FGF7p or vehicle was given subcutaneously (SQ) to female mice subjected to no injury, intraperitoneal (IP) CPP or external beam radiation over the bladder. One day after injury, bladders were harvested. Slides with paraffin embedded tissues underwent H&E staining, TUNEL and Immunofluorescence (IF) assays.

Results:In uninjured control mice, a 20 mg/kg threshold dose induced expression of phosphorylated (activated) FRS2a (pFRS2a) and pAKT in urothelium (consistent with cytoprotective effects of FGF7, albeit at 4x the dose than FGF7). Unexpectedly, activation of urothelial FRS2a and AKT was delayed by 24 hours vs. FGF7. FGF7p (20 mg/kg) or vehicle was given at 72 and 48 hours prior to CPP (150 mg/kg). One day after CPP, TUNEL staining revealed many more apoptotic and sloughing urothelial cells in vehicle-treated mice than in FGF7p-treated mice. IF for pAKT and its targets, pS6K and pBAD, revealed minimal staining in vehicle-treated mice, but strong urothelial staining for all markers in FGF7p-treated mice. Using the same dosing strategy, we subjected anesthetized mice to 10 Gy radiation over the bladder. One day after injury, TUNEL staining revealed many more apoptotic urothelial nuclei in control mice, than in FGF7p-treated mice.

Conclusions: FGF7p appears to block bladder urothelial apoptosis via AKT and its targets, similar to FGF7. FGF7p is 200x less expensive to make vs. FGF7 and is likely to work via direct bladder infusion (avoiding systemic side effects) due to its small size unlike FGF7. Our future studies will clarify long term benefits of FGF7p and assess effectiveness of direct bladder infusion.

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KGF protects against bladder injury from cyclophosphamide but does not impair its chemotherapeutic effects

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Abstract

Background:

Cyclophosphamide (CPP), used to treat lymphomas and other diseases, can cause severe acute bladder injury and can lead to bladder cancer. We previously showed that keratinocyte growth factor (KGF) pretreatment blocks CPP-induced urothelial injury and leads to higher fidelity repair, likely decreasing bladder cancer risks. A concern is that the doses of KGF required to protect the urothelium may stimulate lymphoma growth or block the lymphoma-killing effect of CPP. We sought to determine if doses of KGF required to protect bladder did not stimulate lymphoma growth or impair ability of CPP to kill lymphoma cells.

Methods:

We engrafted 6-week old NOD.Cg-Prkdc^{scid} /J mice with TMD8 cells (human lymphoma cell line). Once tumors were established, we administered KGF (5mg/kg) or vehicle (PBS) followed by CPP (150mg/kg) or vehicle (sham-injured) 24 hours later. We measured tumor growth daily by caliper and at 9 days, sacrificed mice and removed tumors and bladders. We performed H&E staining on all tissues and immunofluorescence for proliferation markers in tumors and urothelial and proliferation markers in bladders.

Results:

Sham-injured mice given KGF or PBS had similar increasing daily tumor sizes and tumor weights by 9 days. Tumors from these mice had similar numbers of proliferating cells, suggesting that KGF did not stimulate lymphoma growth. CPP-injured mice given KGF or PBS had similar rates decreases in tumor size, starting two days after injury, with no obvious tumor remnants in either group after 9 days. Bladders from CPP-injured mice given PBS had severe hemorrhage and urothelial denuding, while injured mice given KGF had much more intact urothelium and limited hemorrhage. Injured mice given KGF or PBS had similar loss of mature superficial cell markers; however, KGF-treated mice had much stronger uroplakin expression than PBS-treated mice, consistent with earlier recovery of barrier function in the former. Injured mice given KGF had a trend toward reduced urothelial proliferation vs PBS-treated mice, consistent with less injury in the former. Injured mice given KGF had limited ectopic lumenal keratin 14 expression vs PBS-treated mice, consistent with better repair in the former.

Conclusions:

KGF pretreatment, at doses sufficient enough to protect the bladder from CPP injury in mice, does not appear to drive lymphoma growth or interfere with the lymphoma killing ability of CPP. Thus, KGF is a good candidate drug that could ameliorate the side effects of CPP in the bladder but be safe to use when treating lymphomas.

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Androgen deprivation therapy (ADT)-induced pro-inflammatory cytokines linked to cognitive impairment in patients with prostate cancer

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Abstract

Androgen deprivation therapy (ADT)-induced pro-inflammatory cytokines linked to cognitive impairment in patients with prostate cancer

Background

Androgen deprivation therapy (ADT) is a commonly used clinical treatment for non-metastatic and metastatic hormone-sensitive prostate cancer. Long-term ADT treatment results in adverse side-effects in patients including depression, cognitive impairment, and dementia. Studies have reported increased levels of pro-inflammatory cytokines and inflammatory markers in older cancer patients, however, the relationship between inflammatory biomarkers and the severity of cognition in prostate cancer patients under ADT has not been investigated. We sought to identify peripheral biomarkers that could provide links between the mental changes and major pathological mechanisms responsible for the development of cognition in these patients.

Methods

Gene expression data (GSE69223) of 30 matched malignant and non-malignant prostate tissue samples from 15 prostate cancer patients receiving neoadjuvant antiandrogen therapy before prostatectomy, were compared in parallel with postmortem brain tissue samples of Parkinson's and Alzheimer patients as an additional neurological diagnosis. IPA analysis was performed in the context of known biological responses and regulatory networks. Fisher’s exact test for each network was converted to a score of −log10 (p-value). Further validation was performed in BT142-neural cells and M059K-glial cells by qRT-PCR with and without antiandrogen (enzalutamide) treatment.

Results

A total of 1952 DEGs were identified in postmortem brain tissue specimens, and 101 DEGs were identified in prostate cancer patients receiving ADT before surgery. IPA analysis revealed 33 commonly expressed genes with changes in cytokine-cytokine signaling network overlapped in both patient cohorts. Pathway analysis showed that the IL17 signaling pathway, regulation of cytokine production, and changes in T-cell subsets by IL-17A and IL-17F were overrepresented. Furthermore, lipopolysaccharide (LPS), TNF, and toll-like receptors were identified as upstream transcriptional regulators of these signaling pathways. Furthermore, gene expression of pro-inflammatory cytokines viz. LIFR, IL1RN, IL6, IL10, and LIF were increased in both neural and glial cells treated with enzalutamide, compared to non-enzalutamide treated cells.

Conclusion

Our results suggest that changes in cytokine signaling under the influence of ADT in prostate cancer patients may be linked with cognitive impairment presenting new areas for diagnostic and therapeutic development in combating brain deficits.

Acknowledgments

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Decreased Stromal Complement Component 7 accelerates prostate cancer progression

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Abstract

Decreased Stromal Complement Component 7 accelerates prostate cancer progression

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BACKGROUND:

The complement system consists of a cascade of plasma serine proteases and membrane proteins. Complement activation involves multiple tightly regulated steps. The final step of complement activation is the formation of the membrane-anchored terminal complement complex called the membrane attack complex (MAC). MAC destroys membrane integrity, causing calcium influx and cell lysis. C7 is a critical component in the formation of MAC and is the only complement component that is not secreted by liver but is expressed by specific cell lineages in individual tissues. We explored where C7 is expressed in the prostate, how its expression is regulated, and how its expression level affects prostate cancer growth.

METHODS:

We analyzed two independent human prostate cancer datasets and checked the correlation between C7 and the clinical patient outcomes. We used quantitative polymerase chain reaction to measure the level of *C7* different prostate cell lineages. The C7 promoter sequence was analyzed with the JASPAR and ALGGEN-PROMO database. C7 was overexpressed in murine primary stromal cells and cocultured with RM1 cells in the syngeneic mouse model and the effect of C7 overexpression on the growth of xenograft tumors was accessed *in vivo*.

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RESULTS:

We found that a higher expression level of C7 is positively correlated with a better clinical outcome in two independent human prostate cancer datasets and the level of C7 was significantly decreased in metastasis tumor compared with primary tumor. We found that C7 is specifically expressed in the prostate stromal cells. C7 expression is higher in human transition zone prostate stroma than in peripheral zone stroma. In mice, C7 expression is higher in the stromal cells in proximal prostatic ducts than in the stromal cells of distal ducts. C7 expression level was tightly regulated by stromal AR expression in both *in vivo* and *in vitro* models. AR activation/overexpression upregulates C7 expression in primarily cultured prostate stromal cells. Conversely, the expression of C7 is downregulated in stromal cells when AR was ablated in murine prostate stromal cells *in vivo*. Overexpression of C7 in stromal cell suppresses the growth of RM-1 cells in vivo.

CONCLUSIONS:

Decreased expression of C7 associates with high grade tumor and shorten patient survival time. Overexpressing C7 inhibits the prostate tumor growth. Stromal AR - C7 axis may be therapeutically exploited to improve the prostate cancer treatment.

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Multi-omics analyses pinpoint PPP1R12B and POSTN as potential prognostic biomarkers in prostate cancer

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Abstract

Background: Prostate cancer (PCa) is a progressive disease with only 30% chances of survival for those diagnosed with distant-stage disease. Some PCas are histo-pathologically grouped within the same Gleason Grade (GG) but can differ significantly in outcome. The aim of this study was to identify molecular biomarkers that can improve risk prediction of PCa with an eye toward those that could behave independently from GG and further recognize GGs that may be more likely to progress.

Methods: An in-depth proteomic analysis was performed on human prostate adenocarcinoma and benign prostate hyperplasia (BPH) tissues. Protein candidates enriched in PCa *vs* BPH were further evaluated *in-silico* using a custom-made webtool platform named *Gene Hunter*: a *Shiny*-based search tool for differential gene expression analysis and visualization across multiple datasets (7 PCa transcriptomic datasets, n = 875). Clinical significance of gene candidates was assessed by patient overall survival (OS). Multivariable analyses were performed in the presence of clinical-pathological parameters previously associated with increased death risk, to validate potential biological makers.

Results: Proteomic analysis rendered 89 proteins enriched in PCa. Of those, 9 showed high expression in PCa datasets and a strong association with a poor prognosis. In particular, *PPP1R12B* (HR = 1.5, p = 0.0078), *FBLN5* (HR = 1.33, p = 0.0305), *CRIP2* (HR = 1.72, p = 9,43 x 10^{-6}) and *POSTN* (HR = 1.47, p = 0.0004) displayed independence from Gleason Score (GS), age and *TMPRSS2-ERG* fusion when performing a multivariable analysis (HR = 1.5, p = 0.0084 ; HR = 1.34, p = 0.035 ; HR = 1.36, p = 0.016 and HR = 1.4, p = 0.0028, respectively). Further, when considering a multivariable Cox-proportional hazard model including age, GS, *TMPRSS2-ERG* and the genes selected, only *PPP1R12B* (HR = 1.5, p = 0,013) and *POSTN* (HR = 1.3, p = 0.025) could be independent predictors of death risk.

Conclusion: PPP1R12B and POSTN proteins were enriched in PCa human tissues and showed increased expression across multiple PCa transcriptomic datasets. Further, they appeared to be critical for OS in PCa patients and independent from known risk factors and predictors of PCa. Thus, these factors rise as potential prognostic markers.

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The expression of estrogen receptors is associated with steroid 5α reductase 2 in prostatic tissue

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Abstract

Background: Benign prostatic hyperplasia (BPH) is a highly prevalent health problem among elderly men. Steroid 5α reductase 2 (SRD5A2) is the predominant enzyme responsible for prostatic development and growth. However, therapeutic responses to 5α-reductase (5ARI) therapies vary widely. Our previous study demonstrated that there is an “androgenic to estrogenic switch” when SRD5A2 is absent in the prostate gland. Here we wished to identify if the expression of estrogen receptors (ER) is associated with SRD5A2 in the prostate.

Methods: 18 prostatic specimens collected from patients who underwent transurethral resection of the prostate were used to determine the histological expression of ERα and ERβ by immunohistochemistry (IHC). Mouse and human single-cell RNA sequencing (scRNA seq) data were analyzed to identify the subpopulation for ERα and ERβ expression. The expression of ERs was correlated to SRD5A2 expression with RNA sequencing (RNA seq) data from GTEx (Genotype-Tissue Expression, n=100) in normal prostate and TCGA (The Cancer Genome Atlas, n=496) databases for prostate cancer. Human prostatic stromal cell line (BHPrS1) and epithelial cell line (BPH1) were transfected with SRD5A2 plasmid or vector to identify the change of transcript and protein levels of SRD5A2, ERα, and ERβ.

Results: ERα and ERβ variably expressed in both the stroma and epithelial compartments of BPH surgical samples and had nuclear expression in human prostatic cell lines. ScRNA seq analysis of human and mouse prostate tissues showed that *SRD5A2* is predominantly expressed in fibroblasts. Meanwhile, ERα *is* expressed mainly in myofibroblasts of human prostate and in luminal cells of mouse anterior prostate. ERβ had minimal expression both in mouse and human prostate. RNA seq analysis demonstrated a significant association between *SRD5A2* and *ERα* in both benign and malignant human prostate tissue. Transcript correlation analysis showed that SRD5A2 was positively correlated with *ERα* (R=0.6797, p=0.0019) and *ERβ* (R=0.7030, p=0.0011). In addition, in BHPrS1 cells, transcript *ERα,* total ERα and phosphorylated ERα protein expressions were all upregulated in SRD5A2 overexpressed cells compared with vector control.

Conclusion: Our study demonstrates that the expression of ERα is associated with SRD5A2 expression. Targeting the estrogenic signaling pathway may serve as an effective treatment strategy in 5ARI-insensitive BPH patients.

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Prostatic luminal epithelial plasticity in absence of SRD5A2

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Abstract

Background: Steroid 5α reductase 2 (SRD5A2) is the predominant enzyme responsible for prostatic development and growth. 5α reductase inhibitors (5ARI) are the only class of benign prostate hyperplasia-related medications that reduce prostate size. However, patients respond variably to 5ARI therapies. We previously demonstrated that 30% of adult human prostatic tissues do not express the SRD5A2 gene and protein via epigenetic modifications. The goal of this study is to characterize the prostate cellular and transcriptomic changes when SRD5A2 is absent by single-cell RNA sequencing (scRNA seq) in a genetically modified animal model.

Material and Methods: Homozygous SRD5A2-/- mice and littermate control heterozygous SRD5A2+/- mice were generated. The intact prostate tissues were collected from 8-16 weeks old male mice and digested for single cells. After transcriptome profiling, cell clusters were generated based on differentially expressed gene profiles from scRNA seq data, a complete transcriptomic profile was obtained to identify cellular subsets and functional differentiation.

Results: Unbiased scRNA seq resulted in transcriptome data for clustering of 23,000 single cells, which were further annotated to 18 subpopulations demonstrating the heterogeneity within the prostate. The *SRD5A2* gene was identified to be exclusively expressed in fibroblasts and myofibroblasts. Deletion of *SRD5A2* induced a significant decrease of luminal epithelial (LE) cells (53.2% vs 31.8%, SRD5A2+/- vs SRD5A2-/-), while a significant elevation of stromal (11.3% vs 18.0%) and immune cells (3% vs 6.6%) were observed. Constructing the potential differentiation trajectories of epithelial clusters suggests a branched structure with two trajectories heading towards Lineage+ (LE1 and LE4) or ESR1+ (LE2) cells. Notably, deletion of SRD5A2 caused LE2, the subpopulation from anterior prostate and ESR1+ cells, to increase (32.3% vs 25.2%, SRD5A2-/- vs SRD5A2+/-), while Lineage+ LEs decreased (29.1% vs 37.6%), and the total LEs decreased (30.2% vs 52.3%), as well. More importantly, ligand/receptor interaction analysis identified that the ligand-receptor pairs of WNT5A/PTPRK, TGFB2/TGFbeta receptor 1 and (NRP1, NRP2, FLT1)/VEGFA are among the nine most significantly changed pairs.

Conclusion: Our data demonstrate that SRD5A2 deletion causes luminal epithelial plasticity with stromal cell interactions. LE2 subcluster, with the signature of estrogen target genes, may play an important role in absence of SRD5A2 and androgen deprivation. Understanding the mechanism(s) by which prostatic stromal cells regulate the development of epithelial luminal cells may pave the way to find new therapeutic targets for the management of a subset of BPH patients who lack *SRD5A2* expression.

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Nitric oxide controls secretion of NGF in bladder cells

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Abstract

Background: Urine of patients with OAB present elevated levels of nitric oxide (NO), decreased concentrations of NGF and lower ratio of NGF/proNGF. Increased NO has been associated to insulin resistance, found to be a potential causative factor of OAB related to metabolic syndrome. This project aims to examine how NO controls the secretion of NGF in bladder cells in culture.

Methods: Primary cultures of urothelial and smooth muscle cells (SMCs) were obtained from rat bladders and grown in vitro. Cell extracts were assessed by RT-qPCR, immunoblotting, ELISA (cyclic GMP, NGF, proNGF) and enzymatic kits (MMP-9). Nitric oxide was measured by the Griess reaction.

Results: Incubation in hyperglycemic medium (27 mM) increased secretion of nitric oxide. Subsequently, sodium nitroprusside acid (SNP) (300 µM) incubated for 24 hours decreased NGF secretion and the ratio NGF/proNGF, while proNGF levels were stable in both cell types. SNP incubated in the same conditions potently increased cyclic GMP levels in SMCs, but decreased it in urothelial cells. Stable permeable analogs of cGMP, 8-(4-Chlorophenylthio)-cGMP (1 mM) and N2,2'-O-Dibutyryl-cGMP (1mM) respectively decreased and increased NGF secretion in SMCs and urothelial cells, mimicking the changes observed with cGMP. On the other hand, these decreases did not involve MMP-9, the major protease degrading NGF, as SNP potently decreased NGF in cells after MMP-9 CrisprCas9 genomic removal. Finally, RT-qPCR revealed stable expression of NGF and MMP-9 mRNA in SMCs, and increased expression of $p75^{NTR}$, ligand of proNGF. In urothelial cells, NGF and MMP-9 mRNAs were increased, while $p75^{NTR}$ remained unchanged.

Conclusions : Nitric oxide controls NGF secretion by cells of the bladder through cyclic GMP, independently of MMP-9. Expression of gene involved in the synthesis and signaling of the couple NGF/proNGF are also regulated by nitric oxide to promote an inflammatory pattern.

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Control of NGF and MMP-9 expression by p75NTR antagonist THX-B in bladder cells in vitro

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Abstract

Background: Urine of patients with OAB is characterized by decreased levels of NGF and high activity of the metalloproteinase 9 (MMP-9). Rodents with type 1 diabetes have similar findings reversed by chronic treatment with the $p75^{NTR}$ antagonist THX-B. This project aims to detect the expression of NGF and MMP-9 by bladder cells and how they are influenced by THX-B.

Methods: Primary cultures of urothelial and smooth muscle cells were obtained from rat bladders. Cell extracts were analyzed by PCRs, immunoblotting, ELISA and enzymatic kits. Confocal immunohistochemistry was performed on cells grown on coverslips. Crispr-Cas9 was used to knockdown MMP-9.

Results: NGF and MMP-9 mRNA are expressed in urothelial and smooth muscle cells. Both cell types were major sources of proNGF and NGF, while MMP-9 enzymatic activity was much higher in urothelial cells. Immunohistochemistry localized both proteins in the cytoplasm of cells. Crispr-Cas9 successfully abolished the expression of MMP-9, leading to a potent decrease in MMP-9 activity and concomitant increase in the secretion of NGF without affecting proNGF levels. On the other hand, incubation with THX-B (5 µg/mL) had no effect on SMCs. However, THX-B increased secretion of NGF by urothelial cells, increasing the ratio NGF/proNGF, and potently decreased synthesis and secretion of MMP-9. Interestingly, α_2-macroglobulin, a protein that binds proNGF and leads to sustained activation of $p75^{NTR}$ receptor was also decreased by THX-B. When grown on insert separating an upper and lower chamber to mimick the in vivo conditions, urothelial cells produced more NGF toward the apical than basal chamber. In the presence of THX-B, NGF secretion was increased solely toward the basal medium. Finally, THX-B was not linked to the pathways usually associated to $p75^{NTR}$, including Erk, Jnk, P38 and phosphodiesterase-4D.

Conclusions : Bladder cells express and secrete NGF, proNGF and MMP-9. MMP-9 activity is directly linked to the amount of NGF secreted by cells. THX-B increases NGF secretion from urothelial cells at least in part by decreasing MMP-9 activity.

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Analysis of candidate genes influencing racial disparity in prostate cancer

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Abstract

Background. African-American (AA) men have higher incidence and mortality from prostate cancer compared to Caucasian-American (CA) men. Increasing evidence suggests that genetic and molecular alterations play important roles. We identified a 5 gene panel viz. p-Akt (Ser473), chemokine (C-X-C motif) receptor 4 (CXCR4), fatty acid synthase (FASN), interleukin-6 (IL-6) and matrix metallopeptidase 9 (MMP-9) highly expressed in prostate cancer and analyzed their expression in AA and CA cohorts.

Methods. IHC of p-Akt, CXCR4, FASN, IL-6 and MMP-9 were evaluated in RRP specimens (n=20) from each ethnic group exhibiting Gleason scores ranging from 6 through 9.

Results. Low to medium staining for p-Akt and weak focal staining for MMP-9 was observed in the cytoplasm of tumor cells (10-20%) in <20% specimens in both groups; whereas moderate to strong cytoplasmic expression of FASN was noted in >80% of tumor cells in both groups. Expression of IL-6 varied from weak to moderate intensity between (20-100% tumor cells) in 85% cases in CA- and 75% in AA- specimens. A marked difference in CXCR4 expression was noted between AA- and CA- cancer specimens. Weak CXCR4 staining was noted <5% of CA- specimens; whereas >85% of AA- prostate cancer exhibited weak to strong CXCR4 expression in between 10-100% of tumor cells localized in membrane, cytoplasm and nucleus in high-grade tumors.

Conclusions. CXCR4 expression appears to be distinctly different in prostate cancers from AA and CA men. Further studies are needed to assess whether this distinction correlates with prognosis between racial groups.

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A Preclinical Investigation of Growth Hormone Receptor Antagonists in Prostate Cancer

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Abstract

Background:

The growth hormone (GH)/insulin-like growth factor 1 axis has been studied in a variety of cancers, including prostate cancer (PCa) where some patients exhibit a dysregulation of the genes making up the axis. These findings and others justify the exploration of GH receptor (GHR) antagonists as potential therapies in cancer. Studies of an FDA-approved GHR antagonist, pegvisomant, in human PCa cell line xenografts in mice showed modest responses to pegvisomant. However, the incompatibility of the human GHR with mouse GH present in the host mice provides an explanation and opportunity to further explore the axis.

Methods:

Mouse PCa cells were maintained in media containing bovine pituitary extract (BPE). Bovine growth hormone (bGH) replaced BPE in viability studies. A scratch assay was used to determine cell mobility across an artificial wound. PTEN-CaP2 and -CaP8 cell pellets were surgically embedded under the kidney capsule of immunocompromised mice. Grafts grown *in vivo* for 3 weeks received 3x weekly doses of either vehicle or 100 mg/kg pegvisomant for 3 weeks. Collected grafts were sectioned and stained using immunohistochemistry for the proliferation marker Ki67 and the apoptosis marker Cleaved Caspase 3. Differential gene expression in grafts was assessed via RNAseq. Prostates of mice possessing the PCa-inducing transgene C3(1) SV40 T-antigen were histologically and immunohistochemically compared to prostate of mice possessing the T-antigen and a transgenic GH antagonist.

Results:

PTEN cells starved of BPE grew *in vitro* less than cells grown in BPE or bGH-containing media. PTEN cell growth due to bGH can be blocked by pegvisomant. Pegvisomant also slowed the migratory behavior of PTEN cells across an artificial wound. PTEN cell tumor growth *in vivo* was inhibited with pegvisomant treatment due to a decrease in proliferation and increase in apoptosis. Several genes are differentially expressed upon pegvisomant treatment. In another model of PCa utilizing T-antigen expressed in the mouse prostate, transgenic GH antagonism slows disease progression and proliferation.

Conclusions:

While human GH can stimulate mouse GHRs, mouse GH cannot do the same to human GHRs. The current studies use models to avoid this discrepancy. We've shown that GH antagonism using pegvisomant can slow GH-dependent PCa cell growth both *in vitro* and *in vivo* while differentially regulating several gene sets. Likewise, congenital expression of a GH antagonist slows disease progression in a genetic model of PCa.

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STAG2 loss alters chromatin accessibility and invasiveness in MIBC

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Abstract

Background

STAG2 (Stromal Antigen 2) functions in chromatid cohesion, DNA damage repair and genome organization, but its impact on chromatin and gene regulation in muscle invasive bladder cancer (MIBC) remains unknown. We found that in MIBC STAG2 is frequently mutated, and its loss is associated with better clinical outcomes. This study aims to determine how STAG2 affects chromatin structure and gene transcription to alter cell phenotype and therapy response in MIBC. We **hypothesize** that STAG2 loss in MIBC slows disease progression by reducing chromatin accessibility and transcription of genes promoting invasion, rendering tumor cells more sensitive to epigenetic drugs.

Methods

To determine effects of STAG2 loss, we used a combination of short hairpin RNA and CRISPR-Cas9 to knock down (KD) or knock out (KO) STAG2 in T24 MIBC cells, respectively. We identified altered chromatin regions using Assay for Transposase-Accessible Chromatin. We determined transcriptomic changes employing RNA-seq to identify STAG2-mediated biological pathways. Utilizing time lapse microscopy and invasion assays we quantitively determined cell movement over time. Finally, we used drug screening to determine if STAG2 loss alters response to an array of anticancer agents including epigenetic and DNA damaging drugs.

Results

STAG2 KD in T24 MIBC cells led to an overall reduction in chromatin accessibility. Since changes in chromatin accessibility can alter gene transcription levels, we investigated if this reduction was associated with transcriptional changes. RNA-seq revealed a downregulation of extracellular matrix (ECM) related gene transcripts. Reduced ECM gene expression corresponded with reduced displacement (78 vs 114 µm, $p<.05$), speed (0.30 vs 0.41 µm/min, $p<.05$) and invasion (137 vs 190 cells/field, $p<.001$) of T24 cells *in vitro*. Screening revealed that combining STAG2 KO with several epigenetic drugs, including histone deacetylase and methyltransferase inhibitors, reduced cell viability up to 19% vs the inhibitors alone.

Conclusions

STAG2 loss alters chromatin accessibility and downregulates ECM gene transcripts, leading to a less invasive phenotype. This may explain how loss of STAG2 can alter cell behavior leading to slower disease progression and positive outcomes in MIBC. The mechanism by which STAG2 alters chromatin, transcription, and invasiveness in MIBC will be dissected by integrating co-immunoprecipitation and chromatin immunoprecipitation results that identify STAG2 binding partners and sites. MIBC patients with known STAG2-null status may benefit from treatment with epigenetic drugs in addition to standard of care.

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Asparagine biosynthesis pathway as a targetable metabolic vulnerability in TP53-deficient castration-resistant prostate cancer

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Abstract

Background: The functional loss of TP53 is often observed in castration-resistant prostate cancers (CRPCs) showing poor clinical outcomes, making it a key target for PC therapy. Metabolic adaptation can contribute to PC progression and treatment resistance. To identify targetable metabolic vulnerabilities associated with TP53 alteration, we integrated gene expression and metabolomics profiles of CRPCs from patient samples, genetically engineered mice (GEM), PDX models and cell lines.

Methods: We performed RNA sequencing (RNA-seq) and liquid chromatography–mass spectrometry (LC–MS)-based metabolomics on GEM and PDX CRPC models with or without TP53 loss/mutation. Our findings were validated using genetic and pharmacologic approaches in relevant in vitro and in vivo models. We assessed the effect of L-asparaginase (ASNase) or/and glutaminase (GLS) inhibitor CB-839 in cell lines, organoids and PDX models. Tumors were characterized by histology and immunohistochemistry for relevant markers.

Results: Using RNA-Seq and IHC analyses on CRPC tumors from GEM and PDX models, we identified asparagine synthetase (ASNS), the enzyme catalyzing the synthesis of asparagine, as the most up-regulated gene in CRPCs with TP53 mutation/loss. In addition to prostate cancers, ASNS expression was significantly upregulated across different types of cancers carrying TP53 alterations. Metabolomics profiling revealed activation of ASNS-mediated asparagine biosynthetic pathway in both mouse and human CRPC with TP53 mutation/loss. Indeed, TP53 depletion by shRNA (KD) or inhibition with small molecule inhibitor enhanced ASNS-mediated asparagine synthesis in PC cell lines. Inhibition of de novo asparagine synthesis by ASNS KD combined with ASNase-mediated depletion of extracellular asparagine significantly impaired cell viability of cells and PDX organoids with TP53 loss/mutation. This effect was rescued by asparagine addition. Notably, pharmacological inhibition of intracellular asparagine biosynthesis using CB-839 and depletion of extracellular asparagine with ASNase significantly reduced aspartate and asparagine production and effectively impaired tumor growth.

Conclusions: These results indicate that enhanced ASNS activity and asparagine metabolism driven by TP53 alteration play a crucial role in facilitating CRPC progression. Thus, therapeutic approaches to inhibiting ASNS-mediated metabolism (e.g. CB-839 and L-asparaginase) may be a potential strategy to treat CRPC with TP53 alterations.

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Exploiting interdata relationships between HO-1 and its interactors in prostate cancer

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Abstract

Background: Prostate Cancer (PCa) cells display abnormal expression of proteins resulting in an augmented capacity to resist chemotherapy and colonize distant organs. We have previously shown the anti-tumoral role of Heme oxygenase 1 (*HMOX1*) in this disease. We propose that HO-1 (protein encoded by *HMOX1*) and its interactors reprogram PCa cells, favoring a less aggressive phenotype.

Methods: In this work, we undertook a mass spectrometry-based proteomics study to identify HO-1 molecular partners which might collaborate with its modulatory function in PCa. PCa cells were transiently transfected with GSTHO-1 or control and treated with the stressor agent H_2O_2. Immunoprecipitated protein complexes were subjected to LC-ESI MS/MS.

Results: Among the HO-1 interactors, we identified TRIM28, HNRNPA2B1, HSPB1, CBX1, CBX3, MATR3, NPM1, DDB1, HMGA1, 14-3-3ζ/δ and ZC3HAV1, with nuclear localization. To assess the clinical significance of these factors we performed correlation analysis between *HMOX1* and the selected candidates, using open-access PCa patient datasets. Results show a significant and positive Spearman correlation between *HMOX1* and 6 of those genes, and an increased relapse-free survival in PCa patients with high expression of those genes. Alternatively, *HMOX1* and *YWHAZ* (14-3-3ζ/δ encoding gene and a strong predictor of PCa aggressiveness) showed a significant negative correlation (Spearman=-.4; p˂.0001) and PCa patients with high expression of *YWHAZ*, showed a higher risk of relapse (HR=3.94; p<.001). We then validated our proteomics approach by co-immunoprecipitation analysis, ascertaining HO-1 and 14-3-3ζ/δ interaction. Immunofluorescence assays provided evidence that HO-1 and 14-3-3ζ/δ co-localize in the cell nuclei under oxidative stress conditions (Manders 1=.57).

Conclusion: In summary, we describe a novel protein interaction between HO-1 and 14-3-3ζ/δ in PCa and highlight the clinical correlation of these two proteins pointing out to a potential inhibitory role of HO-1 on 14-3-3ζ/δ, for future therapeutic avenues.

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Targeting WLS expression suppresses the progression of aggressive neuroendocrine prostate cancer

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Abstract

BACKGROUND: Neuroendocrine differentiation of prostate cancer cells is a phenomenon that can occur *de novo* or in association with the development of resistance to anti-androgen therapy. Neuroendocrine prostate cancer (NEPC) cells often do not respond to AR-targeted therapy and respond only transiently to chemotherapy. Therefore, there is an urgent need to understand the mechanism of NEPC development and develop therapeutic strategies that are independent of the androgen/AR axis. In this study we investigate the role of WLS in NEPC, and test the efficacy of NicS, a potent small molecule derivative of niclosamide, in the treatment of NEPC.

METHODS: To examine the expression of WLS in NEPC, we interrogated two GEO data based and measured WLS expression in prostate adenocarcinoma and small cell neuroendocrine prostate cancer. We also utilized western blotting and qPCR to compare protein and mRNA levels of WLS in prostate cancer cells with neuroendocrine features as well as the established neuroendocrine cell line NCI-H660. The effects of NicS on Wnt and NE signaling were determined by RNA-sequencing analysis. Cell proliferation was assessed by CCK8. In vivo studies were carried out by treating H660 tumor bearing mice with NicS via gavage administration.

RESULTS: WLS expression is significantly increased in NEPC compared with prostate adenocarcinoma in patient data bases. We also found that WLS is overexpressed in the cell model of NEPC NCI-H660 compared to castration resistant prostate cancer cell models. Treatment of NCI-H660 with NicS inhibits the expression of WLS, downregulates Wnt signaling, as well as markers of NEPC differentiation including chromogranin A (CHGA), neuron-specific enolase (NSE) and synaptophysin (SYP). NicS treatment also reduces cellular viability. To examine if NicS can inhibit tumor growth in vivo, we treated H660 tumor bearing mice with NicS and found that it significantly reduced tumor growth of H660 cells.

CONCLUSIONS: Our results show that WLS is overexpressed in NEPC patient data as well as in cell models of NEPC. We also show that NicS decreases viability and NEPC tumor growth, suggesting that NicS could be potentially developed to treat NEPC.

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Exploiting the tumor-suppressive activity of the androgen receptor by CDK4/6 inhibition in castration-resistant prostate cancer

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Abstract

Background: The androgen receptor (AR) plays a pivotal role in driving prostate cancer (PCa) development. However, when stimulated by high levels of androgens, AR can also function as a tumor suppressor in PCa cells. While the high-dose testosterone (high-T) treatment is currently being tested in clinical trials of castration-resistant prostate cancer (CRPC), there is still a pressing need to fully understand the underlying mechanism and thus develop treatment strategies to exploit this tumor-suppressive activity of AR.

Hypothesis: We hypothesize that Rb family proteins play a central role in mediating the transcriptional repression activity and the tumor suppressor function of AR and that treatments enhancing Rb activity can exploit the tumor-suppressive activity of high-T.

Methods: ChIP-seq and RNA-seq analyses were performed in Rb-proficient and Rb-depleted CRPC cells treated with high-T. The combination treatment of CDK4/6 inhibitor with high-T was assessed in a series of preclinical CRPC models.

Results: We found that Rb and an Rb-like protein, p130, are critical for maintaining the global chromatin binding and transcriptional repression program of AR and that Rb inactivation desensitizes CRPC to the high-dose testosterone treatment in vitro and in vivo. We also demonstrated that the efficacy of high-T treatment can be fully exploited by a CDK4/6 inhibitor, palbociclib.

Conclusion: Our study provides strong mechanistic and preclinical evidence on further developing clinical trials to combine high-T treatment with CDK4/6 inhibitors in treating CRPC.

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Identification of transcriptome features of perinephric adipose tissues adjacent *versus* distant from clear cell renal cell carcinoma

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Abstract

Identification of transcriptome features of perinephric adipose tissues adjacent *versus* distant from clear cell renal cell carcinoma

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Background

Perinephric fat invasion (PFI) is an important staging factor for pathologic T3a clear cell renal cell carcinoma (ccRCC) and serves as an independent indicator for worse prognosis of cancer-specific mortality. However, the molecular mechanisms underlying PFI are very limited. Therefore, we performed a comprehensive transcriptome profiling of perinephric adipose tissues (PATs) adjacent to *versus* distant from ccRCC of the same patients to understand molecular features of the PFI tumor microenvironment.

Methods

Nine pairs of PATs taken from area immediately adjacent to and from a position distant from the tumor in patients with pT3a ccRCC were freshly collected from radical or partial nephrectomy surgery at University of California, Irvine Medical Center with IRB approved informed consents. RNAs were extracted from snap-frozen tissues using RNA STAT-60 (Amsbio). Libraries were prepared using Takara SMARTer Stranded Total RNA-Seq Kit and sequenced on Illumina NovaSeq. Paired end reads were aligned to the current human genome (hg19 - GRCh38), and PCR duplicates were removed using Picard tools. Data quality was examined using fastQC, then reads were filtered to remove transcripts <10 counts per 5 samples (of 18 being compared). Principal component analysis and differently expressed genes were identified using R packages glmpca and DESeq2, respectively. Gene Set Enrichment Analysis (GSEA) GO and reactome analyses were performed using WebGestalt and the normalized enrichment score (NES) together with the false discovery rate (FDR) were calculated.

Results

The RNA seq analysis revealed 807 differently expressed genes (DEGs) (*Ps*<0.01) between paired PATs adjacent to and distant from pT3a ccRCC tumors. The majority of the DEGs were found to be hyper-expressed in adjacent PATs (95.4%, 770 out of 807) compared to distant PATs. Reactome GSEA enrichment analyses point to activation of the complement cascade (NES: 2.38, FDR: 0.0051), regulation of the complement (NES: 2.38, FDR: 0.0051), WNT signaling (NES: 2.06, FDR: 0.0350), Beta-catenin independent WNT signaling (NES: 1.99, FDR: 0.048), and semaphorin pathway (NES: 2.18, FDR: 0.0203) in adjacent PATs compared to distant PATs. The significantly up-regulated genes in adjacent PATs include family of *NLRP2, CFH, C3, C4B, ROBO2, ROBO4, SLIT2, DPYSL4, CRMP1, DAK3, DACT1, FZD1, FZD2, LEF1, Wnt3, Wnt5A*, etc.

Conclusions

Our results suggest that ccRCC tumors may interact with PATs to activate WNT, complement cascade and semaphorin pathways to promote inflammation, tumor invasion and suppressive immune microenvironment.

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Clinically obstructive benign prostate hyperplasia tissue contains elevated glucocorticoid levels, which can induce prostatic growth

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Abstract

Background: Other than androgens and the androgen receptor, disease-altering pathways in benign prostate hyperplasia (BPH) have not been well established. 5-alpha reductase inhibitors (5ARI) reduce synthesis of the active androgen metabolite dihydrotestosterone. Treatment failure is common, and progression despite medical management is not well understood. We evaluated the glucocorticoid receptor and glucocorticoids in the progression of BPH.

Methods: Tissue was collected from patients with clinically obstructing BPH requiring surgery (S-BPH) and from controls were incidentally harvested benign tissue collected from patients undergoing radical prostatectomy for localized prostate cancer treatment (I-BPH). Steroid levels were compared between groups. Based upon elevated tissue glucocorticoid levels in S-BPH, 3D organoid culture of BPH cell lines (BHPrE1, NHPrE1, RWPE-1 and PZ-HPV-7) were assessed for branching/budding morphology with and without dexamethasone (Dex), a synthetic glucocorticoid. Bulk RNA-sequencing was performed for S-BPH, I-BPH, and cell lines +/- dexamethasone. Upregulated genes which overlapped in S-BPH and branching/budding cell lines were identified.

Results: Higher testosterone levels and lower dihydrotestosterone levels in S-BPH on 5-ARIs confirmed the success of the 5ARI compared to controls. Corticosterone levels were higher in S-BPH patients on 5ARI compared to I-BPH. Branching/budding morphology was induced in all four cell lines in 3D culture with the addition of Dex compared to controls. On RNA-sequencing, there were 3375 genes upregulated in S-BPH (n=30) compared to I-BPH (n=14). There were 368 genes upregulated in the budding/branching cells treated with Dex. Overlapping genes were narrowed further by proximity to YAP1/TEAD/AR adjacent binding sites based on previous research, and a signature of 9 genes was identified and expanded based on biologic principles.

Conclusions: In clinically obstructive BPH refractory to treatment with 5ARI, glucocorticoidslevels in tissue are elevated. 3D organoid culture showed morphologic induction of branching/budding with synthetic glucocorticoids. An RNA signature identifying candidate genes was developed using bulk RNA-sequencing of patient and *in vitro* benign cell lines.

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Targeting Chromatin effector Pygo2 enhances antitumor immunity and immunotherapy in prostate cancer

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Abstract

Targeting Chromatin effector Pygo2 enhances antitumor immunity and immunotherapy in prostate cancer

Background: Immune checkpoint blockade (ICB) generates durable therapeutic responses across a variety of cancer types. Unfortunately, advanced protate cancer (PCa) shows overwhelming *de novo* resistance to ICB. PCa is generally considered immunologically “cold”. A cold tumor microenvironment (TME) phenotype can be traced back to the genetic alteration and oncogenic pathway intrinsic to cancer cells. To combat resistance to immunotherapy, therapeutic efforts using targeted agents to convert “cold” to “hot” TME are promising approaches, which are currently lacking in PCa. We recently identified Pygopus 2 (Pygo2) as the driver for the amplicon 1q21.3 in PCa, based on its function in promoting PCa progression and metastasis (PMID: 29769196). As a chromatin effector, Pygo2 has both Wnt-dependent and Wnt-independent activities. It remains unclear whether Pygo2’s role in PCa involves the regulation of the TME.

Methods: To determine Pygo2 function during autochthonous PCa development in an immune-intact context, we crossed Pygo2 conditional null allele to the aggressive PB-Cre/Pten/Smad4 (DKO) mice and generated PB-Cre/Pten/Smad4/Pygo2 (TKO) PCa mouse model. DKO and TKO cell lines were established from the models that can establish syngeneic tumors in C57BL/6 mice. Various immune-phenotyping techniques including CyTOF, flow cytometry and immunostaining were performed on spontaneous and syngeneic DKO and TKO tumors, as well as the RM9 syngeneic model with and without Pygo2 knockout. Unbiased transcriptomic and epigenetic profiling followed by gain and loss of function studies were conducted to decipher the underlying mechanisms.

Results: TKO mice significantly decelerated tumor progression and extended survival compared with DKO mice. TKO tumors had significantly increased infiltration of cytotoxic T lymphocytes (CTLs) and total T cells compared with DKO tumors. Depletion of CTLs with anti-CD8 antibody recovered the growth disadvantage of TKO tumors, and DKO cells were more resistant to *in vitro* CTL killing than TKO cells. Consistently, Pygo2 knockout in RM9 diminished tumor formation in C57BL/6 mice but not in nude mice. Surprisingly, this newly identified activity of Pygo2 is independent of Wnt signaling. Through genetic mouse models, we found that the function of Pygo2 in PCa required wild type (WT) p53 status, a finding corroborated by the clinical evidence that Pygo2 amplified PCa patients have worse survival when p53 is WT but not mutated. Mechanistically, through omics analysis and functional validation studies, we established that Pygo2 downregulated T cell infiltration and enhanced PCa resistance to T cell killing through a new p53/Sp1/Kit/Ido1 signaling axis. Importantly, Pygo2 knockout dramatically enhanced the ICB treatment efficacy and eradicated tumor formation in the RM9 model, whereas the myeloid-derived

suppressing cell (MDSC) inhibition alone or in combination with ICB synergistically suppressed TKO tumors growth. Finally, small molecule inhibitor of Pygo2, JBC117, inhibited Pygo2-positive but not Pygo2-deficient tumor growth through downregulating Kit expression, confirming our mechanistic studies and suggesting the potential clinical benefit of pharmacological Pygo2 inhibition.

Conclusions: We discovered a novel immune-related function of Pygo2 in promoting PCa progression and its underlying mechanism through the p53/Sp1/Kit/Ido1 signaling cascade. Our study illuminates a potential strategy to convert immunologically cold TME to hot TME in PCa by targeting Pygo2 and to enhance ICB treatment to benefit patients inflicted with advanced PCa.

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Identification of Serum Based Early Biomarkers for High-risk Prostate Cancer

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Abstract

Background: Prostate cancer is the most common non-cutaneous cancer and the second leading cause of cancer-associated deaths in men in the US and UK. One of the major clinical challenges in prostate cancer is distinguishing clinically significant from indolent disease. Here, we utilized a targeted human protein biomarker discovery approach to identify potential prognostic biomarkers for clinically significant prostate cancer.

Methods: Olink Proximity Extension Assay (PEA) technology was utilized in two independent serum cohorts to identify biomarkers for high-risk prostate cancer: 1. Cambridge prognostic cohort (n=20/group): 1) cancer-free group, 2) Cambridge Prognostic Group 1 (CPG1) disease with 97% 10 year survival, 3) CPG5 disease with 50% 10 year survival, and 4) men with metastatic disease at diagnosis were analyzed using high-multiplex immunoassays from Olink Proteomics (n=20 samples per group). 2. Stanford serum cohort (n=10/group): patient-matched pre-operative and post-operative serum samples from men with high-risk prostate cancer. Potential biomarker candidates were selected for further validation.

Results: We discovered that nine (PTN, MK, PVRL4, EPHA2, TFPI-2, hK11, SYND1, ANGPT2, and hK14) are significantly elevated in metastatic prostate cancer patients when compared to all other groups in Cambridge prognostic group. Likewise, six (CASP8, MSLN, FGFBP1, ICOSLG, TIE2, and S100A4) out of 174 proteins are significantly decreased after radical prostatectomy in Stanford serum cohort. PTN levels are significantly increased in CPG5 and metastatic prostate cancer serum samples in comparison to those from cancer-free and CPG1 groups. Moreover, high levels of PTN in localized prostate cancer tissues are predictive for biochemical recurrence ($p<0.05$). High tissue PTN level is an independent predictor of biochemical recurrence in patients with low pathological Gleason Grades ($p<0.05$). In addition, mRNA levels of CASP8 are elevated in prostate cancer when compared to normal prostate tissue across four clinical datasets. Further, CASP8 mRNA levels are higher in patients with recurrent prostate cancers in comparison of patients who did not recur. CASP8 is highly expressed in prostate cancer tissues when compared to normal prostate tissues by immunohistochemical analysis of prostate TMAs.

Conclusions: PTN and CASP8 may serve as novel biomarkers for high-risk PC. Serum PTN expression may be specific for the presence of poor prognosis local and metastatic disease. Our data justifies their further evaluation as potential early detection biomarkers of lethal disease.

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Disrupted Vitamin D Receptor Signaling in Prostate Cancer Progression

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Abstract

Background: Cell line and epidemiological data support relationships between Vitamin D receptor (VDR) signaling and prostate cancer (PCa) growth restraint. However, clinical exploitation has proved elusive. To understand how altered miRNA:mRNA interactions determine VDR sensitivity in PCa we focused on the miR-106b-93-25 cluster encoded in intron 13 of MCM7, which is an MYC target gene. We hypothesized that MYC regulation of MCM7 leads to increased miR-106b-93-25 cluster expression suppressing VDR's ability to regulate target genes that control cell proliferation.

Methods: We exploited miR-106b-93-25 antagomirs and examined regulation of multiple target genes (TGF-b, PTEN, $p21^{waf1/cip1}$) in nonmalignant prostate HPr1AR and malignant LNCaP cells by Q-RT-PCR and Western immunoblot (WB) alongside cell proliferation (clonogenic) assay and cell viability (ATP) assay to test how antagomirs plus the VDR ligand $1\alpha,25(OH)_2D_3$ (VD3) worked co-operatively on PCa cell growth. Genomically, ChIP-qPCR and RNA-seq were undertaken to examine VD3 regulation on c-MYC binding MCM7 locus, and RNA-Seq to reveal how VD3 and antagomirs converge to regulate target genes.

Results: Cells treated with antagomirs and VD3 displayed strong additive effects in both liquid culture and colony formation assays, suggesting that miRNA regulates VDR cell functions, and these findings were supported by co-operative induction of apoptosis. Q-RT-PCR and WB revealed that individual miRNA antagomirs displayed co-operative actions with VD3 treatment mRNA and protein levels of MCM7 and miRNA cluster and in response to VD3 (100 nM). In HPr1AR, TGFb mRNA levels were most up-regulated, and MYC mRNA levels were most down-regulated following VD3 plus miR-106b or miR-25 antagomirs (p-values <0.05). Similarly, MYC protein down-regulation occurred with VD3 plus miR-93 antagomirs. By contrast in LNCaP cells VD3 and antagomirs co-operatively down-regulated $p21^{waf1/cip1}$ protein, but not c-MYC. These data suggest that the targeting of miRNA and VD3 actions are altered in PCa. The data were supported by partial correlation analyses in PCa TCGA cohorts, revealing that miR-106b expression in high-grade PCa tumors significantly impacted the correlations between VDR and selected target genes. ChIP-qPCR demonstrated that VD3 significantly reduced c-MYC's binding ability on MCM7 in both cell lines, but most pronounced in HPr1AR. For example, VD3 treatment (100nM, 12h) repressed c-MYC binding to MCM7 50-fold in HPr1AR, and < 10-fold in LNCaP (p-values<0.05). The repression was reduced but detected at 72 h in both cell lines (p-values<0.05). We are currently analyzing RNA-seq data from HPr1AR and LNCaP cells treated with antagomirs and VD3 combinations to identify the extent of cooperation the potential footprint of MYC functions in the control of VD3 actions.

Conclusion: Our studies demonstrated that VDR regulates MCM7 as part of a feed-forward loop to control expression of the miRNA cluster and downstream targets, in turn mediating cancer growth progression. This regulation varies across different prostate cell models and stages.

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STROMAL ALTERATIONS OF THE TAG PATHWAY IS ASSOCIATED WITH RACIAL DISPARITIES IN PROSTATE CANCER

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Abstract

Background: Obesity is a risk factor for prostate cancer (PCa). Adult obesity is reported to be higher among the African American (AA) population. The incidence and mortality rate of PCa is higher in AA men compared to European American (EA) men. The mechanisms underlying this racial disparity in PCa is unknown. Our previous studies have shown that cancer associated fibroblasts (CAF) in PCa respond to environmental cues (including obesity) and undergo metabolic-reprogramming resulting in an increased lipid droplets (LD) accumulation. LD formation/degradation is regulated by members of triacyl-glycerol (TAG) pathway where Diacylglycerol O-acyltransferase 1 (DGAT1) plays a pivotal role. The significance of this altered lipid metabolism in PCa CAF, specifically in AA patients is unknown. We postulate that a lipid-rich environment in fibroblasts induces CAF characteristics rendering an aggressive PCa phenotype in AA men.

Methods: DGAT1 expression was assessed by immunohistochemical analysis of radical prostatectomy samples from AA and EA patients. mRNA and protein expression of TAG pathway molecules in primary normal prostate fibroblasts and CAF were accessed by RT-qPCR and western blot. Overexpression of DGAT1 in the benign prostate fibroblasts BHPrS1 ($BHPrS1^{-DGAT1}$) was generated. Phenotypic characteristics of LD accumulation (quantity and size) under basal and obesogenic environment was measured by a combination of flow cytometry and microscopic techniques. The biological effects of lipid-enriched fibroblasts on cancer cells were evaluated *in vitro* and *in vivo*.

Results: Differential expression of DGAT1 was observed in patient tissues and primary fibroblasts between AA and EA cohorts. $BHPrS1^{-DGAT1}$ cells show increased LD accumulation and proliferation compared to BHPrS1 control ($BHPrS1^{-EV}$) cells. DGAT1-engineered fibroblasts altered the expression of CAF markers. *In vitro* and *in vivo* response of prostate cancer cells were significantly affected by $BHPrS1^{-DGAT1}$ cells showing a more aggressive phenotype. Several potential secreted mediators of the pro-tumorigenic effects exerted by $BHPrS1^{-DGAT1}$ cells were identified.

Conclusions: Higher DGAT1 levels observed in prostate CAF induce LD accumulation and is associated with enhanced promotion of tumor progression. The differential expression observed in AA coupled to current evaluation of DGAT1 inhibitors in clinical trials suggest a potential translational utility in PCa racial disparities.

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Mitochondrial dysfunction contributes to fibrosis in aging-associated benign prostatic hyperplasia (BPH)

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Abstract

Mitochondrial dysfunction contributes to fibrosis in aging-associated benign prostatic hyperplasia (BPH)

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Background: Benign prostatic hyperplasia (BPH) is characterized by proliferation, smooth muscle changes, and fibrosis of the prostate. The single greatest risk factor for BPH is age, with 90% of men in their eighties impacted. Many men with BPH will develop lower urinary tract symptoms, which reduce their quality of life as disease severity progresses. Given the multifactorial nature of the disease, treatments have thus far been limited. While aging has been clearly linked to BPH, the molecular mechanisms involved with aging have yet to be fully elucidated. In this study, we specifically examine how mitochondrial dysfunction caused by aging may contribute to fibrosis in BPH. **Methods:** To evaluate how mitochondrial dysfunction may contribute to fibrosis, we used both *in vivo* and *in vitro* models. We examined the complex I protein, NDUFS3 and a mitophagy associated protein, PINK1, via immunohistochemistry in prostate tissue from young (2 months) and old (24 months) C57Bl/6J mice. Additionally, we quantified collagen using picrosirius red as an indicator of prostatic fibrosis. We also assessed loss of complex I function *in vitro* using complex I inhibitor, rotenone, on prostate stromal cells (BHPrS1) and determined collagen gene expression. Complex I rescue experiments using idebenone, a CoQ10 analog, were also tested. **Results:** IHC staining of mouse prostate tissue showed decreased levels of NDUSF3, suggesting a decrease in mitochondrial function, specifically associated with complex I of the electron transport chain. Furthermore, PINK1 was also decreased by IHC, suggesting parkin-dependent mitophagy is reduced. qPCR experiments on the rotenone treated BHPrS1 cells revealed increased gene expression for both *Col1a1* and *Col3a1*, suggesting complex I dysfunction can contribute to increased collagen production, and therefore fibrosis. Furthermore, treatment with idebenone was able to rescue this effect. **Discussion:** Collectively, these *in vivo* and *in vitro* data suggest that mitochondrial dysfunction originating from complex I contributes to the production of collagen, hence the promotion of fibrosis and BPH in men. These data provide new molecular mechanisms and therefore therapeutic targets for the treatment of BPH/LUTS. U54DK104310 (WAR) and K01AG059899 (TL)

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Targeting prostate cancer super enhancers by co-inhibiting LSD1 and BRD4

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Abstract

Background: Lysine-specific demethylase 1 (LSD1) is highly expressed in prostate cancer (PCa) and can promote PCa cells growth and survival. We and others have shown that LSD1 could function as a coactivator with androgen receptor (AR). More recently, we found that LSD1 directly demethylates FOXA1, a “pioneer” factor for AR, and controls the genome-wide occupancy of FOXA1 and concurrent chromatin “openness”. LSD1 inhibition disrupts AR cistrome and synergizes with AR antagonist to suppress PCa xenograft tumor growth. Multiple LSD1 inhibitors have been developed and tested in various types of carcinogenesis. However, the toxicity often thwarts the optimal clinical outputs. Further understanding of the oncogenic functions of LSD1 in PCa cells is required in order to develop combinational therapies to treat the late stage of disease, namely castration resistant PCa (CRPC), where AR targeted therapies often fail.

Methods: Systematic RNA-seq analysis were performed in various CRPC models after LSD1 activity was perturbed by different chemical inhibitors or genetic deletion. Bioinformatic analysis was performed to define the chromatin landscape where LSD1 resides. The synergistic value of combinational treatment was extensively assessed and RNA-seq analysis was used to identify the convergent downstream pathways.

Results: MYC pathway is unanimously repressed after perturbing LSD1 in various PCa models. *MYC* gene emerges to be the direct target of LSD1. Mechanistically, we found that LSD1 genome-wide binding, similar with BRD4 (bromodomain protein 4), is greatly enriched in super enhancers (SEs) identified in PCa cells. We hypothesize that this binding enrichment at SEs may involve phase separation, a typical mechanism of how oncogenic factors form transcription apparatus to drive robust gene expression. Confocal imaging revealed that LSD1 forms puncta in the nucleus, a characteristic behavior of biomolecule condensation. These results rationalize the potential synergism of combined targeting of LSD1 and BRD4. Besides causing a more effective reduction of AR transcriptional activity, the combinational treatment demonstrated great synergism in suppressing CRPC xenograft tumor growth. RNA-seq studies in these animal models showed that MYC pathway was further impaired by the co-treatment.

Conclusions: We demonstrate that LSD1 directly activates MYC pathway through maintaining SEs activation, and targeting LSD1 could synergize with BET inhibitors to suppress CRPC tumor growth.

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Title: Racial Differences in Prostate Stromal EphrinB ligands between African American and European American Populations and their role in Prostate Cancer tumorigenicity

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Abstract

Background: African American (AA) men are at a higher risk of developing and dying from prostate cancer (PCa) compared to European American (EA). Members of the Ephrin family (receptors and ligands) not only regulate a variety of normal biological processes, but are also implicated in cancer. Although there are prominent differences in the tumor microenvironment between AA and EA population, the role of Ephrin ligand (EFN) activation in stromal cells on PCa tumorigenicity is unknown. In this study, we evaluated whether increased EFNB ligands in carcinoma associated fibroblasts (CAF) exert an enhanced pro-tumorigenic microenvironment.

Methods: Expression (mRNA and protein) of Ephrin ligands were assessed in primary prostate fibroblasts of patient samples and compared between two racial (AA vs EA) cohorts. Altered ligand-expressing fibroblasts (benign and CAF) were engineered and their biological effects studied in vitro and in vivo.

Results: Higher expression of Ephrin B1, B2 and B3 (EFNB1, EFNB2 and EFNB3) were found in prostate fibroblasts from peripheral zones (PCa) of AA compared to EA. Overexpression of these ephrin ligands in the benign human prostate stromal cell line (BHPrS1) changed the levels of markers associated with myofibroblast activation (a-SMA, vimentin, TNC) and also increased in vitro cell proliferation of human prostate epithelial cells in a paracrine manner. BHPrS1EFNB1 and BHPrS1EFNB3 significantly increased the tumorigenicity of a premalignant prostate epithelial cell line BPH1 in vivo. Interestingly in the presence of BHPrS1EFNB2, we observed tumor suppressive effects. We also tested the metastatic properties of EA PCa cell lines LnCaP and PC3 and AA PCa cell line MDA Pca 2B in vivo in presence of stromal cells overexpressing the ephrin ligands. Ephrin-B ligands promoted a pro-tumorigenic secretome in BHPrS1 cells, which had various effects on neovascularization, collagen deposition, enhanced inflammation, cancer cell proliferation, and motility, all of which increased PCa tumorigenicity.

Conclusions: Chronic activation of ephrin ligands, especially EFNB1 and EFNB3 in the stroma of prostate cancer have direct implications in tumor progression. Because expression of ephrin ligands shows racial diversity, future research will determine the translational clinical utility in PCa for the AA population.

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The role of protein tyrosine phosphatase 1B in promoting neuroendocrine prostate cancer progression with a new application of targeted therapy

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Abstract

Background

Although androgen deprivation therapy (ADT) is effective for treating hormone naïve prostate cancer (PCa) metastasized to bone, the development of castration-resistant PCa is almost inevitable. Among the current CRPC, the onset of neuroendocrine PCa (NEPC), an aggressive subtype of PCa, becomes evident. Until now, there is very limited agents available for NEPC therapy. The elevated PTP1B gene expression is associated with poor recurrence-free survival of metastatic PCa patients. Also, PTP1B is known to regulate several oncogenic signaling pathways and involved in tumor development and progression. Thus, in this study, we have dissected the underlying mechanisms of PTP1B-elicited NEPC and explored PTP1B as a potential druggable target.

Methods

CRISPR technology was used to knockout (KO) PTP1B gene in Du145 and ARCaP-IIG5 cells and PTP1B cDNA vector was used for overexpression (OE) in LNCaP and LAPC4 cells. The gene expression is measured via qRT-PCR and Western blot. ChIP assay is used to map the binding of androgen receptor (AR) to PTP1B gene promoter. The determination of REST protein turnover in LNCaP cells was treated with either control or PTP1B OE, followed by addition of cycloheximide and western blotting at multiple time points to check PTP1B and AR expression. The SCID male mice carrying ARCaP IIG5 or NCI-H660 tumor are subjected to experimental therapy.

Results

We observed an inverse correlation between AR and PTP1B expression ranging AR-responsive to AR-independent PCa cell lines. Indeed, AR acts a transcriptional repressor on PTP1B expression. Data from PTP1B-KO or -OE cells demonstrated the PTP1B is a major role to up-regulate the master neural transcription factors (NETFs: BRN2, EZH2, FOXA2 and SOX2) and NE biomarkers (Syp and CgA) leading to the onset of NEPC that is resistant to ADT. Whereas REST protein exhibited a turnover half-life of 5.9 h in LNCaP cells treated with control, its degradation was significantly higher in PTP1B OE cells. Likewise, in Du145 cells, PTP1B depletion resulted in significant stabilization of REST protein. Collectively, PTP1B can modulate REST protein stability post-translationally, which is likely to trigger NEPC development. Also, PTP1B inhibitors (MDK3465 and DPM-1001) significantly reduce NE phenotypes and neurosphere formation in PC3, Du145 and ARCaP IIG5 cells.

Conclusions

PTP1B plays a critical role involved in NEPC progression and PTP1B small molecule inhibitors are expected to impact on the clinical application of NEPC therapy.

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MEIS proteins inhibit HOXB13-dependent prostate cancer metastasis and Androgen Receptor signaling

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Abstract

Background: Prostate cancer (PCa) is one of the most frequently diagnosed malignancies in men, and its incidence and mortality continue to be a significant clinical problem. Recent evidence from our lab identified MEIS1, an important HOX protein cofactor, as a potential tumor suppressor. Patients bearing MEIS1-positive prostate tumors were significantly less likely to have biochemical recurrence and metastasis compared to men bearing MEIS-negative tumors. Androgen receptor (AR), the major oncogene in PCa, has been shown to interact with HOXB13 to promote prostate cancer progression, but the function of MEIS proteins to antagonize AR/HOXB13 interactions are unknown. We hypothesize that MEIS1 proteins interact with HOXB13 to suppress cancer initiation and progression, and loss of MEIS1 expression in a portion of prostate tumors enables oncogenic AR/HOXB13 interactions.

Methods: We determined the impact of MEIS1 expression and dependency of HOXB13 on AR signaling using cell lines ectopically expressing MEIS1 and/or CRISPR-mediated HOXB13 deletion in both androgen-sensitive LAPC4 and castration-resistant CWR22Rv1 cells. Western blots, qPCR, Proximity Ligation Assay and co-IPs were performed to evaluate the relationship between expression of MEIS1, HOXB13 and AR. Hormonally-intact and castrated-male nude mice were used to test the *in vivo* capability of MEIS1-mediated tumor xenograft formation and rate of tumor growth in the presence and absence of AR ligand. RNA-Seq and informatics analyses was performed on tumor xenografts to determine the global impact of MEIS expression on AR gene targeting in PCa.

Results: We found that AR expression was significantly increased when MEIS1 was ectopically expressed compared to controls and HOXB13 knock-out lines. Re-expression of MEIS1 enhanced the binding between HOXB13-MEIS and AR-MEIS, and reduced AR-HOXB13 interaction. In castrated mice, re-expression of MEIS1 significantly decreased the tumor formation and tumor growth rate compared to hormonally intact nude mice. Conversely, xenografts of HOXB13-knockout tumors showed an increased rate of tumor growth and tumor formation compared to MEIS1-expressing cells and controls in both hormonally intact and castrated nude mice. Our preliminary RNASeq analysis demonstrate that when MEIS is present, AR pathway activation in response to androgen was associated with increased differentiation and growth suppression.

Conclusion: Our collective data supports our hypothesis that increased MEIS1 expression reduces the AR/HOXB13 interaction and increases the *in vivo* sensitivity to host castration; this suggests that MEIS-positive cells have decreased oncogenic AR signaling. Future RNA-Seq studies combined with AR ChIP-Seq data will enable us to identify the MEIS-AR targeted genes that are involved in AR pathway modulation that inhibit prostate cancer cell proliferation and suppress tumor progression. These data provide us a strong rationale to support the potential utility of MEIS proteins as predictive clinical biomarkers of metastatic progression.

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An association of programmed death ligand 1 with the recurrent therapy- and castration-resistant prostate cancer

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Abstract

Background Interferon-γ (IFNγ) is released by CD4+/CD8+ T cells and Natural killer (NK) cells infiltrated at the tumor microenvironment. Elevated serum levels of IFNγ are detected in metastatic prostate cancer (PCa) patients after receiving radiotherapy. IFNγ facilitates cancer epithelial-to-mesenchymal transition as well as induces programmed death ligand 1 (PD-L1), a predominant immunosuppression mediator that inhibits activated T cell proliferation. IFNγ-induced PD-L1 upregulation via JAK1/2-STAT1 pathway is shown to enhance tumor resistance to T cell-mediated cytolysis. Overall, JAK1/2-STAT1-PD-L1 represents a critical mechanism for tumors to evade immune eradication. Despite immunotherapy targeting PD-1/PD-L1 has shown therapeutic efficacy in many malignancies, the definitive role of PD-L1 in the disease progression of PCa remains unclear.

Methods RNA sequencing, Ingenuity pathway analysis and Gene Set Enrichment Analysis were applied to study clinical correlation of STAT1-driven genes in PCa. *In vitro* tumor sphere assay and i*n vivo* subcutaneous xenograft model were used to examine drug efficacies of small molecules targeting JAK1/2-STAT1 signaling.

Results Based on TCGA dataset, PD-L1 and IFN-inducible STAT1-driven genes are significantly upregulated in higher grade PCa. Parallel to clinical observation, PD-L1 is inversely correlated with Androgen receptor (AR) level among PCa lines examined. Inhibition of AR activities by enzalutamide or androgen-deprived condition enable PD-L1 upregulation in AR-positive adenocarcinoma lines. In contrast, restoration of AR in therapy- and castration-resistant PCa (t-CRPC) lines can suppress PD-L1 induction by IFNγ. Also, an elevation of PD-L1 is observed in androgen-repressed ARCaP subclones exhibiting neuroendocrine (NE) phenotypes, which is consistent with clinical observation that NEPC tumor specimens have higher level of PD-L1 compared to CRPC cohort. Meanwhile, elevation of PD-L1 and STAT1 can be observed in the tumor spheres of several PCa lines with acquired stemness properties. Indeed, targeting JAK-STAT1 signaling can diminish PD-L1 levels and suppress self-renewal capacities in PCa. Thus, we believe STAT1-mediated signaling pathway play a critical role in modulating PD-L1 expression that is associated with t-CRPC progression.

Conclusion: The role of JAK1/2-STAT1 signaling in the induction of PD-L1 could provide a new strategy in combination with immunotherapy to target immune evasive t-CRPC.

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Androgen receptor and DNA repair signaling in prostate cancer of African American men

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Abstract

Background: Even after adjusting for socioeconomic factors, African American (AA) men with low Gleason score prostate tumors have worse survival outcomes compared to European American (EA) men. This suggests that disparate outcomes may be caused by differences in tumor biology. The overall objective of the study is to investigate how AR-mediated DNA repair mechanisms alter biology of low Gleason score prostate tumors and cause worse clinical outcomes in AA men.

Methods: Tissue microarrays of prostate tumor and adjacent non-tumor tissues from AA (n=107) and EA (n=133) men treated at Roswell Park were utilized to detect AR protein expression. AR nuclear positivity in TMAs was quantitated using ImageJ. Prostate tumors from AA (n=43) and EA (n=270) men, in the cancer genome atlas (TCGA) legacy database, were categorized into *AR*-high and *AR*-low expressing tumors (z-score cut-off: 1.5). To investigate AR transcriptional activity *in vitro*, testosterone (T) treated AA-derived (MDAPCa 2a, MDAPCa 2b, referred to as 2a and 2b), and EA-derived (LAPC4) prostate cancer cell lines were used.

Results: AA men had higher percentage of AR-positive nuclei in adjacent non-tumor tissues than EA men (78.16 vs. 70.73%, $p<0.001$). AA men also had higher percentage of AR-positive nuclei in low Gleason score prostate tumors than EA men (77.61 vs. 73.10%, $p<0.05$). Compared to EA men, *AR* high prostate tumors from AA men from TCGA were enriched for genes involved in DNA damage repair pathways, including *PARP2* and *FEN1*. *In silico* analysis revealed that *PARP2* had at least 10 androgen response elements, the 'AGAACA' sequence, suggesting potential transcriptional regulation of DNA repair genes by AR. *In vitro*, T increased *PARP2* and *FEN1* expression at 24 hours in 2a and 2b cells and that of known AR targets, *KLK3* and *TMPRSS2*. Finally, prostate tumors from AA men in TCGA had higher *JMJD6* expression, a chromatin remodeler, than EA men (mean z-score: 9.19 vs. 8.91, $p<0.05$). In the Roswell Park cohort, *JMJD6* expression was higher in adjacent non-tumor tissues from AA and EA men compared to prostate tumors ($p<0.05$).

Conclusions: The results suggest that in AA men, AR-mediated increase in *PARP2* and *FEN1* transcription, can potentially occur through the recruitment of chromatin remodelers, such as JMJD6. This AR-mediated DNA repair pathway could be targeted with a combination of AR and DNA repair inhibitors. Future studies will determine the correlation between AR transcript and protein levels in the same tissue. Studies will also investigate if addition of PARP and AR inhibitors to hormone therapy, particularly for AA men whose prostate tumors recur after radiation, has a greater potential to reduce recurrence of lethal disease compared to hormone therapy alone.

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Somatic point mutation in SPOP prevents N-Myc driven cancer progression to NEPC

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Abstract

Background: Early-onset somatic point mutations in the E3 ubiquitin ligase SPOP (SPOPmut) drive prostate tumorigenesis through modulating stability of oncogenic proteins including androgen receptor (AR) and its cofactors, thereby resulting in reactivation of AR signaling. Accumulating evidence shows that SPOPmut prostate cancer (PCa) respond favorably to AR targeting therapies. Interestingly, SPOPmut is rarely present in neuroendocrine prostate cancer (NEPC), an AR-indifferent, clinically aggressive subtype of castration-resistant prostate cancer (CRPC) with poor survival rate, while prevalent across other PCa subclasses. A mechanistic understanding accounting for the resistance of SPOPmut PCa cells to progress to NEPC would provide novel insight into this progression and potentially benefit patient care.

Methods: We analyzed the frequency of SPOPmut in two large cohorts of well-characterized tumors from metastatic CRPC and NEPC patients. To assess the impact of SPOPmut on NEPC progression and maintenance, we introduced the $SPOP^{F133V}$ mutation into models of NEPC, including genetically engineered mouse (GEM) models with prostate-specific *Pten/Rb1* loss and human *MYCN* expression (PRN) and patient-derived NEPC organoids. We performed large-scale phenotypic and molecular analyses which included single-cell RNA sequencing (scRNA-seq).

Results: Based on analyses from the clinical cohorts, we found that *SPOP* point mutations were exclusively observed in CRPC-Adenocarcinoma, but rare in NEPC patients (SU2C 2019 and Beltran et al 2016). In model systems, our results show that $SPOP^{F133V}$ significantly impaired N-Myc overexpression/Rb1-loss driven growth of GEM-derived prostate organoids. Similarly, subcutaneously injection with $SPOP^{F133V}$-PRN organoids significantly impaired allograft tumor development in nude mice. Intriguingly, scRNA-seq identified that prostates from 6-week-old PRN mice had both luminal (*Ar, Cd24a, Krt8*) and NE (*Ascl1, Chga, Syp, Insm1)* cell populations, while the NE cell population was not present in age-matched $SPOP^{F133V}$-PRN mice. Furthermore, ectopic expression of $SPOP^{F133V}$ reduced mRNA and protein levels of NEPC markers (NKX2-1 and ASCL1) and restored adenocarcinoma markers (NKX3-1 and TMPRSS2) in patient-derived NEPC PM154 cells and organoids.

Conclusions: Our current findings demonstrate that SPOPmut hampers N-Myc overexpression/RB1 loss-driven allograft tumorigenesis, impairs NEPC maintenance, and restores an adenocarcinoma phenotype. Our findings will also lead to the development of critical predictive biomarkers, guiding which CRPC patients should receive additional AR targeting therapy, and which should transition to other forms of treatment and/or develop lethal metastatic PCa.

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Mechanism of PDEF action: Insights from the changes to proteome landscape

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Abstract

Background: Prostate cancer (PCa) is the most non-cutaneous cancer diagnosed in men, and results in approximately 30,000 deaths each year in the United States. Therapy resistant PCa, known as castration-resistant prostate cancer (CRPC), does not fully respond to common therapeutics. Most of the deaths from prostate cancer are due to metastatic CRPC indicating the importance of studying PCa to formulate more effective treatments. Previous studies have shown that the SAM pointed domain containing ETS transcription factor (SPDEF) protein plays a key role in the inhibition of prostate cancer metastasis; however, the mechanism for how SPDEF regulates metastasis is still poorly understood.

Methods: Mass spectrometry technology was used to screen for differentially regulated proteins in response to SPDEF expression in PC3 cells, a CRPC cell line that normally lacks SPDEF.

Results: A total of 1077 proteins were differentially regulated (fold change ≥ 1.5; 371 downregulated and 706 upregulated). Network analysis identified glucocorticoid receptor (GR, or GCR) also known as NR3C1 signaling pathway as one of the top pathways modulated by PDEF. Quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) or Western blot analyses were used to confirm the changes observed in proteomic analysis.

Conclusions: The data provide first insight into the effects of SPDEF at proteomic level in prostate cancer, and the first evidence for modulation of GCR signaling by PDEF. Further studies, underway, are aimed at understanding the mechanism by which SPDEF interacts with glucocorticoid receptor signaling in prostate cancer and may help in the design of novel therapies to tame CRPC.

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Investigating the Role of SOX2 in Cell Cycle Regulation and Enzalutamide Resistance in Prostate Cancer

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Abstract

Background: The central target for prostate cancer therapeutics is the androgen receptor (AR). As prostate cancer progresses, deregulation of AR will result in anti-androgen resistance. Predicting treatment outcome is crucial in creating an individualized treatment plan for increased patient survival. Currently, there are few functionally-defined biomarkers for treatment outcome in prostate cancer, and new clinical strategies are needed to understand, prevent, and overcome therapy resistance in prostate cancer.

SOX2 has been linked to increased disease severity in prostate cancer. Ectopic SOX2 expression in castration-sensitive LAPC-4 cells significantly increased castration-resistance *in vivo*. Further, SOX2-positive prostate tumors demonstrate a more rapid time to metastasis after biochemical recurrence. However, the mechanisms by which SOX2 promotes therapy resistance and metastasis are not fully understood.

Methods: SOX2 expression was knocked out in castration-resistant CWR-R1 cells using CRISPR/Cas9 gene targeting. The loss of SOX2 led to decreased cell growth, and we observed a further reduction in growth when cells were treated with Enzalutamide. Bioinformatic analyses of RNA-Seq datasets from Control vs. SOX2-knockout cells treated with Enzalutamide prioritized cell cycle pathways. This led us to examine E2F1, a cell cycle regulating protein and SOX2 transcriptional target.

Results: We observed increased E2F1 expression in the presence of SOX2 and Enzalutamide compared to controls.

Conclusions: Our data thus far supports a model whereby SOX2 promotes cell cycle progression despite AR-targeted therapies by promoting E2F1 expression. Continued investigations are focused on defining SOX2 control of E2F1, the function of E2F1 during AR-targeted therapies, and the potential utility of SOX2 tumor expression as a biomarker for poor therapeutic outcomes.

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Conflict of Interest Disclosure statement: No disclosures and no conflicts of interest

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MCT inhibition synergizes with tyrosine kinase or mTOR inhibitor treatment in renal cell carcinoma

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Abstract

Background: RCC has been recognized in recent years as a uniquely metabolic disease. All histologic types of RCC exhibit increase in aerobic glycolysis with disease progression. The TCGA-RCC study showed that high grade, high stage, and low survival are associated with a shift to aerobic glycolysis and a decrease in oxidative phosphorylation. Approaches targeting glucose metabolism in RCC such as GLUT1 inhibitors, hexokinase inhibitors, GAPDH inhibitor, and PKM2 inhibitor showed limited clinical benefit. Monocarboxylate transporters (MCTs) play a major role in the export/import of the glycolytic end-product lactate. Different MCT isoforms are differentially expressed in RCC tissues with an increase in the expression of MCT1 and MCT4 in clear-cell RCC and papillary RCC. Here we hypothesized that MCT inhibition may be an attractive therapeutic strategy against RCC cells.

Methods: We analyzed the expression levels of MCTs in RCC tissues using public datasets from Oncomine. We treated normal renal epithelial and renal cell carcinoma cells with varying concentrations of MCT inhibitors AR-C155858, AZD3965, or syrosingopine either singly or in combination with sunitinib or everolimus and assessed cell survival, cell viability, proliferation, clonogenic ability, survival in 3-D models, and glycolytic activity.

Results: We found that the suppression of MCT activity using the MCT antagonists AR-C155858, AZD3965, or syrosingopine not only diminished the proliferation, survival, and clonogenic ability of RCC cells, but also potentiated the response of RCC cells to treatment with sunitinib or everolimus. These findings imply that MCT inhibition may be an effective strategy to develop combinatorial therapies in renal cell carcinoma.

Conclusions: MCT inhibition may improve the anti-tumor effects of tyrosine kinase or mTOR inhibitors in renal cell carcinoma.

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Contraction and Desensitization to Histamine Require Cholesterol in Urinary Bladder Smooth Muscle

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Abstract

Background: Histamine is a vasoactive inflammatory signaling molecule that increases hypersensitivity to other inflammatory mediators and causes pleiotropic effects in various organs depending on the receptor subtype activated. Although histamine is implicated in many urinary bladder pathologies, these investigations focus heavily on nerves instead of on urinary bladder smooth muscle (UBSM) contractility. Histamine-induced contractions rapidly desensitize in the trachea, gut, and urinary bladder, but it is unknown if this is due to encocytosis, ß-arrestin mediated receptor inhibition, or histamine metabolism. We hypothesized that histamine induced UBSM contractions rapidly desensitize via caveolae mediated endocytosis.

Methods: Isometric contractility with urothelium-intact UBSM strips from male C57BL/6 mice was performed in the presence or absence of the following drugs/chemical agents: the cholesterol depleting agent methyl-ß-cyclodextrin (MßCD; 10 mM), cholesterol (5.1 mM), the dynamin inhibitor dynasore (15 µM - 100 µM), and the ß-arrestin/ß2-adaptin interaction inhibitor barbadin (100 µM). We further determined if the UBSM response to histamine was due to metabolism using the histamine-n-methyltransferase (HNMT) inhibitor SKF-91488, HNMT knockout mice, and a histamine buffer exchange bioassay.

Results: MßCD inhibited histamine induced UBSM contractions, which were recovered by reintroducing cholesterol. However, contractions to histamine remained transient. Dynamin inhibition had no effect on histamine-induced contractions, nor did pharmacological inhibition or genetic ablation of HNMT. Also, buffer from strips already contracted and desensitized to histamine still contracted naïve UBSM strips, implying histamine was not metabolized.

Conclusion: These data suggest that histamine-induced contractions in UBSM depend on lipid rafts and/or caveolae to drive both contraction and desensitization. This desensitization is also not due to dynamin-mediated endocytosis or histamine metabolism. The transient UBSM contractile response may indicate that histamine’s role pertaining to contractility is insignificant in the absence of a disease state or chronic exposure. However, future studies will examine potential genotypic changes that could occur to UBSM from prolong exposure to histamine.

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Calculation and Analysis of Bladder Wall Biomechanics During Ex Vivo Filling

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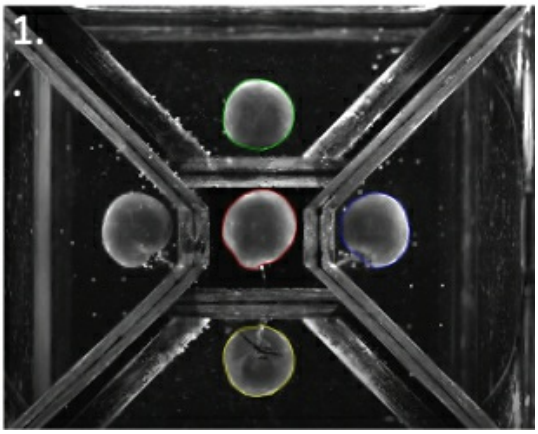
Abstract

Background: Optimal wall compliance is essential to urinary bladder storage and voiding functions. Changes in compliance occur in many lower urinary tract symptoms (LUTS), however compliance is poorly defined. Clinically, bladder compliance is the change in bladder volume per change in intravesical pressure, but without regard to wall structure or wall volume. Thus, it is unknown if the true mechanical properties of the bladder wall affect bladder function during filling. Thus, we developed a novel method to accurately calculate bladder wall compliance as a measure of the mechanical stress *vs* stretch instead of pressure *vs* volume.

Methods: Whole mouse bladders were mounted in our novel Pentaplanar Reflected Image Macroscopy (PRIM) System for simultaneous measurement of intravesical pressure (P*ves*), infused volume and video during *ex vivo* filling. The PRIM allows simultaneous visualization of the bladder in 5 planes (Fig. 1). Mechanical forces acting on the bladder wall were calculated by assuming the bladder to be an ellipsoid formed with planar areas equal to those measured from the PRIM recordings. Values of wall thickness and wall volume were calculated and used to measure wall stress as [(P*ves*)(radius)(stretch^2)]/(2)(Thickness). Stretch was calculated from the infused volume as a function of the relative change in diameter from the start of filling. Volume measurements were validated by comparing the calculated volume in full bladder to the sum of infused and residual volumes.

Conclusions: Accurate analysis of bladder compliance is an essential step towards understanding the underlying changes in wall tension and geometry during distention. This study demonstrates how wall tension, stress and stretch can be quantified and used to define true wall compliance. This method can be used to explore underlying changes in biomechanics of the bladder wall that accompany LUTS.

Results: The calculated volume measurements were comparable to the measured infused volumes. Wall stress only increased rapidly when intravesical pressure exceeded 15 mmHg, implying that wall compliance is maintained until larger infused volumes. At large volumes, bladder wall stress with respect to strain increases rapidly.



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IGF1R Inhibitors Decrease SOX2 and Re-sensitize Castration-Resistant Prostate Cancer Cells to Enzalutamide

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Abstract

Background

SOX2 has been identified to be involved in prostate cancer progression and 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 anti-androgen treatment, resulting in decreased proliferation *in vitro* and increased survival *in vivo*. Identifying a clinically-relevant approach to decrease SOX2 expression in CRPC could reduce enzalutamide resistance in patients, resulting in improved overall survival for men with CRPC.

Methods

Using CRISPR technology and a nano-luciferase (nLuc) reporter system, we created CWR-R1 cells harboring an nLuc-SOX2 fusion protein. Importantly, this reporter approach maintained control of SOX2 under its endogenous promoter and enhancers. A subsequent drug screen of ~400 compounds revealed "hits" that decreased SOX2 protein expression using nLuc bioluminescence as a proxy readout. These hits were normalized to total protein, selecting compounds that decrease SOX2 but were not overtly cytotoxic. Several drugs were further validated to decrease SOX2 by Western blot, and clinical implications for these drugs were assessed by co-treatments with enzalutamide.

Results

Of the 430 compounds screened, 39 compounds were identified to decrease SOX2 with minimal cytotoxicity. Follow-up studies showed that 10 of these compounds acted synergistically with enzalutamide to decrease total protein in combination. Interestingly, pathway analysis of these hits showed that 7/10 target the angiogenesis/tyrosine kinase pathways. Further validation of these drugs by Western blot suggest targeting the IGF1R and FGFR pathways decreased SOX2 most robustly. To confirm these initial findings, several other compounds targeting IGF1R and FGFR were tested; while all IGF1R-targeted compounds tested showed a reduction of SOX2 protein, the FGFR results were more variable. These additional compounds targeting IGF1R were also shown to act synergistically with enzalutamide to decrease proliferation *in vitro*.

Conclusions

Targeting SOX2 pharmacologically has revealed a potential upstream regulatory pathway through IGF1 receptor signaling. This approach provides insight into the upstream pathways that regulate SOX2 in CRPC. Additionally, SOX2 has been identified as a key factor in the development of CRPC and anti-androgen resistance – identifying compounds that target SOX2 in CRPC could potentially be integrated into clinical practice to improve survival of men with recurrent disease.

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Vitamin D receptor signaling is genomically distinct in African American prostate cancer

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Abstract

Background. TMPRSS2 and ERG gene fusions are frequent tumor-driving events in European American (EA) prostate cancer (PCa) but infrequent in African American (AA) PCa. We hypothesized that AA TMPRSS2:ERG fusion negative (FN) PCa is driven by distinct mechanisms centered on the vitamin D receptor (VDR).

Methods. Cells (EA: HPr1-AR, LNCaP, AA: RC43N, RC43T, RC77N, RC77T) were treated (1α,25$(OH)_2D_3$) and/or genetically-modified (BAZ1A). VDR binding (ChIP-Seq) and regulated mRNA and miRNA (RNA-Seq); VDR protein interactions (RIME); nucleosome positioning (ATAC-Seq); 1α,25$(OH)_2D_3$ phenotypic assays; Serum from high grade PIN (HGPIN) patients and two PCa cohorts.

Results. AA cells have highest VDR protein expression and most significantly 1α,25$(OH)_2D_3$ antiproliferative responsive. Cistrome-transcriptome analyses revealed that VDR is most potent in RC43T and RC43N than LNCaP and HPr1-AR. RC43N and RC43T contained ~ 3500 VDR binding sites enriched for a larger and distinct motif repertoire, including ZNFs, than EA peaks. Analyses of ~10,000 publicly available cistromes revealed VDR significantly overlapped with core circadian rhythm transcription factors (e.g. NONO) in AA models (ATAC-Seq is ongoing). RNA-Seq revealed significantly stronger VDR transcriptional responses enriched for circadian rhythm and inflammation in AA models, with VDR binding enriched in Active Enhancers and Polycomb regions. Global coregulator analyses identified significantly reduced BAZ1A/SMARCA5 in AA TMPRSS2:ERG FN PCa, in TCGA and a published cohort of ~1500 AA/EA tumors. We are dissecting differential VDR coregulator interactions using RIME. Restored BAZ1A expression in AA models significantly increased 1a,25$(OH)_2D_3$-regulated VDR targets.

Serum miRNA expression that predicted AA progression from HGPIN to PCa were enriched for 1a,25$(OH)_2D_3$-regulated VDR targets, as were AA tumors in a previously analyzed cohort of 1α,25$(OH)_2D_3$-treated PCa patients. Finally, in a cohort of AA/EA PCa, serum 25$(OH)D_3$ levels was most significantly associated with 1a,25$(OH)_2D_3$ regulated VDR targets in AA tumors.

Conclusion. Together, these data suggest VDR transcriptional control in the prostate is most potent and dynamic in AA men, and is primed to govern inflammatory and circadian pathways. Reduced BAZ1A/SMARCA5 expression and/or reduced environmental-regulated serum vitamin D_3 levels suppress these actions. Therefore, the VDR axis lies at the cross-roads of biopsychosocial processes that contributes to PCa health disparities.

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Cooperative actions of a novel ERβ ligand, OSU-ERB-12, and Enzalutamide in advanced prostate cancer

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Abstract

Background: Early successful therapies in prostate cancer (PCa) included the use of estrogens but were discontinued due to serious side effects. Given estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) appear to have opposing actions, it is unclear how estrogen actions control PCa growth. The OSU Drug Development Institute has developed a novel compound, OSU-ERB-12 (ERB12), that selectively targets ERβ, allowing for the possibility to study ERβ effects in androgen deprivation therapy (ADT) resistant or sensitive PCa.

Methods: A combination robotic high-throughput drug viability screen was undertaken in ADT-sensitive and resistant PCa models that combined multiple concentrations of ERB12 with 30 compounds including Enzalutamide (ENZA), and epigenomic regulators. The ERB12 + ENZA combination was examined in LNCaP, C42 and 22RV1 by RNA-Seq and ATAC-Seq. In 22RV1, liquid and clonogenic assays were undertaken for selected ERB12 combinations.

Results: The drug screen revealed that all cell lines tested were sensitive to high doses of ERB12 alone (1-10μM) whereas each cell line displayed unique responses to ERB12 drug interactions; 22RV1 cells responded with the greatest additive effects to combinations of ERB12 + ENZA and histone deacetylase inhibitors (FK-228) and DNA methyltransferase inhibitors (EGCG). Clonogenic assays revealed significant suppression of colony formation by ERB12 combined with ENZA, FK228, and EGCG. For the RNA-seq analysis, 22RV1 cells treated with ERB12 (100nM) + ENZA (1μM) were uniquely and significantly enriched for inflammatory genes and TNFa signaling and revealed a significant decrease in transcription of AR target genes including *KLK3*, *TMPRSS2*, *NKX3-1* as well as *MYC*. To investigate the Myc status at the protein level, we dosed 22RV1 cells with ERB12 alone or in combination with ENZA and collected lysates for immunoblot analysis, which revealed that only the combination of ERB12 + ENZA led to reduction in Myc protein versus either drug alone. Currently, we are undertaking ATAC-Seq in 22Rv1 to reveal the unique effects on chromatin state by the combination of ERB12 + ENZA.

Conclusion: The possibility of targeting ERβ in the context of PCa is now becoming a reality with development of the novel, highly selective ERβ agonist, ERB12, which has excellent PK/PD properties. Specifically, this ligand is a powerful tool to understand the specific role of ERβ has on transcriptomic and epigenetic regulation. Our initial data suggest that there is a promising cooperative action between ERB12 and the ADT drug ENZA. Overall, using ERB12 may prove to be a powerful tool to study estrogen anticancer functions in PCa.

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RARg bookmarking function determines AR genomics actions in prostate cancer progression and therapy responses

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Abstract

Background: Prostate cancer (PCa) progression is determined by altered androgen receptor (AR) genomic interactions. Previously, we established that miR-96 targets RARg and limits AR control of luminal differentiation. We hypothesize that RARg and the coactivator TACC1 shapes AR enhancer interactions that drive luminal differentiation and androgen deprivation therapy efficacy.

Methods: Cells (HPr1-AR, LNCaP and 22Rv1) were treated (miR-96 mimics/antagomirs, DHT, Enzalutamide (ENZA) or CD437 (RARg ligand)) and/or genetically-modified (TACC1 and RARg). We measured; miR-96 binding sites (IMPACT-Seq) and miR-96 regulated differentially expressed genes (DEGs - RNA-Seq) and proteins (DEPs - mass spectrometry); mRNA m6A (m6A-Seq); RARg protein interactions (RIME); enhancer access (ATAC-Seq and Cut and Run ChIP-Seq); miR-96 antagomir and ENZA phenotypic assays; RARg gene networks outcomes (coxph).

Results: IMPACT-seq identified miR-96 recognition elements (MREs) in HPr1-AR (~1950 at 24h) and LNCaP cells (~ 350) with ~ 150 shared. The epitranscriptome mark m6A differed significantly between cells. For example, m6A was enriched on TACC1 in HPr1-AR not LNCaP, and miR-96 regulated TACC1 mRNA and protein in LNCaP. miR-96 DEG/DEP were enriched for AR transcriptomes. RIME revealed that RARg significantly interacts miR-96 DEPs including coregulators, m6A regulators (METTL3) and QSER1 complex that protects against enhancer methylation (SPEN). In 22Rv1 cells, restoring RARg levels increased AR genomic binding 12-fold to > 9,500 and further by TACC1. These gained AR sites significantly overlapped with H3K27ac and at ChromHMM Poised Enhancers (logPV = 163) and Transcribed regions (logPV = 179), and enriched for GATA and Homeobox motifs. Parallel RNA-Seq experiments revealed that RARg/TACC1 significantly altered the frequency and fold change of DHT-regulated genes. MiR-96 antagomir/ENZA treatments drove a unique transcriptome that triggered cell cycle arrest and significantly associated with worse survival in the SU2C cohort.

Conclusions: Together these findings support the concept that m6A re-distribution directs miR-96 targeting to the RARg/TACC1 complex. In turn, RARg/TACC1 expression significantly shapes enhancer distribution for AR binding and augments ENZA-regulated genes and cell cycle arrest, supporting a bookmarking function that is lost in PCa leading to poor survival outcomes. Ongoing ATAC-Seq analyses is testing the repositioning of nucleosomes at AR enhancers.

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SPDEF expression is reduced in RCC7/T cells, a line of PCa cells from African American Origin: Potential role of DNA methylation.

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Abstract

Background: In African American (AA) men, prostate cancer is characterized by an early onset, higher aggressiveness, more extensive metastases, and increased mortality rates compared to those in Caucasian men. Our previous studies have reported SAM-pointed domain-containing Ets-like factor (SPDEF) as a potential factor to inhibit the metastasis in castration-resistant prostate cancer (CRPC) cells. In the present study, we investigated the role of SPDEF in RCC7/T cells, a line of Prostate Cancer (PCa) cells from AA origin.

Methods: RCC7/T cells were grown in DMEM and maintained at 37°C in a humidified incubator. SPDEF was cloned into pBABE-puro plasmid to generate cell lines with stable expression of SPDEF. qPCR and western blotting were used to assess mRNA/protein levels. Cell migration and invasion were analyzed by wound healing and trans-well invasion assays, respectively. DNA methylation was evaluated by bisulfite sequencing (BSP) technique.

Results: We observed that SPDEF expression was reduced in RCC7/T cells as compared to LNCaP cells. Stable expression of SPDEF in RCC7/T cells resulted in decreased cell migration and impaired invasion. We observed that SPDEF expression in these cells reduced expression of Snail, Slug, Vimentin, etc. (epithelial-to-mesenchymal transition markers). Our analysis of clinical cohorts confirmed that SPDEF expression is lost in patients with metastasis. To further characterize the mechanisms controlling the SPDEF expression during PCa metastasis, we assessed large cohorts of studies from TCGA (GDC TCGA Prostate Cancer, and TCGA Prostate Cancer (PRAD) for DNA methylation. Our analysis revealed that SPDEF expression shows significant inverse correlation with methylation in these clinical cohorts. BSP experiments indicated that SPDEF in RCC7/T cells are hyper-methylated compared to LNCaP and LNCaP-C4 cells at enhancer and promoter regions. Additionally, treatment with a general DNA Methyl transferase inhibitor, 5-aza-2-deoxycytidine, also inhibited cellular growth, colony formation, migration and invasion in RCC7/T cells.

Conclusion: Collectively, these data support the modulatory role of SPDEF in PCa progression in AA cells, suggesting that SPDEF expression is regulated, at least in part, via DNA methylation, and potential therapeutic role for DNMT inhibitors during prostate cancer progression at least in subsets of AA men.

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Soy Isoflavones Enhance the Effects of Src Targeting Agents on Metastatic Activity in Prostate Cancer Cells

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Abstract

Background: In advanced prostate cancer (PCa), cells escape from the primary tumor and enter the bloodstream, preferentially targeting the bone. This results in weakened bones, spinal compressions, fractures and intense pain. Efforts to interfere with early steps in the metastatic cascade by affecting the tumor microenvironment have led to the development of promising small molecule targeting agents. The tyrosine kinase inhibitors, saracatinib and dasatinib, have been shown to inhibit activation of Src and downstream effectors thought to be involved in cancer cell dissemination. As multi-agent treatment regimens are sought to increase anti-metastatic effects while limiting toxicities, soy isoflavones (ISFs), listed by the FDA as Generally Recognized as Safe (GRAS), with known cytostatic effects on PCa cells, could be used to enhance anticancer effects of targeted agents or reduce the effective dose needed.

Methods: PC-3 and LNCaP cells were treated with either a Src inhibitor (dasatinib or saracatinib), a soy isoflavone (ISFs) extract (Novasoy, Archer-Daniels-Midland), or a combination. Modified Boyden transwell chambers were used to evaluate migration and invasion. cell cycle progression and proliferative capacity were measured using FACS analyses and clonogenic cell survival assays, respectively.

Results: ISFs and Src inhibitors reduced proliferation. Src inhibitors increased cells in G1 phase, while ISFs increased cells in G2/M phase. When ISFs were combined with Src inhibitors, both phases were increased with a concomitant decrease in S phase cells. In transwell studies, all agents significantly decreased cell migration: dasatinib>saracatinib>ISFs, with additional reductions observed with the combination. The addition of ISFs reduced the concentration of Src inhibitors needed to inhibit metastatic activity by 30-45%. Therefore, by adding ISFs, lower doses of potentially toxic inhibitors might needed to achieve an optimal response.

Conclusions: In vitro studies suggested that a combination of a Src inhibitors and ISFs results in greater inhibition of metastatic and invasive activities than either alone. Orthotopic in vivo studies are planned to determine if this strategy could be used clinically to treat patients with lower doses of small molecule inhibitors by including soy isoflavone concentrates in the treatment regimen.

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Identification of Androgen Receptor splice variant proteins using Targeted Mass Spectrometry

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Abstract

Castration Resistant Prostate Cancer (CRPC) is a treatment resistant form of prostate cancer (PCa) with no cure. Currently, there is no way to identify which patients will develop this resistance until full blown CRPC develops. Therefore, all PCa is treated the same resulting in tumor regression in some cases and progression in others. Targeting the androgen receptor (AR) is still the main focus of current therapies even in CRPC. Emergence of AR splice variants (AR-Vs) after initial treatment is thought to be one of the primary mechanisms of resistance. AR-Vs lack the ligand binding domain rendering Androgen Deprivation Therapy (ADT) ineffective in tumors expressing these variants. Recent work has identified the DNA and RNA species of ARVs in CRPC but investigation into whether the protein is translated and functional are unknown. One exception is the approval of an antibody test that detects a specific AR-V, AR-V7, from the blood of PC patients and predicts ADT response. However, there are several cases where a patient may not express ARv7 and do not respond to ADT. This may suggest that other AR-V proteins, not currently evaluated clinically, are important predictors for ADT response. We propose to expand the protein identification of known and unknown AR-Vs that may predict response to ADT using targeted mass spectrometry-based proteomics (T-MSP). T-MSP provides accurate, precise, and reproducible detection of a pre-determined set of peptides from multiple samples with high sensitivity without the need for antibody enrichment. We have developed the AR-V targeted MS (ARvT-MS) platform and identified known AR splice variants in models of PCa and we have identified novel AR splice variants via RNA-guided peptide standards. The goal of this work is to generate a robust set of unique peptides that behave as surrogates for AR-V proteins and then evaluate them via ARvT-MS. Using this approach, we have detected 3 known AR-Vs (AR-V7, AR-V9 and AR-V12) and 4 novel AR-Vs in the 22Rv1 PCa cell line. In addition, we assessed patient derived xenografts intact versus castrated samples and detected 3 novel AR-Vs at the protein level that haves not been measured before. The power of this work is that it will provide a platform to set up a system for future functional studies in pre-clinical models and then ultimately clinically to evaluate endogenous AR-Vs levels that may provide clues to therapeutic resistance for patient stratification leading to treatment decisions. Furthermore, the outcomes of these studies will establish a predictive biomarker program that measures AR-Vs proteins in real time from clinical biopsy tissues, circulating tumor cells, or exosomes and informs the clinician on whether ADT may be effective for each patient.

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The essential role of stromal androgen and hedgehog signaling in regulating pubertal prostate epithelial differentiation and development

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Abstract

Background: It has been well demonstrated that stromal androgen receptor (AR) action is essential for early prostate development and morphogenesis. The major studies that established this utilized embryonic tissue recombination experiments to demonstrate a requirement for stromal rather than epithelial AR in early prostate development. These studies were, however, limited to early embryonic timepoints, leaving the role of stromal AR in pubertal prostatic epithelial growth and maturation unknown. For the field to progress, it is crucial to further characterize the role of stromal AR during puberty as circulating androgens rise, stimulating the expansion and maturation of prostatic epithelial ducts. Defining such mechanisms will aid in the effective targeting of prostatic cancers and developmental defects.

Methods: Using tamoxifen-inducible *Gli1*-driven Cre, we assessed the effects of selective deletion of a floxed AR allele in stromal, Shh responsive, *Gli1*-expressing cells during pubertal prostate epithelial growth and development. Single-cell transcriptomic analysis and xenografted tissue recombination assays were then utilized to further characterize cell-types, paracrine interactions, and differentiation trajectories in the AR-knockout mice.

Results: Deletion of stromal AR expression in *Gli1*-expressing cells significantly impeded pubertal prostate epithelial growth and development. Single-cell transcriptomic analyses showed that AR loss in prepubescent *Gli1*-expressing cells dysregulates androgen signaling-initiated stromal-epithelial paracrine interactions, leading to significant growth retardation and epithelial defects in the pubertal prostate. Specifically, AR loss elevates Shh-signaling activation in both prostatic stromal and adjacent epithelial cells, directly inhibiting prostatic epithelial growth. Single-cell trajectory analyses further identified aberrant differentiation fates of prostatic epithelial cells, with apparent inhibition of basal to luminal differentiation. *In vivo* recombination of AR-deficient stromal *Gli1*-lineage cells with wild-type prostatic epithelial cells similarly failed to develop normal prostatic epithelia.

Conclusion: These data demonstrate novel mechanisms underlying how stromal AR-signaling facilitates Shh-mediated cell niches in pubertal prostatic development and establish a new model for future investigation of pubertal androgen signaling.

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FGF-5 stimulates metastasis and anchorage-independence in prostate cancer

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Abstract

Background

While localized prostate cancer has a five-year survival rate of over 99%, five-year survival rates drop to approximately 30% once metastases develop. Fibroblast growth factor-5 (FGF-5) has been shown to be overexpressed in many forms of cancer and has been implicated as a factor promoting metastasis in hepatocellular carcinoma. In this study, we propose that FGF-5 expression stimulates metastasis and increases anchorage-independence, suggesting that FGF-5 may be a suitable therapeutic target for the treatment of metastatic prostate cancer.

Methods

The BCaP prostate cancer progression model was utilized to study changes in FGF-5 expression as cells move towards an increasingly metastatic phenotype. We also utilized an overexpression vector and CRISPR-cas9 sgRNAs to generate multiple prostate cancer cell lines either overexpressing FGF-5 or with FGF-5 loss. Protein expression was verified by western blot. Recombinant human FGF-5 (rhFGF-5) protein was used to treat cells to confirm that increased FGF-5, not just transfection, caused observed changes. To demonstrate the tendency of each cell line towards metastasis, both scratch assays and agar invasion assays were utilized.

Results

Using qPCR, we found that FGF-5 expression is significantly increased in aggressive and metastatic prostate cancer BCaP sublines compared to non-tumorigenic at the RNA ($p=0.001$, $p<0.000$ respectively) level. We also found that FGF-5 expression is generally increased in the tumorigenic BCaP sublines compared to benign prostate tissue. Scratch assays demonstrated increased motility in cells treated with rhFGF-5; untreated cells were not able to close the scratch completely by the observation point, where treated cells (1 nm and 2 nm rhFGF-5) had completely closed it. To bolster this finding, we also performed agar invasion assays and found that cells overexpressing FGF-5 had a modest increase in number of colonies compared to cells transfected with empty vector.

Conclusions

Our study shows that FGF-5 expression increases with prostate cancer progression and promotes invasion and anchorage-independence. While further study *in vitro* and *in vivo* is needed, our results suggest that targeting FGF-5 is a promising strategy for the treatment of metastatic prostate cancer.

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Osteopontin deficiency improves urinary function in a steroid hormone-induced lower urinary tract dysfunction model

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Abstract

Background: Inflammatory and fibrotic processes in the prostate are linked to the development of lower urinary tract symptoms (LUTS) in men, but the molecular mechanism is unknown. We previously established that osteopontin (OPN), a pro-fibrotic cytokine, is more abundant in the prostate of men with LUTS compared to prostate tissue with normal histology. We also identified that the resolution of bacteria-induced inflammation and prostatic fibrosis are accelerated in OPN-deficient mice. The current study investigates whether prostatic OPN levels contribute to steroid hormone-induced lower urinary dysfunction and whether its action is directed via modulating inflammatory and fibrotic processes.

Methods: Male C57BL/6J (WT) or *Spp1*^{tm1Blh}/J (OPN-KO) mice were surgically implanted with slow-releasing subcutaneous pellets containing 25 mg testosterone (T) and 2.5 mg estradiol (E2). Mice were euthanized 2, 6 or 12 weeks later. Urinary function was analyzed with void spot assay, assuming that increased void spot number identify dysfunction. OPN, collagen I and CD45 (immune cell marker) protein expression were investigated using immunohistochemistry (IHC).

Results: WT T+E2 mice exhibited significantly increased voiding frequency two weeks after pellet implantation compared to sham animals and continued to rise afterwards. OPN-KO T+E2 mice produced significantly less void spots on weeks 2, 3 and 4, but the voiding frequency reached that of the WT T+E2 group at week 5. There were almost no significant differences in bladder and prostate lobe masses between the two hormone-implanted strains, which implies that proliferation and obstruction had a similar course in these groups. In contrast, we observed decreased inflammatory cell numbers at week 2 and decreased Col1a1 density at week 12 in OPN-KO mice. This indicates that the early differences in inflammatory processes may contributed to the improved urinary function in OPN-KO mice and OPN deficiency inhibits fibrosis in the progressed stage of the model. OPN protein levels were significantly increased in the ventral prostate 2 weeks after pellet implantation demonstrating the modulation of prostatic OPN levels by steroid hormones.

Conclusions: Our results are the first to demonstrate the hormonal regulation of OPN in the prostate and provides evidence for a direct role in converting hormonal imbalance to the reorganization of the immune environment that ultimately affects urinary function.

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ADT poses a therapeutic advantage against COVID-19 by enhancing the activation of the human myxovirus resistance gene 1 (*MX1*)

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Abstract

Background: Population-based studies have shown that prostate cancer (PCa) patients undergoing androgen-deprivation therapies (ADT) were partially protected from COVID-19. Further, a recent clinical trial demonstrated that proxalutamide reduces the rate of hospitalization for COVID-19 male outpatients.

The human myxovirus resistance gene 1 (*MX1)* participates in antiviral response, and we have previously demonstrated its antitumoral activity in PCa. In this work we: 1) assessed gene expression profiles of the main host cell receptors described for SARS-CoV-2 and potential antiviral genes in response to coronavirus infection, in COVID-19 versus non-COVID-19 patients; 2) evaluated androgen regulation on key host cell receptors and antiviral proteins involved in the SARS-CoV-2 infection

Methods: In a published case-control study (GSE152075) from SARS-CoV-2-positive (n = 403) and negative patients (n = 50), we analyzed the response to infection assessing gene expression of host cell receptors and antiviral proteins from nasopharyngeal swabs at time of diagnosis. We performed multiple bioinformatics analyses (*in vitro and in vivo* transcriptomic studies) to study gene expression changes upon ADT.

Results: we have previously reported that SARS-CoV-2 positive cases had higher *ACE2*, but lower *TMPRSS2, BSG/CD147* and *CTSB* expression. *MX1*/*MX2* were higher in SARS-CoV-2 positive individuals, and negative trends were observed as patients' age increased. Principal Component Analysis determined that *ACE2*, *MX1*, *MX2* and *BSG/CD147* expressions were able to cluster non-COVID-19 and COVID-19 individuals. Multivariable regression showed that *MX1* expression significantly increased with viral load. When analyzing tracheal samples from ferrets intranasally infected with SARS-CoV-2, *MX1* was significantly up-regulated. Similar results were found upon infection with SARS-CoV-2 in A549 and Calu3 lung cell lines.

Since ADT showed a protective effect against COVID-19, we evaluated *MX1* regulation by dihydrotestosterone (DHT). We evidenced comparable *MX1* levels in lung, prostate and salivary gland of healthy humans (GTEx). LNCaP cells treated with DHT showed a decrease ($p<0.05$) in *MX1* mRNA levels. ChIP-seq experiments revealed AR binding sites on the *MX1* sequence in different PCa cell lines under DHT stimulation. Further, when comparing paired samples of locally-advanced/metastatic PCa patients before and after ADT, *MX1* increased expression ($p<0.05$) was observed after ADT.

Conclusion: our results point out to *MX1* as a critical responder in SARS-CoV-2 infection. Further, we showcase *MX1* modulation by DHT. We propose *MX1* as a key player in the therapeutic advantage posed by ADT.

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Frailty is increased in aging mice with lower urinary tract dysfunction

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Abstract

Background: Benign prostatic hyperplasia (BPH) with lower urinary tract symptoms (LUTS) is a disease of aging men that leads to a substantial decrease in quality of life, increased morbidity, and is associated with an increased mortality risk. In patients with BPH/LUTS, frailty may increase the risk of disease progression and serious adverse events. A clinical frailty assessment has been developed for use in mice that exhibits similar features as that established in humans. In this study, we examine young and aging male mice for changes in frailty and urinary function. We hypothesize that the frailty index is increased in aging mice with lower urinary tract dysfunction (LUTD).

Methods: To assess the degree of frailty in young and aged mice, we assessed eight-week-old and two-year-old C57Bl/6J mice using a 31 item frailty assessment. Forelimb grip strength, one measure in the frailty assessment, was quantified using a grip strength meter. Menace reflex, another item assessed in the frailty index, and associated anxiety were examined using an open field test. LUTD was assessed using void spot assays and analyzed with the Void Whizzard software.

Results: As previously described, aging mice develop LUTD when compared to young mice. Assessment of the same mice showed a significant increase in frailty among the aging compared to the young mice. Notably, significant changes with aging included a loss of whiskers, alopecia, malocclusions, loss of forelimb grip strength, and loss of menace reflex. Quantification of grip strength showed a significant decrease in force (Newtons) in aging mice compared to young mice. Menace/anxiety as measured by open field testing showed a decrease in anxiety with aging as compared to young.

Conclusions: Our study shows that a frailty index calculated using a 31 item survey distinguishes aged from young mice. In addition, frailty in aged mice correlates with alterations in urinary function. Thus, mice provide an appropriate model to determine whether interventions that prolong healthy aging (i.e., limiting frailty) will likewise reduce LUTD and enhance the quality of life in older adult males.

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Characterizing Senescence Response to PARP Inhibition May Provide Opportunities for Enhanced Efficacy through Combinations with Senolytic Agents

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Abstract

Background: Inhibition of poly (ADP-ribose) polymerase (PARP) is an exciting treatment strategy recently approved for prostate cancer patients with homologous recombination repair defects. Despite this advance in the field, it remains unclear how PARP inhibitor (PARPi) sensitive cells respond to treatment. We previously demonstrated that treatment with the PARPi olaparib induces not only cell death, but also G2/M arrested senescence characterized by activation of the p53 signaling pathway. We hypothesize that targeting PARPi induced senescence may provide a means to enhance the efficacy of PARPi treatment. In our current work, we sought to 1) understand whether senescence induction is a generalized response to all PARPi's and 2) characterize senescence induction to guide the development of novel treatment strategies combining a PARPi with a senolytic drug.

Methods: PARPi sensitive LNCaP and C4-2B prostate tumor cells were treated with olaparib, rucaparib, niraparib, or talazoparib for 5 days to induce senescence. Both vehicle treated and quiescent cells (LNCaP and C4-2B cultured in FBS-low (0.2%) conditions) were used as controls. Cell viability, flow cytometry, and beta-galactosidase activity assays tested response to PARPi's. Western blot was used to detect PARP activity, apoptosis, and DNA damage response. RNA-sequencing was performed to characterize senescence induced signaling alterations.

Results: We found that exposure to rucaparib, niraparib, and talazoparib all induce a robust G2/M arrested senescence response in LNCaP and C4-2B cells, suggesting that senescence induction is a class effect of PARPi's. PARPi induced senescence is characterized by activation of the p53 signaling pathway and significantly increased expression of the cyclin-dependent kinase inhibitor p21. Furthermore, PARPi induced senescence is distinct from quiescence, suggesting that response to PARP inhibition is phenotypically different from a more general growth arrest. RNA-sequencing revealed several signaling changes associated with senescence which may provide novel treatment opportunities.

Conclusions: Senolytics are a class of drugs thought to specifically target senescent cells. Our results demonstrate that PARPi's induce senescence. Future work will be directed at further characterizing PARPi induced senescence, leading to rationally selected senolytic drug combinations which may enhance the efficacy of PARPi therapy.

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The impact of pharmacological DDX3 inhibition on a metastatic CRPC cell model through integrated omics analyses

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Abstract

The impact of pharmacological DDX3 inhibition on a metastatic CRPC cell model through integrated omics analyses

Prostate cancer presents with a vast heterogeneity in stage and type that makes it difficult to properly classify and treat. The growing emergence of castration resistant prostate cancer (CRPC) underscores the critical need to discover novel biomarkers distinguishing cancer stages. DEAD-box helicase 3 X-linked (DDX3) is involved in RNA metabolism and its overexpression has been implicated in cancer progression and castration resistance. Recently, it was established that DDX3 mediates the formation of castration resistance through posttranscriptional regulation of androgen receptor (AR) in a cellular prostate cancer progression model. Here, we aim to determine the effects of pharmacological loss of DDX3 on intracellular and secreted proteomics in a metastatic CRPC cellular model.

The BPH1 cell line and MT10 line from the BPH1-derived cancer progression (BCaP) model were treated with 2 μM RK-33 for 48 hours, with cells and conditioned media harvested for proteomic analysis immediately following treatment. Cells were homogenized via sonication to extract proteins, then reduced and alkylated with dithiothreitol and iodoacetamide, followed by trypsin digestion. Conditioned media was concentrated and precipitated using 80% acetone, then processed and digested as above. Tryptic peptides were analyzed using LC-MS/MS on a nanoAcquity UPLC coupled to Q Exactive quadruple orbitrap mass spectrometer. Peptides were fragmented using higher-energy collision dissociation (HCD) for identification and quantification using PEAKS software.

Preliminary analyses show an increase in AR protein for the MT10 lines when treated with RK-33, indicating that pharmacological inhibition of DDX3 allows for re-expression of AR. Because of this, we expect significant differences in both secreted and intracellular proteomic expression concerning AR-regulated genes and processes. Additionally, we expect to see significant changes in expression of proteins whose mRNA are bound to DDX3 similar to AR protein.

This study aims to identify proteins and pathways involved with DDX3 that modulate CRPC formation for future pharmacological use in CRPC treatment and prevention. Understanding the differences in proteomic expression between CRPC and benign prostate models allows for the discovery of novel mechanisms that target and prevent CRPC formation in men with hormone sensitive prostate cancers. This work will contribute to increased knowledge surrounding CRPC and other lethal prostate cancer phenotypes that are currently incurable.

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Intratumoral androgen biosynthesis associated with a common polymorphism in 3β-hydroxysteroid dehydrogenase promotes resistance to radiotherapy

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Abstract

Background: Half of all men with advanced prostate cancer (PCa) inherit at least one copy of the HSD3B1 A1245C variant allele (Var_HSD3B1), which increases intracellular levels of the steroidogenic enzyme, 3βHSD1. We investigated whether 3βHSD1 dependent androgen biosynthesis was associated with prostate cancer radiosensitivity.

Methods: Stable PCa lines were generated using HSD3B1 targeting and control shRNA and overexpression constructs. We examined proliferation and clonogenic survival after IR (2-8Gy). DNA DSB formation and resolution kinetics were determined. Gene expression profiling was used to assay DDR pathway gene activation. Patient tissue gene expression profiles were used to validate DDR gene induction. Var_HSD3B1 cells were pretreated with 50 μM enzalutamide and clonogenic survival and DDR gene expression post IR was determined.

Results: Cell lines expressing Var_HSD3B1 had an average 3.13 fold higher proliferation rate (t test, $p<0.01$) and clonogenic survival increased by 2 logs at a dose of 800 cGy (ANOVA $p<0.01$) compared to controls. Var_HSD3B1 cell lines exhibited more efficient DNA repair kinetics with a significant reduction in residual γH2AX foci at 24 hours (t test, $p <0.05$) and reduced COMET Tail moment at 24 hrs. There was increased expression of DDR genes in Var_HSD3B1 cells, including NHEJ, HR, MMR and BER pathway genes. Induction of DDR genes following radiation was significantly more pronounced in Var_HSD3B1 cells. The results observed in-vitro were further confirmed via in-vivo tumor xenograft experiments that showed PCa xenografts expressing var_HSD3B1 gene were more radioresistant compared to control xenografts under to conditions mimicking human adrenal physiology. A positive correlation between AR expression and increased DDR gene expression was confirmed in 680 patient tumor samplesPretreatment with 50μM enzalutamide resensitized var_HSD3B1 PCa cells to 800cGy IR via suppression of DNA DSB repair as evidenced by residual γH2AX foci and increased COMET tail-moment 24hr post IR.

Conclusion: Increased intracellular 3βHSD1 associated with a germline polymorphism promotes prostate cancer radioresistance by upregulating the DNA damage response in the presence of adrenal steroid hormone precursors. This work has therapeutic implications,supporting prospective study of combined androgen blockade (ADT+ARI) and radiation in men harboring the var_HSD3B1 allele.

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Immune cell Single cell RNA sequencing and interaction analyses suggest a prominent role for T cells and T cell-macrophage interactions in Benign Prostatic Hyperplasia

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Abstract

Immune cell Single cell RNA sequencing and interaction analyses suggest a prominent role for T cells and T cell-macrophage interactions in Benign Prostatic Hyperplasia

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Introduction and objective

Benign Prostatic Hyperplasia (BPH) is a common condition among older men. Chronic non-resolving inflammation is frequently associated with BPH; however, the role(s) of specific immune cells in perpetuating this inflammation and the impact on BPH is unclear. Single cell mRNA sequencing (scRNA-Seq) analyses and histologic evaluation of BPH and normal prostate immune cells are used to further define these populations and their interactions and their roles in BPH.

Methods

Small (<50 grams) and large (>70 grams) BPH prostates were collected and evaluated histologically. Immune cells were isolated by fluorescence-activated cell sorting (FACS) for scRNA-Seq on the 10X Chromium platform. Distinct cell clusters were identified based on differential gene expression and known immune phenotypes. These data were combined with previously published scRNA-Seq data from 3 normal prostates. Ligand-receptor interactions were predicted and scored based on ligand and receptor gene expression and cell number referencing databases of known ligand-receptor pairs and were compared between sample types.

Results

Histologically, T cells predominated in BPH specimens followed by macrophages, B cells, natural killer (NK) cells, mast cells, and plasma cells arranged in loose clusters, dense aggregates, or organizing lymphoid structures. The proportion of $CD8^+$ T cells was significantly correlated with IPSS and prostate volume. Unsupervised clustering of combined sample scRNA-Seq data segregated immune cells into 11 clusters. 2415, 959, and 333 significantly altered interactions were identified between small BPH and normal, large BPH and normal, and large BPH and small BPH, respectively. Predicted interactions between $CD8^+$ T cells and macrophages and among macrophages suggest mixed pro- and anti-inflammatory signaling in BPH. Also, myeloid/macrophage subclustering analysis indicate a mix of M1 and M2 macrophage phenotypes in BPH.

Conclusions

These results indicate that T cells, particularly $CD8^+$ T cells, contribute to BPH-associated clinical symptoms, and mixed inflammatory signaling among T cells and macrophages may promote the non-

resolving inflammation observed in BPH. These data suggest that these interactions play a role in the pathogenesis of BPH and identifies potential immune-related treatment targets.

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The Role of Prostaglandin E2 in Prostate Cancer Progression

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Abstract

Background: Clinical management of castration-resistant prostate cancer (CRPC) resulting from androgen deprivation therapy (ADT) remains challenging. Chemotherapy, such as docetaxel, has a long-established position in the treatment paradigm for patients with metastatic (m) mCRPC and neuroendocrine (NE) prostate cancer (NEPC). Still, docetaxel treatment is not curative because drug resistance develops. The molecular signaling pathways that contribute to PC progression, particularly those leading the ADT and chemotherapy resistance in PC, remain elusive. In addition, African American (AA) men have >2.4-fold higher incidence of PC than Caucasian American (CA) men; and AA-PC is more aggressive than CA-PC and the disparity is attributed to both social economic and a genetic difference, but the true cause remains unknown.

Methods: Antiandrogen resistant PC cell model was generated by long-term treatment of androgen-dependent PC cell line with the antiandrogen (MDV3100) for more than 3 months. Human PC tissues were collected from patients undergoing radical prostatectomy. RNAseq analyses was performed to understand the underlying mechanisms. Prostaglandin's expression was measured by LC/MC and qPCR etc. Mechanical and functional analysis were carried out through a variety of approaches and methods.

Results: Long-term antiandrogen treatment of PC cells induces NE differentiation (NED), chemoresistance (docetaxel) and reprograms PC cells to colonize and grow in the bone microenvironment. Further, antiandrogen treatment induces PGE2 expression in PC cells. We demonstrated that PGE2 increases NE markers expression and contributes to chemoresistant in PC cells. Interestingly, antiandrogen induces rapid progression to NEPC in AA-PC cells and expression of PGE2 synthesis associated factors/enzymes in AA-PC tissues is higher than that in CA-PC tissues with matching relative age/grade. Mechanically, we demonstrated that PGE2 contributes to chemoresistant in PC cells by inducing stemness property through activation of YAP1 signaling. In addition, PGE2 increases osteotropism genes expression in PC cells and stimulates M2, tumor associated macrophages (TAM), polarization in both of autocrine and paracrine manners.

Conclusions: PGE2 induced by antiandrogen can feed-forward to affect PC cells in an autocrine manner to induce chemoresistance by acquiring stemness property through activation of YAP1 signaling. Further, PGE2 increases osteotropism genes expression in PC cells and stimulates M2/TAM macrophage polarization which benefit cancer cell survival, colonization and growth in the bone microenvironment. Based upon these findings, PGE2-YAP1 axis is a novel therapeutic target with enormous potential to define a new clinical treatment for chemoresistant bone mCRPC. Improving the clinical response to docetaxel would have immediate impact on treatment of aggressive disease that can progress rapidly in AA men during ADT while it will also benefit all PC patients that eventually develop resistance to treatment.

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Targeting Wnt5a/FZD2 signaling overcomes resistance to enzalutamide in advanced CRPC

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Abstract

Background Androgen receptor (AR) blockade using antiandrogens is a mainstay for the treatment of castration-resistant prostate cancer (CRPC). Unfortunately, drug resistance occurs frequently due to mechanisms that are not completely understood. Wnt5a, a representative ligand of non-canonical Wnt signaling, is expressed in circulating tumor cells from CRPC patients treated with enzalutamide. FZD2, the cognate frizzled receptor for Wnt5a, is the most commonly co-upregulated non-canonical Wnt receptor in prostate cancer. Here we determine the contribution of non-canonical Wnt5a/FZD2 to enzalutamide treatment resistance and explore the potential of targeting Wnt5a/FZD2 to overcome antiandrogen resistance in castration-resistant prostate cancer.

Methods Wnt5a/FZD2 expression was examined in enzalutamide resistant C4-2B MDVR cells. Wnt5a and FZD2 expression were modulated using specific siRNAs. Cell growth, colony formation, migration and invasion were determined in vitro. RNA sequencing was analyzed on C4-2B MDVR cells with WNT5a/FZD2 knocked down; gene expression of non-canonical Wnt signaling, AR and AR-V7 signature genes were analyzed. A novel RNA bioengineered Wnt5a siRNA (tRNA-siWnt5A) was developed to target Wnt5a/FZD2 signaling. The effect of tRNA-siWnt5a on tumor growth and sensitivity to enzalutamide was evaluated in vitro and in vivo.

Results Wnt5a and FZD2 are highly co-upregulated in CRPC patients and enzalutamide resistant C4-2B MDVR cells compared to parental C4-2B cells. Knocking down Wnt5a and FZD2 abrogates the increase of full-length AR and AR variant expression and diminishes the enrichment of genes involved in the non-canonical Wnt signaling pathway. Blocking Wnt5a and FZD2 using specific siRNAs suppresses prostate cancer cell growth, colony formation, migration and invasion. Wnt5a and FZD2 knockdown with siRNA resensitized C4-2B MDVR cells to enzalutamide treatment. Targeting Wnt5a using the bioengineered tRNA-siWnt5A inhibited the growth of enzalutamide resistant prostate cancer cells and resensitized cells to enzalutamide in vitro, and resistant CRPC LuCaP35CR PDX tumor growth in vivo.

Conclusions Our studies suggest that Wnt5a/FZD2 confers enzalutamide resistance and prostate cancer survival and proliferation. Targeting the non-canonical Wnt5a/FZD2 pathway suppresses tumors expressing a high level of Wnt5a and FZD2, not only overcoming resistance but potentiating anti-tumor effects of enzalutamide in CRPC patients.

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Vitamin D sufficiency enhances luminal differentiation of mouse prostate organoids

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Abstract

Background:

Prostate Cancer (PCa) is a prevalent disease in men worldwide, however there is a large racial disparity between white and black men in age of onset, disease aggressiveness, and associated mortality. Black men are often vitamin D (vitD) deficient due to decreased cutaneous vitD synthesis, since they have more melanin in their skin. VitD is a steroid hormone well-studied for its role in calcium homeostasis, however it also plays an important role in cell fate decisions, proliferation, and differentiation. The role of vitD in the prostate and prostate differentiation is not well understood, though our lab previously published that vitD accelerated human epithelial organoid differentiation. Herein, we describe the effect of vitD on mouse prostate organoids, which, unlike human organoids, are able to sufficiently reach secretory, luminal differentiation.

Methods:

Primary prostate mouse organoids (morgs) will be used, due to limited epithelial differentiation observed human organoids, and treated with various concentrations of vitD and androgens. Morgs were imaged using EVOS Auto FL2 software to assess changes in size, growth, and lumen formation. Differentiation was assessed visually throughout the experiment and via gene expression results. RNA was extracted and cDNA synthesized to serve as input for qPCR to analyze relative gene expression of vitD response and pro-differentiation genes after treatment with vitD compared to ethanol controls.

Results:

Morgs treated with vitD show accelerated growth, size, and lumen formation compared to ethanol controls representing enhanced differentiation. VitD response genes are increased in a dose-dependent manner and pro-differentiation genes are also increased in vitD-treated morgs.

Conclusions:

VitD enhances epithelial differentiation in morgs suggesting that vitD plays an important role in overall prostate differentiation. Differentiation is crucial in the prostate for normal prostate turnover and maintenance of glandular structures; a hallmark of PCa is de-differentiation leading to loss of defined histology and glandular structures. Therefore, vitD deficiency may abrogate prostate differentiation leading to increased risk of aggressive PCa due to the loss of normal epithelial differentiation. Defining the role of vitD in morgs may provide the field with rationale to increase vitD in culture methods to induce differentiation allowing for a more applicable, differentiated model.

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LLS80 is a novel inhibitor of Galectin-1 nuclear localization, promoted by inhibition of the androgen receptor, and associated with prostate cancer progression.

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Abstract

Background: Because the Androgen Receptor (AR) plays a very important role in the development and progression of prostate cancer (PCa), advanced PCa is initially treated with androgen deprivation. We previously demonstrated that resistance to AR-based therapies in PCa cells is often caused by an upregulation of Galectin-1 (gal1). We initially identified LLS30, a benzimidazole-based ligand, as an allosteric inhibitor of gal1. Further optimization of LLS30 led to the development of LLS80, with a distinctive structure. Gal1 is expressed in the nucleus, cytoplasm and the extracellular space, and here we describe the effect of LLS80 on nuclear Gal1.

Methods: Gal1 localization was determined in 162 prostatectomy samples from patients with localized PCa and in fractionated cell lines and xenograft models. LC-MS/MS was used to identify the binding targets of LLS80. LLS80 efficacy and maximum tolerated dose (MTD) was assessed in 22Rv1 and CWR-R1 xenograft models. Quant-Seq analysis was performed in LLS80-treated xenografts and differentially expressed genes were validated through quantitative PCR (qPCR).

Results: Immunohistochemical analysis of prostatectomy samples showed that nuclear gal1 expression was associated with shorter time to progression ($p=0.022$) and reduced survival ($p=0.015$). Nuclear translocation of gal1 was promoted by AR inhibition, which was reversed by stimulation of AR activity. Pull-down analysis using biotinylated LLS80 showed binding to gal1 while LLS80 prevented gal-1 nuclear translocation. LLS80 caused a reduction in cell viability alone and in combination with the AR inhibitor enzalutamide by inducing apoptosis in gal1 expressing cells. LLS80 was well-tolerated in Balb/c mice with a MTD of 40 mg/kg. LLS80 caused a dose-dependent decrease in 22Rv1 xenograft size in nude mice compared to controls ($p<0.001$). In CWR-R1-derived xenografts, the combination of LLS80 and enzalutamide was effective in reducing tumor growth ($p=0.00122$). Quant-Seq analysis identified 57 genes differentially expressed after LLS80 treatment. QPCR analysis validated the downregulation of KLK3 ($p=0.0004$), KLK4 ($p=0.0009$), and various other AR targets.

Conclusion: The above result indicates that loss of AR promotes gal1 nuclear translocation which causes PCa progression. The novel gal1 inhibitor LLS80 prevented gal1 nuclear translocation, and countered the effects of AR inhibition therapy. Thus, LLS80 may effectively block PCa progression following AR inhibition.

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AR 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. Truncated AR variant (AR-V) proteins are broadly enriched in CRPC cell lines and clinical samples, and can function as ligand-independent, constitutively active transcription factors. Our studies highlight the importance of mRNA polyadenylation in AR-V expression.

Methods:

To understand the mechanism of *AR* polyadenylation in greater detail, we conducted biochemical assays and global sequencing techniques to examine the role of candidate trans-acting factors and cis-regulatory elements in regulation of *AR* alternative polyadenylation and growth of CRPC cells. We used siRNA, shRNA, PAC-Seq, RNA-Seq, and eCLIP-seq techniques to examine the function of candidate trans-acting factors and used antisense-oligomers to disrupt cis-regulatory sequences of *AR*.

Results:

Several well-characterized AR-Vs, such as AR-variant-7 (AR-V7) and AR variant-9 (AR-V9) arise from splicing of different cryptic exons (CEs), located within intron 3 of the *AR* gene to *AR* exon 3. We found that blocking the alternative poly(A) site in *AR* exon CE3 reduced expression of AR-V mRNA and protein and increased expression of full-length (FL) AR mRNA and protein in 22Rv1 and LNCaP95 CRPC cells, suggesting this single alternative poly(A) site in exon CE3 is utilized to generate AR-Vs, including AR-V7 and AR-V9. We have nominated CPSF1 as a factor that regulates usage of this poly(A) site based on the finding that knockdown of CPSF1 in 22Rv1 and LNCaP95 cells reduced expression of AR-Vs and increased expression of FL-AR. We have performed Poly(A)-ClickSeq (PAC-Seq), enhanced CLIP-seq (eCLIP-seq), and RNA-Seq in 22Rv1, LNCaP95, and LNCaP cell lines to determine novel pathways that are manipulated by CPSF1 in prostate cancer for hormone resistance and continued growth.

Conclusions:

These results have identified a single alternative poly(A) site in exon CE3 of *AR* that regulates expression of FL-AR and AR-Vs in CRPC cells and have determined CPSF1 as a trans-acting regulator of the poly(A) site, highlighting novel drug targets for CRPC treatment.

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Interleukin-4 Represses Extrinsic Pathway-Mediated Apoptosis in Human Prostate Fibroblasts

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Abstract

Background. Myofibroblasts, major cellular agents of fibrosis, are resistant to apoptosis, and instead persist, accumulate, and promote pathological deposition of the extracellular matrix (ECM). IL-4, an inflammatory mediator that is abundant in the aging prostate microenvironment, represses Fas-ligand (FasL)-mediated extrinsic apoptotic pathways in Th2 macrophages. IL-4 and IL-13 signal transduction occurs through a shared axis, suggesting that both interleukins may play key roles in myofibroblast resistance to apoptosis and continued persistence. Based on these studies, we hypothesized that the IL-4/IL-13 axis may repress myofibroblast apoptosis in fibrotic tissues, thereby contributing to lower urinary tract dysfunction (LUTD).

Methods. Primary human prostate fibroblasts were serum-starved for 24 hr then grown in serum-free media with or without 2 hr pre-treatment with pro-apoptotic TRIO cocktail (TNFalpha, Trail, and Fas ligand @50ng/ml each) followed by growth for an additional 2hr or 48hr with or without added IL-4 (40ng/ml) or IL-13 (40ng/ml). Cells were then photographed and/or lysed and subjected to immunoblotting for pro- and cleaved (activated) caspase 3 or caspase 8, GAPDH or tubulin (loading controls), or FasL receptor.

Results. Primary prostate cells expressed high levels of FasL receptor. When treated with vehicle or IL-4, cells exhibited no caspase cleavage and low levels of cell death. When pre-treated with TRIO followed by supplementation with vehicle, cells exhibited high levels of cell death and caspase cleavage/activation ($p<.0001$) compared to non-TRIO treated. Cells pre-treated with TRIO followed by supplementation with IL-4 demonstrated significantly less caspase 3 ($p<.001$) and caspase 8 ($p<.01$) cleavage/activation and reduced levels of cell death.

Conclusions. Low concentrations of IL-4 protected primary prostate fibroblasts from undergoing complete programmed cell death in response to activation of extrinsic apoptotic pathways and caspase 8 activation. Prostate fibroblasts abundantly expressed FasL receptor, and further investigation should reveal whether other extrinsic pathway death receptors are similarly expressed. IL-4 also repressed activation of caspase 3, which can be activated by caspase 8 and help induce intrinsic pathway-mediated (mitochondrial) apoptosis. Future studies will elucidate potentially targetable signaling mechanisms coupled to IL-4/IL-13-mediated repression of apoptotic pathways contributing to myofibroblast persistence, pathological ECM deposition, and fibrosis contributing to LUTD.

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Interleukin-4 Promotes Human Prostate Fibroblast Extracellular Matrix Protein Deposition

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Abstract

Background. Pathological deposition of extracellular matrix (ECM) by peri-urethral prostate fibroblasts and myofibroblasts contributes to lower urinary tract fibrosis and consequent urinary voiding dysfunction. Inflammatory mediators, such as chemokines and interleukins, can promote ECM accumulation. IL-4 and IL-13 are abundant in the prostate microenvironment and their cognate receptors are expressed in association with high levels of collagen deposition in periurethral prostate tissue. Moreover, IL-4 and IL-13 signal through a shared axis, suggesting that may coordinately play key roles in the promotion of fibrotic pathology. Based on this data, we hypothesized that the IL-4/IL-13 axis may promote expression of ECM proteins through JAK/STAT activation, thereby contributing to lower urinary tract dysfunction (LUTD).

Methods. N1 or SFT1 immortalized human prostate fibroblasts were serum-starved for 24 hr then grown in serum-free media with or without 2 hr pre-treatment with antibodies against IL-4Ra or IL-13Ra1, then supplemented with vehicle (PBS or citric acid), IL-4 (40ng/ml), IL-13 (40ng/ml), or TGFB (4ng/ml, as positive control). Cells were then lysed for protein or RNA purification, and subjected to immunoblotting, sircol assays, or qRT-PCR analysis.

Results. Sircol assays demonstrated significantly higher levels of soluble collagens I-V produced by N1 and SFT-1 cells treated with IL-4 ($p<.001$) or IL-13 ($p<.0001$) compared to vehicle-treated cells. qRT-PCR and immunoblot assays showed that IL-4 and IL-13 robustly up-regulated COL1 ($p<.001$) and moderately up-regulated COL3 transcripts and protein ($p<.05$) compared to vehicle-treated cells. Pre-treatment with antibodies against IL-4Ra or IL-13Ra1 ablated the observed IL-4 or IL-13-mediated collagen transcript and protein expression to levels similar to those of vehicle-treated cells ($p<.05$). IL-4 and IL-13 also promoted robust phosphorylation of STAT6, which can induce the expression of the IL-4, IL-13, IL-4Ra and collagen genes.

Conclusions. IL-4 and IL-13 signal through the JAK/STAT pathway to phosphorylate STAT6, suggesting that the observed IL-4/IL-13 induced expression of high levels of extracellular matrix transcripts and proteins by human prostate fibroblasts likely occurs through JAK/STAT signaling. Several JAK/STAT inhibitors are FDA approved and are in current clinical use to treat human disease. Therefore, further studies that positively link ECM deposition with IL-4/IL-13 mediated JAK/STAT signaling may provide new therapeutic approaches to treat LUTD.

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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 deaths in prostate cancer, highlighting the urgent clinical need to determine the mechanisms underlying cancer progression. Trop2, an oncogenic transmembrane cell surface protein, is highly expressed in metastatic prostate cancer and is a prognostic biomarker for early detection of clinically significant localized prostate cancer. Trop2 is cleaved via A disintegrin and metalloproteinase 17 (ADAM17) resulting in the release of shed Trop2 extracellular domain (TECD) into the extracellular environment. Here, we define the functional role of shed TECD in prostate cancer tumor growth and metastasis, and further identify shed TECD as a potential prognostic liquid biomarker for prostate cancer.

Methods: Shed TECD was determined in prostate cancer cell culture media, serum, and urine of normal vs prostate cancer patients by Western Blot and ELISA. Prostate cancer cell lines (22Rv1, DU145, and PC3) treated with vehicle or TECD were tested in migration, invasion, proliferation, and tumorsphere assays *in vitro*. To determine the effect of TECD on tumor growth *in vivo*, DU145 xenografts bearing mice have been treated with vehicle or TECD and the tumors were measured every five days. To identify the functional role of TECD in metastasis, DU145 and 22Rv1 cell lines were utilized to generate intracardiac injection metastatic model and spontaneous metastasis model. Label-free proteomic profiling was performed on DU145 cells treated with TECD to identify TECD function. The top targets were validated by Western Blot.

Results: Our results demonstrate that shed TECD can be detected in cell culture media, serum, or urine samples from prostate cancer patients compared to cancer free patients. Moreover, treatment with TECD significantly increases cell migration and invasion *in vitro* and *in vivo,* while did not affect prostate cancer cell growth, and tumor growth. Proteomics profiling reveals that TECD modulates a set of proteins associated with invasion and migration.

Conclusions: Our study reveals a new function of TECD in prostate cancer migration and metastasis that is independent from the full-length Trop2. Furthermore, our study suggests that TECD could be potentially used as a liquid biomarker for clinically significant localized and metastatic prostate cancer.

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Identification of novel TROP2 functions in prostate tumorigenesis and metastasis utilizing membrane interactome profiling

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Abstract

Background: Prostate cancer is the most common non-cutaneous cancer and second leading cause of cancer related deaths in men in the United States. The first line of treatment for men with advanced prostate cancer is androgen deprivation therapy. Although initial responses are observed, prostate cancer commonly relapses in its lethal metastatic form referred to as castration resistant prostate cancer (CRPC) with 1-2 years mean survival time. The cell surface receptor, TROP2 , is significantly elevated in metastatic prostate cancer. Our recent findings demonstrated that TROP2 induces prostate tumor growth and metastasis. In this study, we set out to delineate the molecular mechanisms through which TROP2 regulates prostate cancer growth and metastasis with its membrane binding proteins.

Methods: Proximity-dependent biotin identification (BioID) and rapid immunoprecipitation mass spectrometry of endogenous protein (RIME) were performed to identify TROP2 interactome in prostate cancer cells utilizing pull-down methods following the mass spectrometry. Lentiviral infection was used to generate prostate cancer cell lines with over-expression of TROP2 and knock-down of NOTCH1 and SLC4A7 shRNA to modulate gene expression levels. *In vitro* functional assays were performed including colony formation and Matrigel drop 3D cell invasion assays. *In vivo* metastasis ability was assessed via intracardiac injection of cancer cells with Luciferase/RFP reporter into NSG mice and quantified via whole body bioluminescence or RFP signals from collected organs. *In situ* Proximity ligation assay (PLA) was used to determine TROP2-SLC4A7 interaction in patients' clinical samples or patient-derived xenografts (PDX).

Results: The TROP2 membrane interactome in living cells was identified utilizing Proximity-dependent biotin Identification (BioID) and rapid immunoprecipitation mass spectrometry of endogenous protein (RIME). Our findings uncover that TROP2-mediated prostate cancer growth and metastasis are orchestrated by distinct downstream pathways including Notch signaling (NOTCH1) and a control protein of intracellular pH (SLC4A7). Co-localization of TROP2 binding partners including NOTCH1, SLC4A7, SLC3A2 and OCLN with TROP2 were further validated by confocal microscopy. Moreover, knockdown of SLC4A7 in TROP2 overexpressing prostate cancer cells suppressed TROP2-driven clonogenic and invasion ability, and inhibited *in vivo* tumor growth and metastasis. We further confirmed that the TROP2-SLC4A7 interaction is naturally occurred in patients' clinical prostate cancer sections or patient-derived xenografts (PDX).

Conclusions: We previously identified that the cell surface receptor TROP2 is a novel driver of aggressive prostate cancer. Herein, our new findings reveal that TROP2 interacts with NOTCH1, SLC4A7, SLC3A2, and OCLN which may highlight novel biological functions of TROP2 in prostate tumorigenesis and develop new therapeutic strategy for metastatic CRPC.

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FOXA1 chromatin binding is repressed by MLL1/SETD7-mediated lysine methylation

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Abstract

FOXA1 (Forkhead Box Protein A1) acts as a critical lineage-specific pioneer transcription factor for the subsequent chromatin binding of androgen receptor (AR) in normal prostate and prostate cancer cells. Recent studies have indicated that increased or reprogrammed FOXA1 activity is a major mechanism contributing to prostate cancer resistance to the AR signaling-inhibition therapies. However, the extent to which FOXA1 binding is dynamic, and mechanisms that may regulate its reversible binding, have been obscure. We have recently reported that FOXA1 chromatin binding is stabilized by LSD1 (Lysine Specific Demethylase 1) through demethylating its lysine 270, adjacent to the wing2 region of the FOXA1 DNA-binding domain. However, the methyltransferase that can directly methylate FOXA1 and thus negatively regulates its chromatin binding remains to be identified. In this study, we have identified MLL1 (Mixed lineage leukemia protein-1) and SETD7 (SET Domain Containing 7, Histone Lysine Methyltransferase) as the specific methyltransferases for FOXA1. The expression of MLL1 and SETD7 is strongly correlated with FOXA1 expression in prostate tumor samples. Inhibition of MLL1 or SETD7 in prostate cancer cells globally antagonized the inhibitory effect of LSD1 inhibition on FOXA1 chromatin binding. More importantly, silencing or inhibition of MLL1/SETD7 increased the expression of FOXA1-regulated genes and promoted prostate cancer cell proliferation and migration. Overall, our study provides novel mechanistic insights into the molecular mechanisms regulating FOXA1 activity in prostate cancer.

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MYC drives aggressive prostate cancer by disrupting transcriptional pause release at androgen receptor targets

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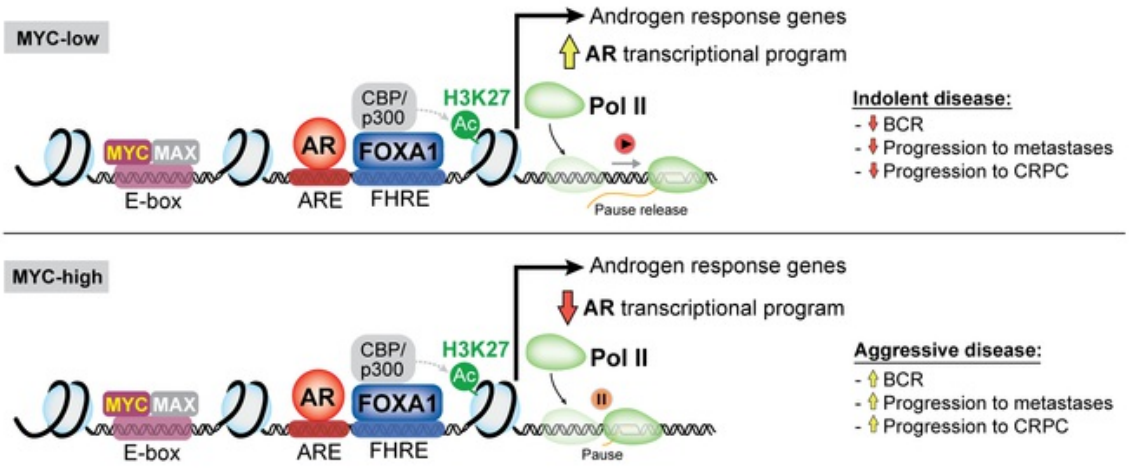
Abstract

Background: Prostate cancer initiation and progression involves the corruption of the normal prostate cancer transcriptional network, following deregulated expression or mutation of key transcription factors. c-MYC (MYC) is a major driver of prostate cancer tumorigenesis and progression. Although MYC is overexpressed in both early and metastatic disease and associated with poor survival, its impact on prostate transcriptional reprogramming remains elusive.

Methods: We used a genetically engineered mouse model of prostate cancer driven by c-MYC overexpression (Hi-MYC) and patient-derived xenograft models (LuCaP). We performed transcriptomics (bulk and single-cell RNA-seq) and chromatin immunoprecipitation followed by sequencing (ChIP-seq) experiments and analyses along with analyses of clinical specimens.

Results: We demonstrate that MYC overexpression significantly diminishes the androgen receptor (AR) transcriptional program (the set of genes directly targeted by the AR protein) in luminal prostate cells without altering AR expression. Importantly, analyses of clinical specimens revealed that concurrent low AR and high MYC transcriptional programs accelerate prostate cancer progression toward a metastatic, castration-resistant disease. Data integration of single-cell transcriptomics together with ChIP-seq revealed an increased RNA polymerase II (Pol II) promoter-proximal pausing at AR-dependent genes following MYC overexpression without an accompanying deactivation of AR-bound enhancers.

Conclusions: Altogether, our findings suggest that MYC overexpression antagonizes the canonical AR transcriptional program and contributes to prostate tumor initiation and progression by disrupting transcriptional pause release at AR-regulated genes (**Graphical Summary**)



Graphical Summary

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Inhibition of KDM5 isoforms decreases proliferation and alters growth signaling pathways in castration-resistant prostate cancers

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Abstract

Background: The Jumonji AT-rich interactive domain 1 (JARID1) or lysine demethylase 5 (KDM5) family of lysine demethylases is comprised of four isoforms (KDM5A, KDM5B, KDM5C, and KDM5D) known for their ability to decrease methylation of histone H3 lysine 4 (H3K4). KDM5D functions as a tumor suppressor. However, the other three isoforms are amplified in metastatic prostate cancers. The role of these KDM5 isoforms in prostate cancer growth and development is not fully understood. The goal of this study was to characterize the effects of KDM5 family inhibition in castration-resistant prostate cancers. The LNCaP-MDV 3100, C4-2B, and PC-3 human prostate cancer cell lines were used as models of castration-resistant prostate cancer.

Methods: KDM5 function within human prostate cancer cells was reduced via two strategies. The compound 2-4(4-methylphenyl)-1,2-benzisothiazol-3(2H)-one (PBIT) was used to inhibit the activity of all KDM5 isoforms, while short interfering RNA (siRNA) SMARTpools targeting KDM5A, KDM5B and KDM5C were used to lower KDM5 protein levels. KDM5 mRNA levels were measured via quantitative reverse transcription-polymerase chain reaction (qRT-PCR). The Proteome Profiler Human XL Oncology Array was used to examine alterations in the level of 84 cancer-related gene products following PBIT treatment. Presto blue assays were used to detect alterations in cell proliferation.

Results: PBIT inhibited proliferation of LNCaP MDV3100, C4-2B, and PC3 cells. In each cell line, significant decreases in proliferation were noted following treatment with PBIT concentrations greater than or equal to 5 uM. Furthermore, siRNA knockdown of KDM5C as well as combined knockdown of KDM5A, KDM5B, and KDM5C, reduced proliferation of PC3 cells. Data from Oncology protein arrays demonstrated that PBIT altered the expression of proteins involved in the epidermal growth factor receptor (EGFR) signaling pathways in C4-2B and PC3 cells. PBIT also altered the expression of androgen receptor (AR) target genes in C4-2B cells.

Conclusions: The growth of castration-resistant prostate cancers can be controlled via strategies that reduce KDM5 activity. Inhibition of KDM5 function may reduce the proliferation of castration-resistant prostate cancer cells by altering activation of both the AR and EGFR signaling pathways.

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Patterns of Failure by Gleason Score at Diagnosis Following Intensity-Modulated Radiation Therapy for Prostate Cancer

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Abstract

Background:

Intensity-modulated radiation therapy (IMRT) is the most common type of external beam radiation therapy for prostate cancer. Its conformal dose distribution helps minimize irradiation of normal tissue while also permitting dose escalation for improved local control. The objective of this study was to assess how Gleason score at diagnosis of biopsy-proven prostate cancer (PCa) correlates with trends of IMRT failure.

Methods:

We retrospectively reviewed the electronic medical records of all patients (n=5259) at our institution who underwent IMRT for PCa between 2008 and 2017. We identified 308 patients who experienced biochemical failure (BF), which was defined as a PSA rise of 2.0 ng/mL over the nadir post-RT or a clinical failure (e.g. initiation of salvage therapy, radiographic evidence of progressive disease). Gleason score at diagnosis was collected for each patient and the scores were stratified to assess failure at intervals of ≤1, ≤3, ≤5, and ≤10 years post-IMRT.

Results:

At ≤1 year post-IMRT, the percentages of overall failures were 1.8%, 1.6%, and 1.0% for Gleason 6, 7, and 8+ diagnoses, respectively. At the ≤3 year interval, 2.6% of Gleason 6's, 3.5% of Gleason 7s, and 5.6% of Gleason 8+'s had failed. At the ≤5 year interval, 3.1%, 5.4%, and 8.1% failed per Gleason category. We observed the starkest failure discrepancy at the ≤10 year interval with 4.1% of Gleason 6, 7.1% of Gleason 7, and 9.9% of Gleason 8+ patients having failed.

Conclusions:

Our data suggests a trend of men with Gleason 7 and 8+ PCa being more inclined for overall IMRT failure. However, there was no significant differences in failure rates between Gleason 6, 7, and 8+ patients at the ≤1 year post-RT interval over this span, suggesting that higher Gleason scores may not necessarily be a true independent predictor of early failure. A more extended follow-up is necessary to determine whether failure predilection trends remain similar in coming years.

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ACAA2 is a Novel Molecular Indicator for Cancers with Neuroendocrine Phenotype

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Abstract

Background: Cancers with neuroendocrine (NE) phenotype share similar clinical features due to common genetic programs and molecular mechanisms that underly their development. Neuroendocrine prostate cancer (NEPC) and small cell lung cancer (SCLC) are two common subtypes of cancers with NE features and are characterized by a highly aggressive clinical course and unfavorable outcomes.

Methods: Protein expression of ACAA2 were assessed by immunohistochemistry (IHC) and western blot (WB) in cancer xenografts and cancer cell lines respectively. ACAA2 mRNA expression was analyzed in clinical datasets to compare NEPC vs. castration-resistant adenocarcinoma (CRPC) and SCLC vs. non-SCLC (NSCLC). ACAA2 expression was determined in prostate cancer tissue microarrays (TMAs), LuCaP PDXs TMAs, and lung cancer patient tissues by IHC staining.

Results: ACAA2 is highly expressed in NEPC cell lines and tumor xenografts, which includes H660 and Trop2-driven NEPC. In addition, ACAA2 mRNA level is elevated in NEPC patients relative to CRPC patients across three independent clinical datasets. More importantly, ACAA2 is highly expressed in advanced prostate cancer, especially NEPC, when compared to benign prostate (N=35) and localized prostate cancer (N=18). Likewise, we observed high levels of ACAA2 in SCLC cell lines and xenografts in comparison with those from NSCLC, and ACAA2 is upregulated in SCLC (N=10) patient tissues compared to NSCLC (N=15) patient tissues.

Conclusions: Our study demonstrates that ACAA2 is highly expressed in cancers with NE phenotype such as NEPC and SCLC. This suggests ACAA2 as a molecular indicator for NE cancers and may also serve as a potential therapeutic target. Our results support further validation in larger, independent cohorts to evaluate ACAA2 as a potential biomarker for cancers with NE features.

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Radiotherapy Inhibits the Antitumor Immune Response Through Release of Immunosuppressive Tumor-Derived Extracellular Vesicles in Prostate Cancer

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Abstract

Stereotactic ablative radiotherapy (SABR) has shown durable response rates in a subset of prostate cancer (PCa) patients with low metastatic burden (oligometastasis). By delivering a highly focused, intense dose of radiation, SABR optimizes local control and induces a systemic antitumor immune response at non-irradiated distant metastatic sites (called abscopal response). However, cases of abscopal response remain scarce and the majority of patients progresses by developing wide-spread metastasis. Therefore, it is crucial to elucidate the underlying mechanisms of resistance to SABR in non-responders and develop more effective combination therapies.

By analyzing plasma samples from metastatic castration-resistant prostate cancer (mCRPC) patients, we have observed that radiotherapy induces a significant increase in levels of tumor-derived extracellular vesicles (tdEVs). High levels of tdEVs were associated with higher risk of developing metastases following SABR. Patients with high blood concentration of tdEVs tend to have significantly lower circulating levels of tumor-reactive CD8 T cells. Based on these clinical observations, we hypothesized that radiotherapy can induce the release of immunosuppressive tdEVs resulting in impaired systemic antitumor immune response.

CRPC cell lines PC3 and DU145 were treated with single dose or fractionated radiotherapy. Levels of EVs were measured with Apogee Micro60-Plus nanoscale flow cytometer. To assess the immunoregulatory function of tdEVs, we co-cultured tdEVs with human $CD8^{+}$-T cells and analyze T cell phenotype and cytotoxic function. Finally, molecular composition of cancer cell surface and EVs was analyzed by mass spectrometry, western blotting and flow cytometry to uncover key players of EV-mediated immunosuppression upon radiotherapy.

First, we found that not only did radiotherapy cause release of tdEVs, but also induce overexpression of the immune checkpoint molecule B7-H3 on surface of EVs. Accordingly, cell-surface protein profiling showed radiotherapy-induced B7-H3 enrichment on cancer cell surface. Co-culture of CD8 T cells with tdEVs from irradiated cells inhibited T cell proliferation and cytotoxic activity. Genetic deletion of B7-H3 expression was associated with partial recovery of T-cell function.

This study unveils a novel cellular mechanism that can impair with radiotherapy-induced antitumor immune response. Targeting immunosuppressive tdEVs (i.e B7-$H3^{+}$) represents a promising strategy to elicit the radiation-induced anti-tumor immune response and abscopal response in oligometastatic cancer patients treated with SABR.

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Androgen receptor represses the expression of ligands that activate EGFR leading to resistance upon androgen receptor inhibition

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Abstract

Background:

Prostate cancer (PCa) strongly depends on androgen receptor (AR) transcriptional activity, making AR inhibition the mainstay of treatment for advanced PCa, but resistance to this treatment is common. We previously showed that AR inhibition leads to increased activity of the epidermal growth factor receptor (EGFR) family, and this upregulation is a major cause of PCa recurrence. However, the mechanism causing the increase in EGFR activity is not known. Here, we investigate how AR inhibition increases the activity of the EGFR family and resistance in PCa.

Methods:

PCa-derived cell lines were treated with the AR inhibitors (enzalutamide, darolutamide, apalutamide, and bicalutamide) and EGFR family inhibitors (erlotinib, dacomitinib, and afatinib). Protein expression and phosphorylation were determined by immunoblotting. Luciferase via a PSA-Promoter reporter plasmid was used to analyze AR transcriptional activity. RT-qPCR was used to compare mRNA levels. Drug combinations were tested on patient-derived-organelles (PDO) and patient-derived-xenografts (PDX) in immunocompromised mice.

Results:

Enzalutamide-resistant cell lines had elevated levels of EGFR phosphorylation and EGFR ligand mRNA compared to enzalutamide-sensitive lines, and this increase in ligands was associated with increased EGFR phosphorylation and enzalutamide-resistance. Additionally, AR inhibition, AR knockout, and reduction in AR ligand concentration increased EGFR ligand mRNA levels. Although EGFR knockout and stimulation increased AR transcriptional activity, EGFR inhibition and overexpression reduced it. Furthermore, treatment with an EGFR inhibitor, erlotinib, prevented enzalutamide-induced resistance from EGFR activation, and the pan-ErbB inhibitor, dacomitinib, had an even better effect. The combination of erlotinib and enzalutamide was effective in PDOs. In a PDX tumor model resistant to AR inhibitors, enzalutamide increased levels of the EGFR ligand, TGF-α. Additionally, dacomitinib reduced these enzalutamide-induced effects and tumor growth in combination with enzalutamide.

Conclusions:

Our results indicate that AR normally suppresses expression of EGFR family ligands, and AR inhibitors

used in PCa standard of care relieve this suppression. This release leads to an increase in EGFR family ligands, EGFR activation, downstream signaling, and resistance to AR inhibitors. However, combining AR inhibition with an EGFR inhibitor overcomes this form of resistance in PCa.

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Microbial Exposure Reduces Susceptibility to Localized Uropathogenic E. Coli Infection in the Bladder and Urosepsis

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Abstract

Urinary tract infections (UTI) are the most prevalent infectious disease of the bladder-affecting >130 million people worldwide annually. Uropathogenic *E. coli* (UPEC) causes most UTI, starting with bladder colonization and then ascension to the kidneys. In severe cases of pyelonephritis, bacteria can enter the bloodstream, causing urosepsis. The immune system is involved in the development of these pathologies and response to treatment, but relatively little is known about how the basal state of the immune system influences the ability to respond to local or systemic UPEC infection. The difficulty in performing hypothesis-driven research in humans justifies the need for a clinically relevant experimental UTI/urosepsis model. Most preclinical studies are conducted with specific pathogen-free (SPF) mice, which possess a largely naïve immune system equivalent to neonatal humans. Cohousing (CoH) SPF mice with microbially diverse pet store mice matures the immune system to resemble that of adult humans. Therefore, the objective of these studies is to determine how prior microbial exposure affects the immune response to local and systemic UPEC infections.

For these studies, we infected SPF and CoH mice with UPEC strain UTI89 locally via bladder instillation or systemically via intravenous injection. Immune cell composition in the bladder or in circulation was determined before and after infection, and bacterial clearance and survival following infection was monitored.

Microbial exposure altered the baseline immune cell composition in the bladder, with CoH mice showing increased numbers of CD4 T cells and neutrophils. Interestingly, CoH mice were better able to clear UPEC infection following local infection of the bladder. Following systemic infection to model urosepsis, greater numbers of immune cells were found in the circulation of CoH mice, and CoH mice showed faster clearance of infection in the blood, spleen, and kidney. Importantly, all SPF mice succumbed to infection within 48 hours, while all CoH mice survived beyond 72 hours.

Our results have important implications for models of UTI/urosepsis. They suggest a general history of microbial exposure reduces susceptibility to localized bladder infection and urosepsis. While these studies importantly further our understanding of the immune response initiated after local or systemic UPEC infection, they also suggest mouse studies incorporating exposure to diverse infections may provide a more clinically relevant experimental UTI/urosepsis model.

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Taxol-elevated PLK1 Overcomes BETi-Resistant in Prostate Cancer via Triggering Phosphorylation-dependent Degradation of BRD4

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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 (PCa). Currently, a number of BRD4 inhibitors or degraders have been discovered. However, recent studies revealed that *SPOP* mutant of prostate cancer patients leads to BRD4 stabilization and resulted in JQ1-resistant. Therefore, revealing the mechanism underlying BRD4 stability may improve the clinical response rate and efficacy of BRD4-targeted therapy in PCa patients.

Methods: To evaluate the level of BRD4 during the mitosis phase, we applied a Confocal microscope and WB. *In vitro* kinase assay and Mass spectrometry were applied to test the phosphorylation of BRD4 by PLK1. A homemade phosphorylation-specific antibody was used to verify p-BRD4 in PCa cells and a tissue micro-array. A PDX-tumor model was used to evaluate the combination efficacy of Taxol to overcome JQ1-resistant.

Results: We found that the level of BRD4 obviously decreased during the mitosis phase of PCa cells. Upon release from M-phase arrest, the BRD4 level increased along with PLK1 decreasing in PCa cell lines, suggesting PLK1 might be involved in the degradation of BRD4 in the M-phase. We found that the PBD domain of PLK1 was responsible for interacting with the CTD of BRD4. Enforced overexpression of PLK1 promoted BRD4 dramatically reduced. Reversely, tet-induced depletion or inhibition of PLK1 led to BRD4 stabilization. Furthermore, applying gain or loss of function assay, we found that PLK1 directly phosphorylated BRD4 and triggered its phosphorylation-dependent degradation in the M-phase, which was confirmed in clinical samples that a high level of PLK1 was negatively correlated with BRD4. Accordingly, we found that overexpression of PLK1 lowered the stabilized BRD4 caused by *SPOP* mutant in PCa cells and consequently made cells sensitive to JQ1. Intriguingly, upon being treated with Taxol, a commonly used medicine for PCa patients, the PLK1 level was dramatically elevated as well as p-BRD4 status but total BRD4 downregulated in PCa cells. Moreover, sequential treatment of Taxol and JQ1 resulted in significant inhibition of proliferation, colony formation, and PDX tumor carrying *SPOP* mutant, indicating the application of Taxol overcame JQ-1 resistance.

Conclusions: Collectively, our results suggested that PLK1 phosphorylates BRD4 and consequently triggers its phosphorylation-dependent degradation in PCa cells. Sequential treatment of Taxol and JQ1 overcomes *SPOP* mutant-related BETi-resistant in prostate cancer.

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PIK3R1-alteration augments insulin- Akt-glucose metabolism and act as a key driver for aggressive prostate cancer

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Abstract

Bac

Background: Prostate cancer is one of the leading causes of cancer deaths among men, with 34,000 deaths projected for the US in 2021. Once prostate cancer becomes castration-resistant and metastatic (mCRPC), it is incurable. Oncogenic alteration of the phosphatidylinositol-3-kinase (PI3K)/AKT pathway occurs in >40% of patients with mCRPC. Alterations in the PI3K-signaling pathway in cancer have led to a surge in the development of PI3K/Akt inhibitors, and many of these targeted therapies are currently in clinical trials. Therefore, in precision oncology, identifying advanced prostate cancers with high PI3K activity is critical for treatment selection and eligibility into clinical trials of PI3K/Akt inhibitors.

Methods: We analyzed panel sequencing data from 1417 samples obtained from prostate cancer patients: 825 (58%) samples from primary tumors and 592 (42%) samples from metastases. We discovered a significant enrichment of PIK3R1 (regulatory subunit that codes for p85a protein and modulates the catalytic activity of PI3K-pathway) alterations in metastatic prostate cancers compared to primary cancers. We used siRNA-based knockdown and cells growth assay to validate the effect of PIK3R1 alteration in prostate cells experimentally.

Results: We observed that PIK3R1, a tumor suppressor gene located on the chromosome 5q13.1 locus, was more frequently lost in metastatic prostate cancer samples than in primary prostate cancers (36% vs 24%, $p<0.001$). Also, PIK3R1 driver alterations (5% of metastasis samples vs. 2.7% of primary samples; $p = 0.030$) were significantly enriched in metastatic samples. PIK3R1 oncogenic alterations or reduced mRNA expression were associated with worse clinical outcomes in the prostate and several other cancers. In prostate cancer cell lines, PIK3R1 knockdown resulted in increased cell proliferation and AKT activity (including insulin-stimulated AKT activity). In cell lines and organoids, PIK3R1 loss/mutation was associated with increased sensitivity to AKT inhibitors. Importantly, PIK3R1-altered patient prostate tumors had increased uptake of the glucose analogue 18F-fluorodeoxyglucose in PET imaging, suggesting increased glycolysis.

Conclusion: Our findings uncover a novel subtype of lethal prostate cancer and suggest that PIK3R1 alteration may be a key event for insulin-PI3K-glycolytic pathway regulation in prostate cancer. Thus, PIK3R1 alterations identify patients with worse prognosis, but more importantly, they identify patients for PI3K/AKT-inhibitor-based clinical trials.

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Nuclear/perinuclear localization of ErbB3 in prostate cancer and its role in regulating ligand-dependent activation of ErbB2/ErbB3 heterodimers and its downstream targets.

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Abstract

Background: Prostate cancer (PCa)-specific nuclear expression of ErbB3, a receptor tyrosine kinase of the epidermal growth factor receptor (EGFR) family, has long been recognized (Koumakpayi et al. Clin Cancer Res. 2006;12:2730-7). While an 80kDa nuclear variant of ErbB3 has been identified (El Maassarani et al, PLoS One. 2016;11(5): e0155950), full-length 185 kDa ErbB3 also translocates to the nucleus in PCa in a bone microenvironment and androgen status-dependent manner (Cheng et al, Mol Cancer Res. 2007;5(7):675-84). However, the mechanism by which nuclear ErbB3 regulates PCa progression has not been elucidated.

Methods: ErbB3 localization was investigated *in vitro* (human prostate cancer cell lines, LNCaP, C4-2, 22Rv1) using amiloride or heregulin-1β (ErbB3-specific ligand). ErbB3 expression and subcellular localization were analyzed using immunofluorescent microscopy and subcellular fractionation followed by immunoblot. Physiological readouts included viability, migration and reporter gene assays.

Results: Here we show that ErbB3 can occupy a nuclear/perinuclear location in prostate cancer cells. The hormone sensitive PCa line LNCaP expressed higher levels of nuclear ErbB3 compared to castration resistant lines C4-2 and 22Rv1. The diuretic amiloride was previously shown to inhibit ErbB3 internalization by macropinocytosis (Koumakpayi et al, Mol Carcinog. 2011;50(11):901-12); thus, we investigated whether it could also modulate ErbB3 nuclear localization in PCa cells. In LNCaP cells, amiloride dose dependently prevented ErbB3 nuclear transport, whereas in C4-2 and 22Rv1, no such effect was observed. Further analysis revealed that amiloride promoted ErbB3 expression in the membrane, where ErbB3 underwent ligand-dependent phosphorylation, formed ErbB2/ErbB3 dimers, and in turn phosphorylated the downstream targets AKT and ERK1/2 (p42/p44MAPK). On the other hand, EGFR phosphorylation and dimerization were unaffected by amiloride. Our results suggest that ErbB3 is stored in the nuclear region in an inactive form, whereas upon stress induction, including androgen deprivation, ErbB3 is translocated to the plasma membrane, where it is activated by ligand binding, and causes downstream signaling.

Conclusion: We previously showed that the EGFR/ErbB2 dual inhibitor lapatinib failed to affect PCa (Jathal et al, Br J Cancer. 2019;121(3):237-248); we now show that this may be due to modulation of nuclear and cytoplasmic ErbB3 expression. Amiloride prevented translocation (and thus expression of) ErbB3 to the nucleus and retained this RTK in the membrane, resulting in the formation of active ErbB2/ErbB3 dimers. Lapatinib was then able to inhibit and inactivate its specific target, ErbB2, thereby inducing significant apoptosis. The above indicates a novel role for nuclear/perinuclear ErbB3 in PCa that can be regulated by the combination of amiloride and lapatinib.

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Evaluating the Synergy between Enzalutamide and SRC Kinase Inhibitors in Castration-Resistant Prostate Cancer

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Abstract

Background: Prostate Cancer (PCa) remains the most diagnosed non-skin cancer amongst the American male population. Treatment for localized prostate cancer consists of Androgen Deprivation Therapies (ADTs) which inhibits the androgen receptor (AR). Though initially effective, a subset of patients will develop resistance to ADTs and the tumors will transition to Castration-Resistant Prostate Cancer (CRPC). Treatment of CRPC consists of newer hormonal therapies such as enzalutamide, but they are not curative. Two mechanisms contributing to enzalutamide resistance are the presence of androgen-independent truncated AR proteins called AR splice variants (ARVs) and increased tyrosine kinase activity. Previous work has shown that tyrosine kinases, such as SRC, can bind to and phosphorylate critical residues on full-length AR (AR-FL), increasing AR activity in the presence of low androgens. What is unknown is how kinase signaling directly contributes to ARV function and whether this mechanism aids in the observed resistance with newer hormonal therapies.

Methods: We have begun evaluating synergy between enzalutamide or chemotherapy with SRC kinase inhibitors, saracatinib and dasatinib, in prostate cancer cell line models using Bliss Independence and the Combination Equation via Chou-Talay method. We have chosen four cell lines, DU145, AD-1, R1-D567, and 22Rv1, all of which have SRC and have variable AR genetic background. We also have begun to define the mechanism of synergy via changes in AR phosphorylation and target gene expression after addition of the inhibitors.

Results: We observed synergy between enzalutamide and SRC kinase inhibitors in cells that express AR-FL (independent of ARV status) and antagonism between docetaxel and SRC kinase inhibitors in cells that express both AR-FL and ARVs. We also found that the combination of enzalutamide and SRC kinase inhibitors were able to reduce the effective dose of each drug up to ten-fold, thus reducing toxicity. This fold reduction was not seen in the docetaxel and SRC kinase inhibitor combinations. Lastly, a key SRC substrate on AR, ARY534, was significantly reduced after enzalutamide and SRC kinase inhibitor treatment versus docetaxel and SRC kinase inhibitor treatment.

Conclusions: By elucidating this synergy, we will provide critical pre-clinical data that could influence how future clinical trials are designed based on AR status when combining SRC kinase inhibitors with enzalutamide, leading to the goal of increasing survival for men suffering from lethal CRPC.

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Prostatic androgen receptor expression decreases with age and may be regulated by DDX3X in mice.

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Abstract

Background: Studies suggest that the prostate diseases, such as benign prostate hyperplasia (BPH) and prostate cancer, are closely linked to hormones/receptor changes and aging. However, these detailed cellular and molecular mechanisms remain unclear. Our previous studies indicated that the RNA helicase, DDX3X, binds to the androgen receptor (AR) mRNA at stress granules and inhibits AR translation, which may lead to a failed response to androgen deprivation therapies. Whereas prostatic AR is primarily localized to the nucleus, DDX3X can be found in both the cytoplasm and nucleus. Both AR and DDX3X can be found in both stromal and epithelial tissue compartments. We have previously shown that DDX3X regulates AR protein expression in castration resistance however, it is unknown whether DDX3X regulates AR protein translation in normal aging. Here, we evaluated the localization/expression of cytoplasmic DDX3X and nuclear AR in normal aging in the male mouse prostates.

Methods: We examined the anterior prostate (AP) from 2-month-old (n=9) and 24-month-old (n=7) C57Bl/6 mice. AR localization/expression was examined via immunofluorescence and DDX3X was detected via immunohistochemistry. The percentages of AR and DDX3X positivity were determined by Inform software. The mean of optical density (OD) was also determined for DDX3X by Inform software. Through comparison of the cytoplasmic DDX3X and nucleic AR levels among different age groups, we ascertained the relationships between DDX3X/AR and aging.

Results: More prostatic AR was expressed in 2-month-old (young) mice (92.21% positivity), while 24-month-old (old) mice showed reduced AR expression (54.09% positivity). In terms of cytoplasmic DDX3X, similar levels of DDX3X were detected in 2-month-old (46.75% positivity, 0.047 mean OD) and 24-month-old mice (59.74% positivity, 0.055 mean OD). However, in aged but not young mice, DDX3X had an epithelial-cytoplasmic and punctate pattern, consistent with stress granule localization. This punctate pattern is consistent with DDX3X mediated protein translation inhibition, suggesting DDX3X may be involved in decreased prostatic AR protein expression in aged mice.

Conclusions: Aging is associated with decreased prostatic AR localization/expression and may involve regulation through the DDX3X mediated mechanism.

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Epigenetic change in the KCNB2 promoter during obstruction and chronic bladder obstruction

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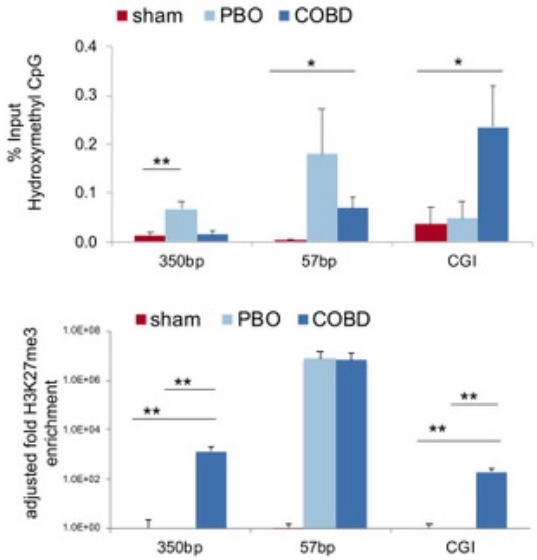
Abstract

Background: **Partial bladder outlet obstruction (PBO) is a widespread cause of urinary dysfunction, patient discomfort, resulting in immense health care costs. The functional and pathologic aspects of obstruction remain even after ablation of the physical obstruction as a chronic obstructive bladder disease (COBD). Chronic disease in general is often associated with altered DNA methylation and histone modifications. Here we examined if COBD alters regulation of the epigenetic marks in a proof of principle gene.**

Methods: **COBD was created in 200-250g female Sprague-Dawley rats by surgical ligation of the urethra for 6 weeks, followed by removal of the suture and following the animals for 6 more weeks. PBO is the 6 week ligation. Sham ligations were performed by passing the suture behind the urethra. Bladder function was non-invasively tested using metabolic cages, both one day prior to de-obstruction at 6 weeks and prior to sacrifice at 12 weeks. RT-PCR for mRNA and pyrosequencing for DNA methylation was performed for KCNB2. To further understand the pyrosequencing results which does not differentiate between DNA methylation and DNA hydroxymethylation, chromatin IP/PCR for hydroxymethylDNA (Hme) was assessed in several regions of the KCNB2 promoter. As another mark of epigenetic repression, histone3 lysine27 trimethylation (H3K27me3) marks were also performed. ANOVA and post-hoc t-test were performed with $p<0.05$ considered significant.**

Results: **RT-PCR revealed that KCNB2 was significantly downregulated during obstruction and COBD coordinate with altered DNA methylation, that correlated with bladder function. Hme and H3K27me3 were both significantly increased during COBD in the CpG Island (CGI). 350 bp upstream of the CGI, fold enrichment of Hme was significantly increased during obstruction by >5 fold but not in COBD, vs. sham. In contrast, the same region showed a fold enrichment of H3K27me3 >20-fold during only COBD and not obstruction vs. sham.**

Conclusions: **Regulation of KCNB2 at the promoter demonstrates dynamic changes in DNA methylation, as well as hydroxymethylation. H3K27me3 of the promoter during COBD but not obstruction suggests this is a later change in the regulation of the gene expression.**


sham
PBO
COBD
% Input Hydroxymethyl CpG
0.4
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0.1
0.0
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350bp
57bp
CGI
sham
PBO
COBD
adjusted fold H3K27me3 enrichment
1.0E+08
1.0E+06
1.0E+04
1.0E+02
1.0E+00
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**
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350bp
57bp
CGI

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Urinary Bladder Global Transcriptomics by Bulk RNA-Seq in Prune Belly Syndrome

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Abstract

Prune Belly Syndrome (PBS) is characterized by bladder dysmyogenesis and dysfunction. At present, our understanding of the molecular mechanisms causing and regulating tissue-specific manifestations of PBS are still limited. We used bulk RNA-Seq to gain deeper insight into the molecular mechanisms that are characteristic of the PBS bladder.

Using UTSW IRB-approved methods, bladder RNA was extracted, the Illumina cDNA library was prepared, and bulk RNA was sequenced from 4 unrelated PBS males and 2 non-PBS control males. The raw reads were trimmed using TrimGalore and aligned to the GRCh38 human genome. Gene counts were generated to identify PBS differentially expressed genes (DEGs) and to perform Gene ontology (GO) and PROGENy (Pathway RespOnsive GENes) analyses. PBS and control bladder tissues were formalin fixed, paraffin embedded, stained with Masson's trichrome for extracellular matrix (ECM) components.

Transcriptome analysis identified 97 DEGs in PBS bladders, of which the top and down 10 are shown (Fig 1A). The highest upregulated DEG in PBS bladders was the Delta Like Non-Canonical Notch Ligand 1 (DLK1). During embryogenesis, the Dlk1-DIO3 imprinted locus regulates placental, mesodermal and ectodermal differentiation. DLK1 is upregulated in Muscular Dystrophy where DLK1 expression reflects skeletal myotube immaturity. GO analysis of the PBS DEGs revealed significantly enriched terms including: “ECM form, function” and “Collagen production”, which was confirmed on PBS bladder sections (Fig 1B-C). To further characterize the molecular signature of PBS bladder, PROGENy analysis, which estimates the activity of 14 relevant signaling pathways based on consensus gene signatures obtained from perturbation experiments, identified activation of Wnt, VEGF, PI3K, TGF-β and Estrogen signaling pathways (Fig 1D).

We, for the first time, have interrogated alterations in PBS bladder global transcriptional networks. This PBS bladder transcriptomic data is a step toward for identifying the molecular basis of PBS and potential markers for diagnosis. Additionally, we can explore the heterogeneity of PBS gene expression analyses for each individual PBS bladder sample, opening the door for future personalized therapeutic interventions.

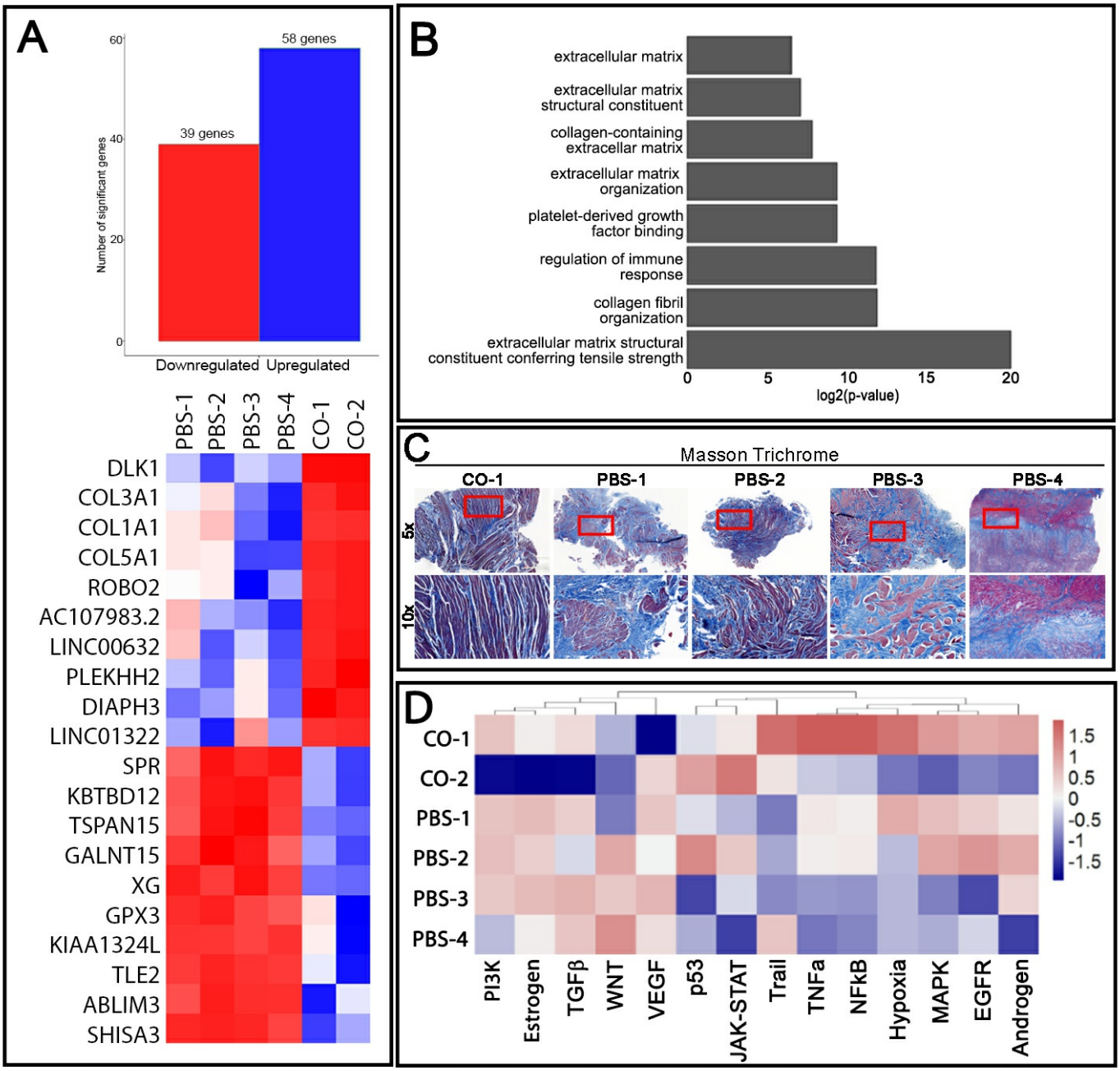

Figure 1: Bulk RNA-Seq analysis PBS vs control urinary bladder.A. Differentially expressed urinary bladder genes included 39 downregulated and 58 upregulated genes. demonstrates 10 most up and downregulated genes in PBS versus controls. B. Gene ontology pathway analysis demonstrating 8 significant biological processes and molecular functions altered in PBS versus controls. C. Masson Trichrome stain of urinary bladders showing cellular disorganization and collagen deposition. D. PROGENy signaling pathway analysis demonstrates alterations in PBS.

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Functional co-dependency analysis identifies ZBTB7A as a therapeutic target with RET in neuroendocrine prostate cancer

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Abstract

Functional co-dependency analysis identifies ZBTB7A as a therapeutic target with RET in neuroendocrine prostate cancer

Background: Neuroendocrine prostate cancer (NEPC) is a highly aggressive subtype of prostate cancer that is characterized by the loss of androgen receptor (AR) signaling and induction of neuroendocrine features, resulting in resistance to AR-targeted therapy. The limited targeted therapeutic options and heterogeneity of NEPC tumors make treatment difficult for NEPC patients. We have recently reported the enrichment of RET kinase activity and its overexpression in NEPC cell lines and tumors, suggesting that RET may be a promising therapeutic target in this lethal variant. Searching for other vulnerabilities in NEPC that may function with RET, we identified ZBTB7A, a transcription factor consisting of four zinc finger and one BTB domains. In cancer, ZBTB7A has been reported to play oncogenic or tumor suppressive roles depending on the tissue type and molecular interactions. ZBTB7A is involved in biological processes including cell proliferation, differentiation, metabolism, apoptosis, and metastasis. However, its function in NEPC has not been investigated to date.

Methods: We took advantage of publicly available datasets of genome-wide RNAi screening and small-cell neuroendocrine (SCN) phenotype evaluation in cancer cell lines to identify a potential target with similar function to RET, such as ZBTB7A. We performed genetic perturbation to validate the effect of ZBTB7A and its combination with a RET inhibitor on cellular growth of NEPC cell line, NCI-H660. In addition, we considered approximately 200 oncogenic signatures in the Gene Set Enrichment Analysis (GSEA) and examined the potential differences of ZBTB7A function that are robust in NEPC than adenocarcinoma.

Results: From the initial analyses, we confirmed that cell lines with higher SCN-like characteristics (SCN HI) are more dependent on RET expression for cell proliferation and observed a strong correlation between dependencies of RET and ZBTB7A. The genetic knockdown of ZBTB7A inhibited the growth of NCI-H660 cells, and reduced the size of cell clusters without altering the activity of the RET pathway. Pharmacological inhibition of RET in stable ZBTB7A-knockdown NEPC cells further decreased cell viability as a result of an additive combination effect. Additionally, GSEA identified a number of positively enriched gene sets involved in cell cycle, including E2F targets and genes upregulated by RB loss, and DNA repair, regulated by MYC and upregulated by increase of EIF4E, a component of the translation initiation complex.

Conclusions: Collectively, our results suggest ZBTB7A as a potential candidate in the development of a treatment strategy for NEPC. Moreover, the antiproliferative activity of targeting RET and ZBTB7A may engage two independent mechanisms, which need to be further elucidated.

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Heterozygous (het) nonsense mutations in Tropomyosin 2 (TPM2) in 3 human males with Prune Belly Syndrome (PBS)

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Abstract

PBS is a rare congenital myopathy with incomplete molecular understanding. Thin filaments of smooth and skeletal muscle cells include TPM2 which regulates actomyosin contraction/relaxation and cell morphology/adhesion. Given the significant smooth/skeletal muscle phenotype in PBS, we hypothesized TPM2 may be deranged in PBS.

IRB-approved PBS probands and relatives were prospectively enrolled and phenotyped. Proband DNA underwent paired-end Whole Exome Sequencing (WES) with data pipeline analysis. WES variants were filtered for allele frequency and predicted mutation impact/type and Sanger validated with segregation tested in available familial DNAs. mRNA and protein were extracted from PBS and non-PBS control abdominal wall (AW) samples, analyzed by qPCR and descriptive statistics, and western blotted (WB). C2C12 mouse cell lines were co-transfected with 1ng of siRNAs (mouse TPM2 or negative control) and 500 ng of plasmid [Empty Vector (EV) GFP, TPM2 WT Flag, or TPM2 R238* Flag]. 3 days after co-transfection, cytoplasmic:nuclear area spreading assays were performed and protein was extracted for WB.

WES identified 2 het TPM2 nonsense mutations [c.70 C > T (p.Q24*) (n=2) and c.712 C > T (p.R238*) (n=1)] in 3 unrelated sporadic PBS males; asymptomatic maternal inheritance was confirmed in 2. AW tissue from the het TPM2 R238* PBS proband revealed a 90.5% reduction in TPM2 transcripts and essentially undetectable TPM2 protein (3.6% vs 203% ± 80.5 in non-PBS controls) (Fig 1A,B). Het TPM2 Q24* PBS probands had no available tissue. In the C2C12 transfections, 4 conditions interrogated how TPM2 silencing and R238* overexpression affect protein expression and spreading properties. WB indicated reduced TPM2 for EV+TPM2siRNA (50%) and R238*+TPM2siRNA (89%), and Flag expression only in WT TPM2 (Fig 1C). Over time, spreading decreased in cosiRNA cells and increased in TPM2siRNA cells. After 60 minutes, TPM2 WT rescued but R238* failed to rescue the endogenous, non-spreading phenotype (Fig 1D).

Human TPM2 het mutations cause distal arthrogryposis and CAP/nemaline myopathies. We now add to the phenotypic spectrum of TPM2 mutations, identifying that never reported TPM2 truncations are a rare cause for PBS and reduced TPM2 protein carries functional cellular consequences.

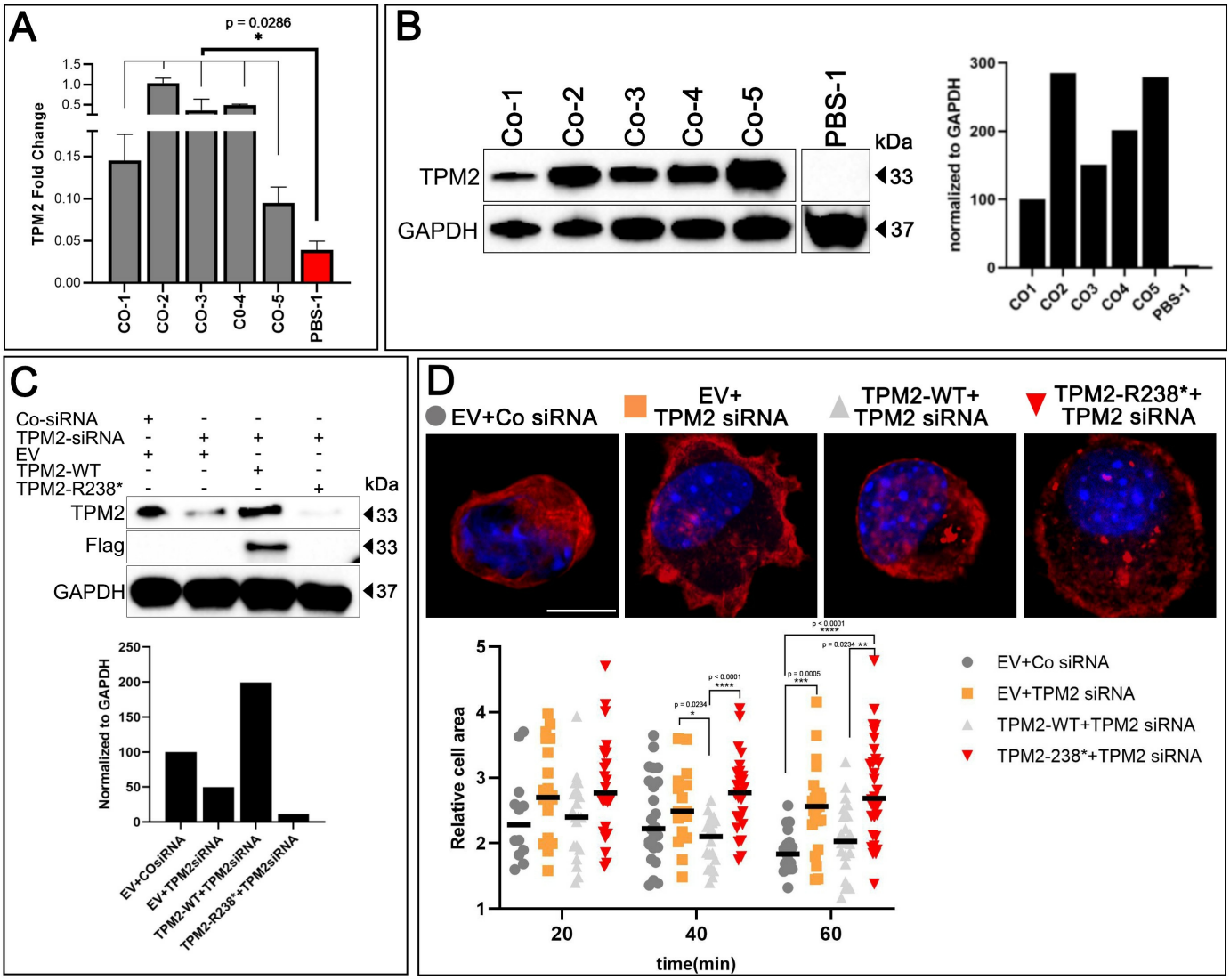

Figure 1: Heterozygous TPM2 premature stop diminishes mRNA and protein expression in PBS abdominal wall muscle; reduced TPM2 protein expression and increased cytoskeletal spreading in C2C12 co-transfected cells. A-B Non-PBS controls (Co-1 through Co-5) versus PBS subject harboring TPM2-R238* premature stop (PBS-1) abdominal wall muscle. **A** qPCR displays profoundly reduced TPM2 mRNA in PBS-1 versus controls. **B** Western blot and corresponding densitometry show essentially no TPM2 protein expressed in PBS-1. **C-D** Differentiated C2C12 mouse skeletal muscle cells co-transfected to establish four conditions: one control (EV+Co siRNA) and 3 TPM2 siRNA knockdown (EV+TPM2 siRNA, TPM2-WT+TPM2 siRNA, and TPM2-238*+TPM2 siRNA). **C** 4 C2C12 cell conditions western-blotted for TPM2, Flag, and GAPDH. Flag detection in TPM2-WT indicates successful transfection. TPM2 endogenous expression in overexpressed TPM2-238* cells attenuated beyond siRNA-induced knockdown alone. **D** C2C12 spreading assay images at t = 60min, 60min, 60min, and 20 min (left to right); blue DAPI-stained nucleus and red phalloidin-stained cytoskeleton. Cytoplasmic:nuclear ratio at 20, 40, and 60 minutes. Significant differences between group-mean relative cell areas identified at 40 and 60 minutes.

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Is Prune Belly Syndrome (PBS) a form of Myosin Heavy Chain 11 (MYH11) and Myosin Light Chain Kinase (MYLK) Smooth Muscle Myopathy (SMM)?

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Abstract

SMM are a group of autosomal dominant or recessive genetic disorders leading to dysfunctional vascular and/or visceral SM, presenting in neonates to adults. Depending on affected gene and specific mutation, the phenotypic spectrum encompasses different diseases including Thoracic aortic aneurysm (TAA) and MMIHS. Many cases of SMM are caused by DNA variants in MYH11 and MYLK. Using a biased approach, we investigated whether PBS might be associated with MYH11 and MYLK variants.

With IRB-approval, PBS probands were prospectively enrolled. Proband blood lymphocyte DNA underwent paired-end Whole Exome Sequencing (WES) using the Illumina SureSelect kit and HiSeq2500 sequencer, with data analysis. Variants were filtered for rare minor allele frequency (<0.001) and their potential functions were predicted using in silico-based computational analysis. Variants meeting at least five of the following criteria were selected as suspected functional variants: SIFT < 0.1, Polyphen2 > 0.9, CADD score > 20, GERP > 4, MutationTaster = “A” or “DC”, Mutation Assessor= “M” or “H”, and Vest > 3.00. After Sanger sequencing confirmation, familial segregation was investigated on available samples.

132 PBS probands underwent WES. After variant filtering, 13 probands demonstrated 12 rare or novel heterozygous missense variants in MYH11 or MYLK (Table1). Previously, 8 of 12 variants have been associated with TAA and classified as uncertain significance on ClinVar due to lack of functional characterization. Four MYH11 variants (R108W, A699V, T1565M and K1628Q) are maternally inherited from asymptomatic mothers and one variant (A699V) was also found in a non-affected brother, suggesting incomplete penetrance or additional modifier genes in the proband.

Different mechanisms of MYH11 or MYLK disruption, tissue specific regulation and unique mutant protein interactions may yield distinct patterns of SM dysfunction and can lead to distinct rare pathologies. Thus, longitudinal studies on PBS cases harboring these SMM genes are needed to assess risk for life-threatening TAA for long-term health counseling. Our data suggest that PBS is a heterogeneous and complex disorder that can be explained in part by mutations in MYH11 and MYLK.

TABLE 1: MYLK and MYH11 PBS variant

Patient ID	Gene	Ethinicity	Amino Acid Variant	Prior Disease Report	Familial Inheritance	Conservation	MAF	SIFT	PolyPhen-2	Vest	Mutation taster	Mutation Assessor	GERP	CAAD
PBS-1	MYH11	WNH	R108W	NPR	MAT-MP	High	~0	DE(0)	DA(1)	-	-	-	-	22.8
PBS-2	MYH11	WNH	R676C	TAAD	NA	High	0.0008369	DE(0.016)	DA(0.954)	0.878	DC(1)	M(3.17)	4.43	31
PBS-3	MYH11	WNH	A699V	TAAD	MAT	High	0.0003897	DE(0)	P(0.561)	0.595	DC(1)	M(2.02)	4.94	25.2
PBS-4	MYH11	WNH	T1565M	TAAD	MAT	Moderate	0.00068	DE(0.081)	P(0.896)	0.326	DC(0.998997)	M(1.96)	4.97	24.1
PBS-5	MYH11	WNH			MAT									
PBS-6	MYH11	WNH	K1628Q	TAAD	MAT	High	0.00009942	DE(0.027)	P(0.732)	0.455	DC(1)	M(2.91)	4.95	24.6
PBS-7	MYLK	WH	R86W	TAAD	NA	High	0.0001433	DE(0.013)	DA(0.997)	0.606	DC(0.980753)	L(1.64)	4.31	25.8
PBS-8	MYLK	AA	E98A	NPR	NA	High	~0	T(0.15)	P(0.644)	-	-	-	-	23.1
PBS-9	MYLK	WNH	E424V	NPR	NA	High	0.000007982	DE(0.044)	DA(0.963)	0.206	DC(0.948512)	NE(-0.05)	5.43	28
PBS-10	MYLK	WNH	R538W	TAAD	NA	Low	0.0000279	DE(0.013)	P(0.599)	0.213	N(0.995127)	L(1.43)	3.9	23.3
PBS-11	MYLK	WH	A1160P	NPR	NA	Moderate	0.00001193	DE(0.005)	DA(0.975)	0.621	DC(0.999835)	L(1.73)	5.01	26.8
PBS-12	MYLK	WNH	R1250H	TAAD	NA	Moderate	0.00005965	T(0.06)	DA(0.978)	0.574	DC(0.999591)	M(2.515)	5.54	25.4
PBS-13	MYLK	WNH	E1446K	TAAD	NA	High	0.0001086	DE(0.036)	-	0.487	DC (0.999997)	L(0.805)	4.82	23.5

WNH=white non-hispanic		WH=White hispanic		AA=African American		TAAD=Familial thoracic aortic aneurysm and dissection		NPR=not previoulsy report	MAT=maternal
MP=multiplex	NA=not available	MAF=Minor allele frequency		DE= deleterious		T=tolarated	P=probally damaging	DA=Damaging	Vest=variant effect scoring too
DC=disease causing		N=polymorphic	L=low	M=medium	NE=neutral	CAAD=Combined Annotation Dependent Depletion		GERP=Genomic Evolutionary Rate Profiling	

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SF3B1 mutation and over-expression causes AR variant formation and drug resistance in mCRPC

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Abstract

Background: AR antagonists which inhibit the full-length AR failed to show significant improvement in overall survival of castration-resistant prostate cancer (CRPC) patients. It is now widely believed that the resistance to AR-targeted agents is due to the presence of AR spliced variants.

Objective: SF3B1, an essential spliceosome component, modulates the mRNA splicing process in normal tissues; however, its role in prostate cancer (CaP) remains understudied. We posit that increased levels and hotspot mutations in *SF3B1* cause (i) variants generation of AR and (ii) drug-resistance emergence in CRPC.

Result: SF3B1 mRNA and protein are highly expressed in CaP and associated with decreased survival in patients. The analysis of genomic data of <4,000 patients show missense, the gain of function, and amplification-mutations in SF3B1 gene in (10 %) pT3b stage cases and neuroendocrine CaP. High-grade CaP exhibits hot-spot mutations (K66N, K700, N626S, K741) in the SF3B1 gene. Notably, RP-resected tumors of GS (3+4; 4+4) exhibited a high mutation count (20-50) in SF3B1. The microsatellite instability score of <10 was noted for SF3B1 mutations in CaP. We asked if SF3B1 induction is related to the therapy (Docetaxel, Estramustine, Mitoxantrone, Carboplatin, Abiraterone) exposure. SF3B1 was elevated in cases receiving Mitoxantrone, and hotspot mutations were detected in 15% of patients receiving Abiraterone. Notably, <25% of samples with SF3B1 mutation exhibited NEPC features. Whereas tumors harboring SF3B1 mutation exhibit alterations in AR, BMI1, TTN, and HSPD1, the cases with SF3B1+/BMI1+ show increased AR-V splicing. Functional studies were conducted to measure the impact of SF3B1 overexpression or mutation showed that SF3B1-overexpression promotes the growth of drug-resistant (Docetaxel, Cisplatin) CaP cells. Although CaP models exhibited elevated levels of SF3B1, none of the models (PC3, LNCaP, DU145, VCaP) exhibit SF3B1 mutation except the RCCT7/E model. SF3B1-overexpression is positively correlated with the formation of splicing forms (variants) of *AR*, MDM2, and MYC in cells. Notably, the induction of SF3B1 decreased the full-length AR and increased the ARVs. SF3B1-mutant clones exhibit NECP features. Activated SF3B1 in co-operation with BMI1 sustains the AR signaling and chemoresistance in CRPC cells. Finally, we showed that genetic and pharmacological targeting of SF3B1 inhibits metastasis of CRPC cells.

Conclusion: SF3B1-targeted therapies as an adjuvant could improve the outcome in mCRPC.

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Myeloid Differentiation Protein 2: Driver of metastasis and immunosuppression in prostate cancer

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Abstract

Background: Metastatic prostate cancer (mCaP), usually treated with androgen deprivation therapy, acquires resistance to develop into lethal castration-resistant prostate cancer (CRPC). The cause of resistance and CRPC development remains elusive, and the lack of an ideal biomarker predictive of CRPC emergence is a stumbling block in the management of the disease.

Objective: We provide strong evidence that Myeloid Differentiation Protein 2 (MD2) plays a critical role in CaP progression, metastasis, and generating an immunosuppressive tumor microenvironment (TME).

Method and Results: The analysis of tumor genomic data and IHC of tumors showed a high frequency of *MD2* amplification and its association with poor overall survival in CaP patients. The Decipher-genomic test of RP-patients validated the potential of *MD2* in predicting metastasis. Functional studies conducted using *in vitro* CaP progression models showed that MD2 confers invasiveness by activating MAP Kinases and NF-kB signaling pathways and the expression of pro-metastatic cytokines. Furthermore, we show that mCaP cells secrete MD2 (sMD2) protein into the TME. Next, we measured serum-sMD2 in CaP patients using ELISA. We show that the serum-sMD2 level is correlated to the disease progression in patients. We determined the functional role of secretory MD2. We found that extracellular MD2 protein induces high levels of CCL2 chemokine in endothelial cells. We show that MD2 induces drug resistance by inducing PARP activity and the release of HMGB into the TME. Notably, PARPi-resistant CaP cells secreted high levels of sMD2 protein. Because HMGB and CCL2 are known to cause immune suppression, we asked if MD2 has a role in immunosuppression in CaP. We measured the immune cell profile in prostate tumors exhibiting different levels of MD2 by using confocal microscopy and found that high levels of MD2 promote increased recruitment of immunosuppressive MDSCs and Tregs in the tumor zone. Next, we determined the significance of MD2 as a therapeutic target by employing genetic modification (overexpression or knockdown) and pharmacologic inhibition in tumor cells. The targeting of MD2 was observed to inhibit metastasis of CaP cells in a murine model. **Conclusion:** We conclude that serum-MD2 is a non-invasive biomarker for mCaP, whereas biopsy-*MD2* predicts the disease outcome in CaP. We suggest that MD2-targeted therapies could be developed as potential treatments for aggressive types of metastatic CaP, and current data warrants further investigation to study the role of MD2 in PARPi-resistance.

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Characterization of Androgen Receptor inverse agonists in prostate cancer

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Abstract

Background: Castration-resistant prostate cancer (CRPC) is a lethal disease driven by amplified androgen receptor (AR) expression and activity. The mainstay clinical therapeutic, enzalutamide, acts as an AR antagonist in order to prevent AR transcriptional activity. While effective for a time, resistance will inevitably occur, largely through genetic mechanisms that drive higher AR expression. In order to target the AR more effectively, we designed the therapeutic BG-15n. We hypothesize BG-15n acts with a unique mechanism of action as an AR inverse agonist to promote AR localization to the nucleus, downregulation of AR-target genes, and decreased prostate cancer cell growth.

Methods: Structure-guided organic chemistry paired with AR response element driven luciferase measurements were utilized to design the lead BG-15a molecule. A diverse panel of 10 CRPC cell models were used in cell growth inhibition experiments. Chemical informatics and pharmacokinetic studies *in vivo* were used to optimize pharmacodynamics of BG-15a to produce the BG-15n molecule. LAPC4 xenograft models, and a panel of LuCaP patient-derived xenograft (PDX) models were further utilized to assess BG-15n efficacy and mechanism of action. RNA-seq in PDX models and CRPC cell lines was used to measure AR transcriptional output and paired with ChIP-seq and CUT&RUN to characterize chromatin changes. AQuA-HiChIP was used to determine BG-15n induced changes to the 3D folding of active AR-bound enhancers to AR target genes.

Results: We found BG-15a/n bind to AR with a higher efficacy than enzalutamide, and decrease cell and PDX growth, even in models harboring AR amplification or point mutations. Treatment with BG-15a promotes localization of the AR into the nucleus. Despite nuclear localization, RNA-seq data and genset enrichment analysis demonstrate downregulation of androgen response pathway genes and MYC pathway genes in response to BG-15n. AR inverse agonist activity is further supported by ChIP-seq data indicating increased AR binding at AR-target gene enhancers in BG-15n treated cells. AQuA-HiChIP revealed 3D contacts between AR bound enhancers and AR targets influenced by BG-15n.

Conclusions: These studies indicate BG-15n as an effective therapeutic for the treatment of CRPC with a novel mechanism of action as an AR inverse agonist. Ongoing studies of coactivator and corepressor recruitment, 3D genomic folding, and potential mechanisms of resistance are aimed at elucidating the BG-15n mechanism of action in preparation for clinical application.

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Novel dual inhibitors of AKR1C3 and AR/AR variants reduce androgen signaling and inhibit tumor growth

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Abstract

Background: AKR1C3 and AR/AR-Variants play key roles in prostate cancer progression and drive resistance to antiandrogens. There are no clinically available therapies that specifically target either AR-Variants or AKR1C3, nor are there dual inhibitors for concurrent targeting of AR/AR-Variants and AKR1C3. We have developed a number of novel small molecules (LX) that are dual inhibitors of AR/AR-Variants and AKR1C3.

Methods: A library of novel compounds (LX) was designed and synthesized according to structure based computer modeling. Cell growth assays were used to assess their efficacy at inhibiting CRPC growth. The effects of the LXs on AR/AR-Variants and AKR1C3 expression were evaluated by Western blot. PSA-luciferase assays were used to determine effects on AR signaling activity. RNA-seq was performed on selected LXs (LX-1 and LX-5). Resistant cell sublines generated from C4-2B cells resistant to enzalutamide (MDVR), apalutamide (ApalR), darolutamide (DaroR), or abiraterone (AbiR) were treated with LX-1 or their respective antiandrogen and cell number was determined. Mice bearing VCaP xenograft tumors and LuCaP35CR PDX tumors were treated with LX-1 and effects on tumor growth were assessed.

Results: Of the 14 LX compounds, LX-1, followed by LX-5, had the greatest effect at reducing cell number, AR/AR variant expression, and AKR1C3 activity. PSA-luciferase activity was greatly reduced by both LX-1 and LX-5. RNA-seq analysis showed a robust reduction in AR and AR-V7 signaling gene expression by LX-1 and LX-5. MDVR, ApalR, DaroR, and AbiR cells all had a reduction in cell number when treated with LX-1. Co-treatment of LX-1 and anti-androgens in resistant lines improved treatment response. LX-1 inhibited conversion of the testosterone precursor androstenedione into testosterone in AKR1C3 overexpressing C4-2B and LNCaP cells. Additionally, LX-1 treatment reduced testosterone production by LUCaP35CR tumor cells which express high levels of AKR1C3 in the presence of androstenedione in a dose-dependent manner *ex vivo*. Treatment with LX-1 reduced tumor growth in both VCaP and LuCaP35CR PDX models and reduced intratumoral testosterone.

Conclusions: We generated novel small molecule inhibitors that dual target AKR1C3 and AR/AR variants. These compounds, specifically LX-1, effectively reduce CRPC growth *in vitro* and *in vivo,* suggesting potential for treating advanced prostate cancer.

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Targeting citron kinase activity overcomes prostate cancer treatment resistance

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Abstract

Background: Mechanistically novel therapies are needed to overcome treatment resistance that causes >30,000 American prostate cancer (CaP) deaths annually. We explore citron kinase (CIT), well-known for controlling cell division, as a target for such therapies.

Methods: Cell viability, cell proliferation, cell cycle analyses, or CaP take rate was assessed in cell lines, xenografts and organoids. CIT was studied in patient CaP specimens representing treatment-naïve or -resistant CaP. Genes of interest were silenced, overexpressed, mutated or pharmacologically inhibited. RNA-Seq and mass spectrometry were used to define CIT-dependent genes, interactors and substrates. PCTA, TCGA, rMATS, GSEA and MSigDB analyses were performed to validate their biological and clinical relevance. Kinase assays were done to isolate CIT inhibitors.

Results: CIT expression, which increases during CaP progression, was induced by growth stimuli, and CIT overexpression caused CaP tumor formation, growth and treatment resistance. In benign prostate epithelial cells, CIT induced cell proliferation and stem cell and pluripotency markers. Conversely, CIT silencing in treatment-naïve, castration-resistant and treatment-induced neuroendocrine CaP cells delayed cell proliferation in vitro and CaP progression in vivo but did not impact benign prostate epithelial cells. CIT and CIT-dependent genes and splicing events associated with aggressive CaP progression were enriched in treatment-resistant CaP and correlated strongly with adult stem cell signatures. Induction of CIT depended on cell cycle progression, revealing a novel role for CIT in interphase where expression was detectable in S phase and increased further in G2/M phase. CIT protein overexpression occurred via a novel signaling axis involving the cell cycle regulators E2F2, Skp2 and p27; a decrease in p27 after E2F2-Skp2 transduction increased CIT. CIT's control over cell proliferation relied entirely on its intact kinase domain. Combining analyses of the target spectrum of clinically evaluated kinase inhibitors and in *in vitro* CIT kinase assays, we isolated the kinase inhibitor OTS-167 as a CIT inhibitor with an IC50 of ~10nM that mimics the effects of CIT silencing on CaP cell proliferation, CIT-dependent gene expression, and phosphorylation of newly identified CIT substrates.

Conclusions: CIT is a previously unrecognized but critical determinant of CaP cell proliferation and a novel druggable target for CaP treatment, suggesting the possibility of entirely new CIT-targeting therapies.

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The role of DNA methylation during chronic obstructive bladder disease (COBD) in function and pathology.

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Abstract

Partial bladder outlet obstruction (PBO) is a widespread cause of urinary dysfunction, patient discomfort and leads to immense health care costs. Even after removal of obstruction, the pathophysiology continues as a chronic obstructive bladder disease (COBD). Previously we found that DNA methylation inhibition exacerbates pathology of 6-week obstruction. Here, we tested the effects of inhibition after relief of obstruction in COBD. METHODS: COBD was created in female Sprague-Dawley rats by partial urethral ligation for 6 weeks, followed by removal of the suture for another 4 weeks. Sham ligations were performed by passing the suture behind the urethra. Post-obstruction, animals were randomized to either DAC treatment (1 mg/kg/3-times/week IP) or normal saline (NS) for 4 weeks. Bladder function was non-invasively tested in metabolic cages, both 1 day prior to de-obstruction and prior to sacrifice. Residual volume and bladder mass were measured after sacrifice. Collagen type I, cell signaling and in situ gelatinase activity were examined by immunostaining. DNA methyltransferase (DNMT)-3A overexpression in bladder smooth muscle cell (SMC) was followed by gel contraction assays and immunostaining. ANOVA was performed with post-hoc t-tests, with $p<0.05$ considered significant. RESULTS: Compared to COBD NS, COBD DAC treatment induced a significant recovery of voiding efficiency. DNMT3A overexpression led to a loss of contractile function. Deposition of collagen and SMC area was reduced by DAC, but bladder mass alongside ERK signaling remained elevated even with DAC. Conclusions: The effect of DNA methylation inhibition during COBD (vs. 6 week obstruction) enhances pathologic and functional recovery of persistently altered smooth muscle.

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Prostate epithelial cell-derived DAMPs drive mast cell activation to release type-2 cytokines associated with prostatic inflammation

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Abstract

Background: Prostatic inflammation is a crucial contributor to various benign prostatic diseases leading to lower urinary tract symptoms (LUTS). An intraurethral infection with a uropathogenic isolate of *E. coli* (CP1) results in clinical signs of benign prostatic hyperplasia (BPH) and LUTS as demonstrated by increased urinary dysfunction, prostate inflammation, and fibrosis associated with type-2 cytokine secretion. Mast cells are elevated in the prostate and therapeutic inhibition of mast cell function alleviates inflammation as well as symptoms suggesting that mast cells play a critical role in the pathophysiology of urinary dysfunction. In this study, we dissect the molecular triggers and signaling events leading to the activation of mast cells in the context of prostate inflammation.

Results: We observed that stimulation with conditioned cell supernatants (CCs) from prostate epithelial cells infected with CP1 showed activation of mast cells as seen by increased production of TNFα, IL4, and IL13. Furthermore, this effect was unique for stimulation using CCs from live bacterial infection of prostate epithelial cells compared to heat-killed (HK) CP1. To understand unique molecular triggers in live CP1 infected prostate epithelial cell supernatants, proteomic analysis of the supernatants was performed comparing them to supernatants from HK CP1 treated epithelial cells. We identified a series of danger-associated molecular pattern (DAMP) ligands that were shown to be capable of triggering mast cell activation and the release of TNFα, IL4, and IL13. We further demonstrated using small molecule inhibitors that cognate receptors for DAMPs on mast cells were critical for these molecular ligands to drive mast cell activation.

Conclusions: These results demonstrate an important connection between prostate epithelial cell damage and activation of prostate mast cells thereby resulting in the regulation of prostate inflammation. An understanding of the role played by endogenous DAMPs in triggering this process would be very informative in understanding the development of prostate inflammation in men with BPH/LUTS.

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Stromal- Epithelial Crosstalk in Benign Prostatic Hyperplasia

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Abstract

Background: The prevalence of benign prostatic hyperplasia (BPH) is approximately 50% for men between the ages 51-60, and 90% for men over 80 years. The molecular effectors/signaling mechanisms that orchestrate BPH are largely elusive. Therefore, a detailed understanding of the molecular mechanisms that underlie BPH development and progression will shed light on the potential therapeutic modalities to target BPH.

Methods: Immunohistochemical (IHC) analysis was performed on prostate tissue sections obtained from the transition zone (TZ) of patients who underwent surgery to relieve LUTS (surgical BPH, S-BPH), or TZ from patients who underwent radical prostatectomy to remove low-grade prostate cancer (incidental BPH or I-BPH).

Results: Our analysis revealed upregulation of Early Growth Response Gene-1 (EGR1) - a transcription factor in the S-BPH samples as compared to I-BPH. Further, EGR1 expression was detected in both - epithelial and stromal components of BPH. In an effort to investigate the downstream effectors of EGR1 in BPH, we performed shRNA-based loss-of-function studies to knock-down EGR1 expression in BPH-1 cells. Our results suggest that knock-down of EGR1 in BPH-1 cells reduces the expression of IL-6 and TGF-β1, that are known to play prominent roles in BPH pathogenesis.

Conclusions: EGR1 signaling promotes the development and progression of BPH potentially via regulating IL-6 and TGF-β1, and in turn orchestrates proliferation, inflammation, and fibrosis – the hallmarks of BPH.

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Osteoblasts induce prostate cancer cell dormancy via Cldn19-dependent physical contacts

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Abstract

Background: Advanced cancers progress and relapse after remission. It was believed that cancer cells disseminated and maintained dormant during remission. Therefore, keeping tumor cells dormant can be an “operational cure” for these patients. We found disseminated prostate cancer (PCa) C4-2B cells in various organs of mice xenografted with primary tumors, but only in bones of the mice from which the xenografted tumors were removed. No bone metastatic symptoms were detected in our hands, suggesting that the disseminated C4-2B cells are dormant in the bone microenvironment (BME). Thus, we aim to determine how the BME causes PCa dormancy.

Methods: We injected C4-2B cells subcutaneously and randomized the mice into two groups, where the tumors were either surgically removed or not at week 2. At the end time point of week 6, we semi-quantified C4-2B cells in various mouse organs via human-specific genomic PCR. To delineate the type(s) of cells in the BME that caused C4-2B dormancy, we co-cultured C4-2B cells in direct contact with either osteoblasts MC3T3-E1, macrophages Raw264.7, mesenchymal progenitors OP-9, or fibroblasts NIH3T3, and compared them with the C4-2B cells cultured alone. We analyzed the transcriptomes of the co-cultured C4-2B/MC3T3-E1 cells and cells cultured alone via RNA-Seq and analyzed published RNA-Seq datasets of MC3T3-E1, Raw264.7, OP-9 and NIH3T3. Representative signature genes extracted from the analyses were confirmed via real time qPCR.

Results: Compared to C4-2B cultured alone, the proliferation of C4-2B cells were inhibited only when co-cultured with MC3T3-E1, but not when co-cultured with other stromal cells. Increases of dormancy markers such as NR2F1 and AXL, decreases of proliferation markers such as Ki67 and Cyclin D1 were observed in the C4-2B/MC3T3-E1 co-culture, suggesting that osteoblasts induced C4-2B cells into a dormancy-like state. Consistently, the transcriptome analyses showed that the significantly down-regulated genes were enriched in the mitochondrial ATP synthesis, oxidative phosphorylation, and electron transport chain; the significantly up-regulated genes were enriched in the epithelial mesenchymal transition and responding to stimulus or stresses. Transwell coculture (no direct contact) did not recapitulate the gene expression changes induced by direct co-culture; however, treatment of C4-2B cells with conditioned medium (CM) from C4-2B/MC3T3-E1 direct co-culture, but not CM from transwell co-culture or MC3T3-E1 alone, partially decreased the proliferation of C4-2B cells, suggesting the physical contact with MC3T3-E1 is necessary to induce C4-2B dormancy. *In silico* analyses of MC3T3-E1 cells vs. other stromal cells that did not induce C4-2B cell dormancy suggested that tight junction genes such as *Cldn19* were enriched in MC3T3-E1. *Cldn19* knockdown in MC3T3-E1 partially attenuated C4-2B dormancy in the co-culture.

Conclusions: The BME can keep the disseminated C4-2B cells dormant, at least within the examined time duration. Osteoblasts induce and maintain C4-2B cell dormancy, which requires the physical contacts between osteoblasts and C4-2B cells. The required physical contacts are partially mediated by CLDN19 in osteoblasts.

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Loss of Thrombospondin-1 in Macrophages as a Promoter of Benign Prostatic Hyperplasia

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Abstract

Background: Benign prostatic hyperplasia (BPH) affects the vast majority of aging men, with nearly half of men exhibiting moderate to severe lower urinary tract symptoms (LUTS) before age 80. Prostate inflammation is linked to fibrosis, hyperplasia, and reduced responses to BPH-related medical therapies. Thrombospondin-1 (TSP1) is a matricellular glycoprotein known to have anti-angiogenic, anti-inflammatory, and extracellular matrix remodeling functions with TSP1 knockout mice displaying greater prostatic hyperplasia compared to control mice. These studies were conducted with the hypothesis that TSP1 may be a mechanism of inflammation-mediated BPH progression.

Methods: Single-cell mRNA-sequencing (scRNA-seq) studies were conducted on the CD45+ inflammatory cells from the transition zone of small (<40 grams) and large (>90 grams) human prostates. Analysis of differentially expressed genes (DEGs) and evaluation of primary macrophage, fibroblast, and epithelial cell types *in vitro* were used to identify novel pathways that may be linked to promoting inflammation in BPH tissues. TSP1 expression was evaluated with immunofluorescence and ELISA, and modulation of TSP1 expression was completed using siRNA or LSKL peptides.

Results: TSP1 was identified as one of the top 10 downregulated DEGs when comparing leukocytes from large *versus* small prostates, suggesting loss of this molecule as prostate size increases and LUTS worsen. Immunofluorescence staining indicated that TSP1 is primarily secreted by BPH macrophages. *In vitro* analysis of TSP1 function in THP-1 cells revealed that pro-inflammatory M1 macrophages secrete less TSP1 than anti-inflammatory M2 macrophages. Additionally, inhibition of TSP1 in M1 macrophages using LSKL peptides induced higher secretion of pro-inflammatory cytokines IL1b and IL6 *versus* control SLLK peptides.

Conclusions: TSP1 is secreted by BPH macrophages but downregulated during clinical BPH progression. TSP1 loss may aid in promoting a pro-inflammatory macrophage phenotype and stimulate additional inflammation within the BPH microenvironment. Ongoing work is aimed at determining the consequences of TSP1 loss on stromal and epithelial proliferation or exacerbation of LUTS.

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Circular RMST may regulate ASCL1 in neuroendocrine tumours of the prostate and lung

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Abstract

Background

Neuroendocrine prostate cancer (NEPC) and small cell lung cancer (SCLC) are two poorly differentiated neuroendocrine tumours that share many common genetic and epigenetic alterations. Both have poor clinical outcome and require better disease management. Circular RNA (circRNA) is a class of noncoding RNA that has been reported to drive the development of multiple cancer types including localized prostate cancer. However, the function in NEPC and neuroendocrine tumours remains unknown. The goal of the study is to investigate the functions of circRNA in driving aggressive neuroendocrine tumour phenotype.

Methods

We conducted total RNA sequencing of 5 castrate-resistant prostate cancer (CRPC) and 5 NEPC. circRNA was annotated by circExplorer and differential analysis was done. Candidate circRNAs were selected for downstream functional studies including shRNA/siRNA knockdown and overexpression in *in vitro* cell lines to study changes in proliferation, invasion/migration and gene expressions.

Results

We identified circular RMST (circRMST) as one of the most differentially expressed circRNA with very high expression in NEPC. We further found that it is also highly expressed in SCLC and other neuroendocrine tissues. RMST has been previously studied as a long noncoding RNA that regulates neurogenesis and stem cell differentiation. However, our bioinformatic analysis and RNase R treatment indicate that more than 90% of RMST transcripts predominantly exist in the circular form. circRMST knockdown reduced growth in NEPC and SCLC models and overexpression promoted invasion and migration. ASCL1, a well-known driver of multiple neuroendocrine tumours, was identified to be a downstream target of circRMST through RNA-sequencing and was validated with western blots. In addition, ASCL1-high or circRMST-high tumours were found to have low immune activation, suggesting a potential immune suppressive role.

Conclusion

circRMST was identified to be highly expressed in NEPC and SCLC and can regulate ASCL1 levels. Since ASCL1 is reported to drive other neuroendocrine tumours such as small cell cancer of esophagus,

circRMST may be functional in a pan-neuroendocrine tumour context. For future experiments, we aim to develop lipid nanoparticle-packaged siRNA to target circRMST *in vivo* and study its effect on growth and immune activation. We aim to reveal new therapeutic strategies and biomarkers for neuroendocrine tumours.

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Identification of novel combination therapies for neuroendocrine prostate

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Abstract

Identification of novel combination therapies for neuroendocrine prostate

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Background: Approximately 34,130 men are projected to die from prostate cancer in the U.S. in 2021, making it the second leading cause of male cancer death. Advanced prostate cancer, whether present at the time of diagnosis or arising post treatment of localized disease, responds to androgen deprivation, but invariably fails and recurs as castration-resistant prostate cancer (CRPC) which is the main cause of prostate cancer-associated mortality. Current therapies for CRPC extend the patient life expectancy by only a few months. Heavily treated tumors, particularly those treated with secondary hormone therapies, frequently acquire a neuroendocrine phenotype (NEPC), which currently accounts for 15-20% of CRPC. NEPC is commonly characterized by expression of neuroendocrine markers, an aggressive clinical course, and downregulation or loss of androgen receptor (AR) that diminishes responsiveness to androgen deprivation therapies, making it the most lethal and currently incurable subset of prostate cancer. Utilizing proteomic profiling, our recent studies identified PARP1 as the most up-regulated druggable protein in a model of NEPC and human treatment-induced NEPC. Moreover, we demonstrated that PARP1 inhibitors suppress tumor growth, inhibit metastasis, and reverse of neuroendocrine features of NEPC. Herein, we further designed and performed an *in vivo* time-lapse proteomic profiling in NEPC xenografts to determine dynamic protein changes and lineage shift under PARP1 inhibition, and to identify novel combination therapies for NEPC.

Methods: To gain insights into the mechanisms underlying the reversible lineage phenotype of NEPC under PARP1 inhibition, we performed a time-lapse analysis of global and dynamic protein changes over the course of treatment of NCI-H660 NEPC tumors with PARP1 inhibitors using mass spectrometry. Individual protein waves and patterns were clustered and plotted as trend diagram based on up-regulation and down-regulation over the time-course of the treatment with PARP inhibitors. Global protein changes were analyzed upon treatment with PARP inhibitors. The effect of PARP inhibitors alone or in combination therapy settings on prostate cancer growth, metastatic colonization, and neuroendocrine phenotype was evaluated in vivo by subcutaneous xenograft implantation and intracardiac injection in male NSG mice.

Results: We performed an *in vivo* time-lapse proteomic profiling in NEPC tumor xenografts to delineate dynamic protein changes upon PARP1 inhibition and to identify new combination therapies for NEPC. Functional network analysis of the up-regulated proteins revealed dramatic changes in a time-dependent manner upon treatment with PARP inhibitors in multiple pathways such as mitotic spindle

microtubule organization, ATAD2-hedgehog signaling along with reversal of NE features, and restoration of AR expression and thus new potential therapeutic strategies to target treatment-induced NEPC.

Conclusions: We previously demonstrated that PARP1 inhibitors, olaparib and talazoparib, suppress tumor growth, inhibit metastasis, and reverse of neuroendocrine features of NEPC. Herein, our new findings reveal effective combination therapies with PARP inhibitors for NEPC including taxanes, second-generation antiandrogens, and pre-clinical ATAD2-hedgehog signaling inhibitors.

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The Role of AR-V12 in Prostate Stroma on Androgen Activity and Epithelial Differentiation

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Abstract

The Role of AR-V12 in Prostate Stroma on Androgen Activity and Epithelial Differentiation

Author:

Candice Loitz

Background:

Many androgen splice variants have been identified in cancer cell lines and prostate epithelium, both malignant and benign. We discovered that androgen receptor variant V12 (AR-V12) mRNA and protein are present in benign patient-derived prostate stroma and at concentrations which suggest functionality. AR-V12 differs from endogenous AR in that it is lacking exons 5, 6 and 7 (the ligand binding domain). Herein, we will examine the biological role of AR-V12 in patient-derived prostate stroma and how it affects the androgen activity within stromal cells as well as paracrine effects on prostate epithelial organoid growth, differentiation and morphology.

Methods:

Patient-derived stromal cells were cultured for no more than 5 passages. RT-qPCR and immunoblotting was used to determine AR full length and AR-V12 mRNA and protein levels.

Results:

AR-V12 and AR-FL mRNA was detected in prostate stromal cell lines. We further observed AR-V12 mRNA and protein in patient-derived stromal cells from ten patients. Treatment with androgen increased the levels of AR-V12 protein, but not mRNA, suggesting increased protein stability.

Conclusions:

A plethora of research has been conducted looking at androgen receptor transcriptional activity in epithelium and cancer cell lines. Yet, even though it is known that stromal androgen receptor activity is required for the development of the prostate and maintenance of epithelium, there is a paucity of research investigating the role of androgen receptor signaling in prostate stroma. Furthermore, it has been demonstrated in recombinant mouse models and in *in vitro* cell culture models that androgen receptor positive stroma is required for the malignant transition of epithelium. Ongoing experiments include identifying the role of AR-V12 in benign stroma and an organoid model to determine how stromal AR-V12 affects the growth/proliferation/differentiation of epithelium. Investigating the role androgen receptor variants play in benign stroma is an important biological question which may reveal pathways implicated in carcinogenesis of epithelium.

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Basal-luminal hybrid cells emerge as a new dormant, androgen pathway directed therapy-resistant population in patient-derived prostate cancer organoids.

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Abstract

Background: Androgen pathway directed therapy (APDT) can help maintain remission in advanced prostate cancer (PCa) patients with bone metastases, however, growth and metastatic spread often recur.

Objective: To address the need for more predictive pre-clinical research platforms and to identify new targets and therapies for bone metastatic castration-resistant prostate cancer (CRPC).

Methods: We used patient-derived xenograft (PDX) tumors from bone metastatic prostate cancer patients to establish three-dimensional (3D) organoids and investigated their response to APDT by either withholding di-hydro-testosterone (no DHT) or treating with enzalutamide. Cyst/spheroid quantitation, immunohistopathology, cell viability assay, qRT-PCR, RNA sequencing, gene set enrichment analysis (GSEA) and live cell cycle tracking using *Fucci2BL* were performed in PDX derived tumor organoids and compared to PCa Cell lines: PC3, DU145 and LNCaP.

Results: APDT resulted in CRPC spheroids with $CK5^+$ $CK8^+$ cells, up-regulated stem-cell transcription factors, steroidogenic and neurogenic pathways and down-regulated AR-target genes, interferon, cell cycle, cell division and circadian pacemaker pathways. Enzalutamide-treated spheroids transitioned to G_0 and AR protein was decreased but not AR mRNA. Moreover, APDT decreased both ACE2 and TMPRSS2, host cell viral entry factors for the severe acute respiratory syndrome (SARS) SARS-CoV-2.

Conclusions: In organoids, or mini-tumors, established from prostate cancer bone metastasis PDXs, a novel type of dormant APDT-resistant cell with specific gene changes emerged which may be targeted in order to eradicate dormant metastases before they can progress. This study identified a new dormant CRPC basal-luminal hybrid prostate cancer cell and gene signature which may be therapeutically targeted to eradicate dormant CRPC bone metastases in order to prevent disease recurrence. ADT also reduced the cell factors required for SARS-CoV-2 or its variants to infect its host cells and thus may reduce COVID-19 disease severity. The PDX organoid models can be used to screen for therapies that target the dormant CRPC cells and that reduce ACE2 and TMPRSS2 expression to suppress viral load of SARS-CoV-2 and its variants.

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Activation of PPARG Signaling Facilitates Renal Urothelium Remodeling Following Urinary Tract Obstruction in Mice

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Abstract

Urinary Tract Obstruction(**UTO**) is a leading cause of pediatric kidney disease. No treatments can prevent obstruction-induced kidney injury, but heterogeneous outcomes implicate unknown protective mechanisms. The kidney adapts a bladder-like renal urothelium during congenital and acquired UTO, whereby the renal urothelium forms an apical layer of urothelial plaque producing uroplakin+ (**Upk**) cells which preserve renal parenchyma and kidney function during UTO. The cellular and molecular basis of renal urothelium adaptation is unclear. The PPARG signaling pathway regulates bladder and ureteral urothelial differentiation, but a role for this pathway has not been investigated in renal urothelium. Here, we investigated the PPARG signaling pathway during UTO. We conducted lineage analysis studies (using *Upk2*^{CreERT2};*R26*^{tdT}mice) and inducibly depleted Upk cells (using *Upk2*^{CreERT2};*R26*^{DTR} mice) in mice subjected to unilateral ureteral obstruction or Sham surgery. Compared to unobstructed controls, tdT+;Upk+ cells in obstructed kidneys express *de novo* PPARG and FABP4 - a transcriptional target of PPARG signaling and Umbrella cell marker. Failure to activate the PPARG signaling pathway is observed in obstructed mice with genetic depletion of Upk cells. Our results indicate that UTO-induced bladder-like remodeling is achieved through activation of the PPARG signaling pathway in the Upk-lineage. A thorough understanding of UTO adaptation may reveal mechanisms with therapeutic utility for mitigating obstructive kidney injury in children with UTO.

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IFN-g IS A POTENTIAL REGULATOR OF HLA-E-MEDIATED IMMUNE EVASION IN BCG-RESISTANT NMIBC

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Abstract

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Co-senior authors

Background:

Bacillus Calmette-Guérin (BCG) is the standard of care for non-muscle invasive bladder cancer (NMIBC), but mechanisms of resistance are poorly understood. We investigated the effects of chronic IFN-g production on tumor adaptive resistance in BCG-resistant NMIBC. We analyzed single-cell (sc) and bulk

RNA sequencing (RNA-seq) data from BCG-resistant tumors and studied interactions of IFN-g-producing cells and tumor cells to identify relationships between IFN-g and tumor expression of immunosuppressive molecules such as *HLA-E*.

Methods:

Analyses were performed in RStudio (R v4.0). scRNA-seq samples were collected from 1 treatment-naïve tumor (later progressed to BCG-refractory) and 3 BCG-resistant tumors, and 19,072 cells containing 26,819 gene features passed quality control. Ligand-receptor interaction predictions were done using the NicheNet R package. Bulk RNA-seq (HTG Molecular^{TM}) was performed by Illumina NextSeq on FFPE sections of NMIBC tumors from patients before (n=20) or after (n=20) intravesical BCG, with median follow-up time of 36.7 months.

Results:

IFNG was near exclusively expressed in CD8+ T cells, including tissue resident memory cells, NK cells, and CD4 T cells (Fig. 1A), and was the top predicted ligand to induce tumor expression of MSigDB's Hallmark "Interferon Gamma Response" pathway (Fig. 1B). *IFNG* receptors *IFNGR1* and *IFNGR2* were expressed on tumor cells, suggesting the presence of IFN-g signaling (Fig. 1B). Ligand-target analysis showed enhanced *STAT1* expression on tumors due to IFN-g signaling (Fig. 1C), and NicheNet predicted *HLA-E* to be regulated by *STAT1*, *MYC*, and *NFKB1*, with *STAT1* being the strongest regulator of *HLA-E* (Fig. 1D). We confirmed the proposed *STAT1-HLA-E* axis in bulk RNA-seq data, which demonstrated a strong positive correlation (Spearman rho = 0.455, p = 2.74e-3) between *STAT1* and *HLA-E* expression on BCG-resistant NMIBC tumors (Fig. 1E).

Conclusion:

We demonstrated that *HLA-E* expression on BCG-resistant bladder tumors is under direct regulation of *STAT1*, which is induced by upstream IFN-g signaling. The source of IFN-g is almost exclusively limited to NK and T cells, suggesting that chronic IFN-g production could drive immune evasion and act as a mechanism for BCG-resistance via HLA-E-mediated NK and CD8 T cell inhibition.

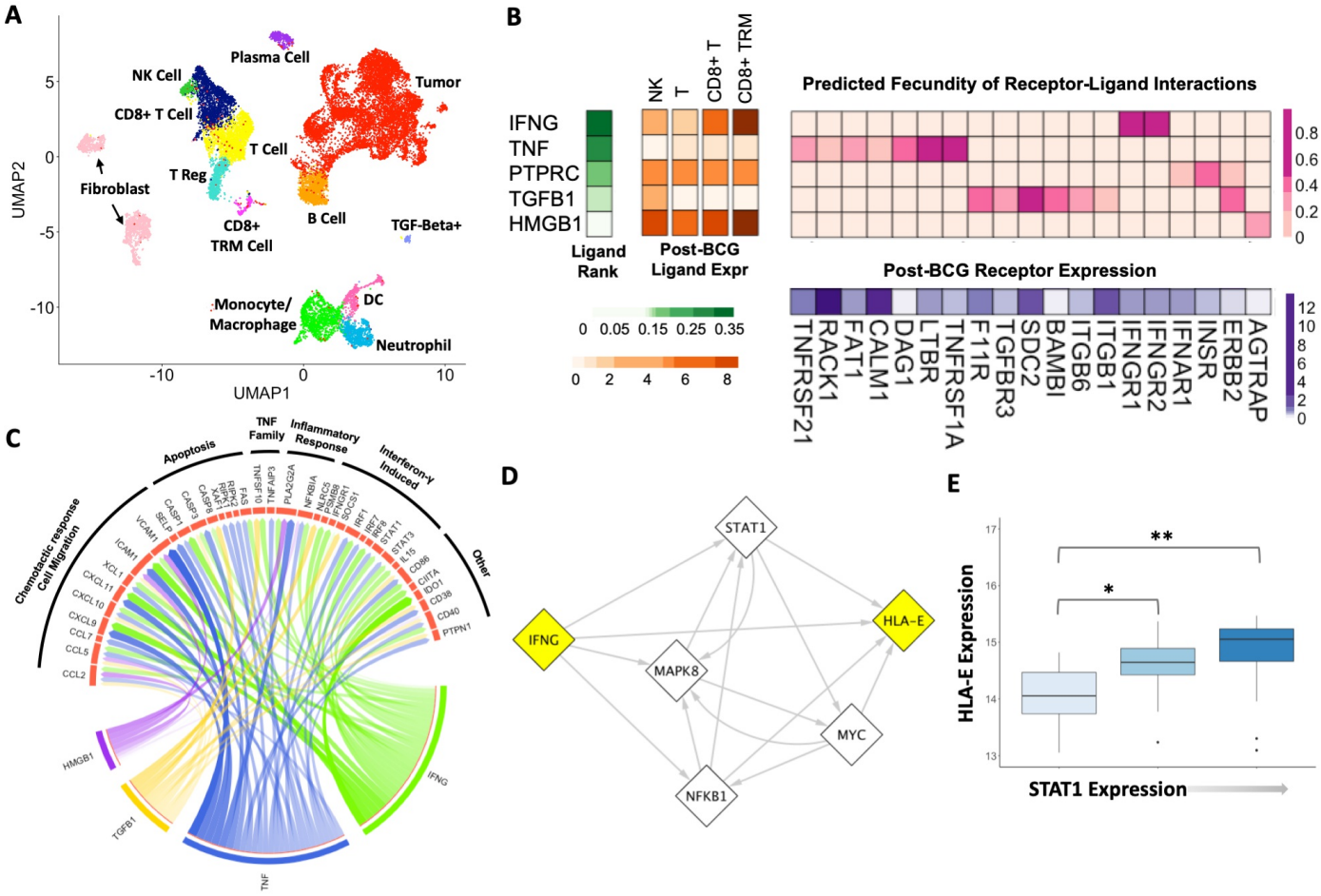

Figure 1

***IFNG* signaling is predicted to drive downstream *HLA-E* expression via *STAT1* in tumor cells.**

(A) UMAP visualization of cell types from scRNA-Seq analysis of urothelial tumor samples.
(B) NicheNet predictions of upstream ligand-receptor interactions showing IFNG, TNF, PTPRC, TGFB1, and HMGB1 (left) as the top 5 ligands expressed in NK, T, CD8+ T, and CD8+ TRM cells (middle) that most likely influence tumor cell expression of interferon-gamma signaling genes. Strength of ligand-receptor interactions (top right) and expression of the receptors in tumor cells (bottom right) are also shown.
(C) Circos plot showing links between NicheNet-predicted ligands and downstream interferon-gamma pathway genes. Strong relationships between ligand-target gene pairs are demonstrated by both wider arrow width and solid arrow colors.
(D) NichetNet-predicted regulatory network between IFNG and HLA-E.
(E) Expression of HLA-E in HTG bulk RNA-Seq urothelial cancer cohort with respect to STAT1 levels. (*p = 0.0274; **p = 0.00674)

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ANPEP: A potential regulator of tumor-immune metabolic interactions in African American men with prostate cancer

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Abstract

Background

In prostate cancer (PCa), lack of TMPRSS2-ETS gene fusion ($ETS^{negative}$) has been a hallmark of tumor in African American (AA) as compared with European American (EA) men. To elucidate biologic features associated with $ETS^{negative}$ PCa in AA men, we identified aminopeptidase N (ANPEP) as ETS-dependent gene, preferentially overexpressed in AA. We sought to investigate the role of ANPEP in a PCa development and exploit its role in PCa as a therapeutic vulnerability, particularly in AA tumors.

Methods

To understand the role of ANPEP in PCa progression and therapeutic vulnerability, we used data driven and experimental approaches. First, we explored ANPEP expression related to a range of genomic classifiers in multiple datasets. We further correlated ANPEP expression with various signatures related to immune and metabolic activity, available within GRID (Genomic Research Information Database) platform. We also experimentally examined the expression of ANPEP in different components of tumor microenvironment.

Results

Using publicly available datasets, we showed that prostate tumors form AA men harbor the highest expression of ANPEP as compared with other groups. Aminopeptidase N is involved in endocytosis, cholesterol, amino acid transport and peptide hydrolysis. Our data driven approach revealed that aminopeptidase N correlates with signature of cholesterol transport and androgen signaling. Based on our recent work on role of macrophage cholesterol in androgen receptor (AR) activation, we explored the expression of ANPEP in different immune cells. Interestingly, we found that ANPEP is predominantly expressed in macrophages compared with prostate tumor cells, T cell, B cells, neutrophils, and dendritic cells. In addition, expression of ANPEP predominantly correlated with a range of nutrient transporters which also tend to be differentially expressed by ETS status.

Conclusion

We have identified ANPEP as a macrophage-related gene with a potential role in cholesterol homeostasis and AR signaling. Future work will focus on the functional role of ANPEP activity in the tumor immune microenvironment using unique metabolomic approaches and explants derived from AA and EA prostate cancer patients.

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mRNA and cytokine analyses reveal a pattern of chronic immune activation in all patients undergoing BCG treatment for non-muscle-invasive bladder cancer

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Abstract

Introduction: BCG is the current standard-of-care for medium and high-risk non-muscle-invasive bladder cancer (NMIBC). BCG has been used for ~40 years, with a five-year recurrence rate of ~40%. Resistance mechanisms to BCG are poorly understood, and the only frontline-approved treatment is radical cystectomy. This may be, in part, because few studies have attempted to understand the

relationship between recurrence timing, reasoning for recurrence, and the immune system state at the time of recurrence.

Methods: *mRNA* sequencing of 2,892 genes was performed on tumor samples from 35 NMIBC patients prior to and following BCG (n=35). The average age at induction was 67.5; recurrence occurred on average 4.5 months after induction; median follow-up for sensitive patients was 34 months. Recurrence was any tumor confirmed pathologically on TURBT after induction. Olink Proteomics (inflammation panel) was performed on urine from a cohort of 20 NMIBC patients, observing 92 cytokines from pre-BCG through first cystoscopy. A gene-set enrichment analysis (GSEA) was performed on the mRNA data. mRNA levels were compared for statistical significance using the Kruskal-Wallis test. Longitudinal data was compared between each timepoint and the pre-BCG timepoint using the Kruskal-Wallis test.

Results: Olink and *mRNA* analyses are shown in *Figure 1*. Among the observed urine cytokines: CXCL9, CXCL10, IL 2RB, IL18 R1, PD L1, TGF-Beta, IFN-Gamma, and TNF were significantly upregulated following BCG administration. When compared to pre-BCG levels, recurrent post-BCG tumor *mRNA* saw significant enrichment in pathways related to interferon-gamma, B-cell markers, cytotoxic CD8-T cells, cytotoxic receptors and functions, T-cell checkpoint modulation, tumor inflammation, Th1 response, and lymphocyte trafficking.

Discussion: These results suggest that all patients, regardless of recurrence, encounter a robust immune response to BCG, mediated in part by natural killer (NK) cells, $CD8^+$ T-cells, and IFN-gamma production. Observed upregulated cytokines all play core roles in inflammation, including T and NK cell recruitment, differentiation, and proliferation. The inflammatory signature persists over the studied period, suggesting that tumor recurrence is not due to a lack of response to BCG. We postulate that functional status, particularly exhaustion and overstimulation, may drive failure of immune clearance to recurrent tumors

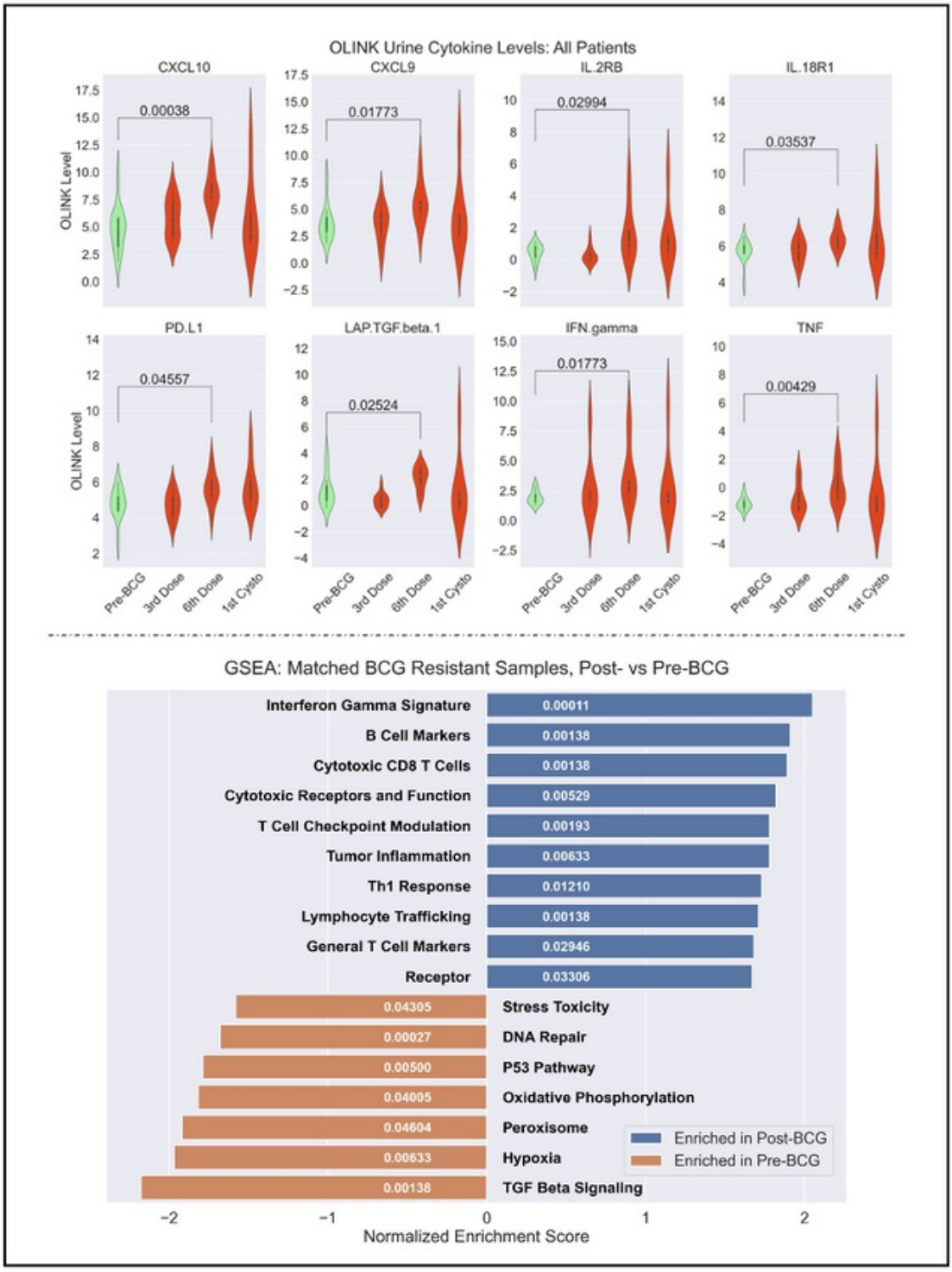

Figure 1: *mRNA* and Cytokine Levels in pre-BCG and post-BCG tissue and urine specimens

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Overcoming Therapeutic Resistance in Models of Lethal Prostate Cancer

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Abstract

The plasticity of prostate tumors contributes to the heterogeneity in response and acquisition of therapeutic resistance in advanced prostate cancer. Cabazitaxel (a 2nd generation FDA-approved taxane) in the treatment of castration-resistant prostate cancer-[CRPC]) causes phenotypic reprogramming of the tumor landscape via reversal of epithelial mesenchymal transition (EMT) to mesenchymal-epithelial transition (MET) in prostate cancer. We examined the effect of sequencing cabazitaxel chemotherapy and antiandrogen therapy on prostate tumor re-differentiation and kinesin (HSET) targeting and its impact on overcoming resistance in pre-clinical models of advanced prostate cancer. To interrogate clinically relevant mechanisms contributing to therapeutic resistance of prostate cancer patients with advanced disease we utilized pre-clinical, PDX models from patients with metastatic CRPC resistant to cabazitaxel. These PDX specimens are from patients who have metastatic lethal CRPC, treated with radiation, ADT, antiandrogens and chemotherapy including 2nd line taxane chemotherapy, cabazitaxel. The tumors were profiled for EMT phenotype, and expression and cellular localization of HSET, and SLCO1B3 transporter. *In vivo* therapeutic targeting by cabazitaxel in combination with antiandrogens was investigated using the androgen sensitive LNCaP and CRPC 22Rv1 xenografts. We also use *in vitro* models of therapeutically resistant human prostate cancer C4-2B cells to analyze mechanisms of cross-resistance between anti-androgens and taxane chemotherapy and RNASeq to determine gene changes/signatures underlying the phenotypic reprogramming during emergence of therapeutic resistance.

Tumor-bearing male nude mice were treated with cabazitaxel (3mg/kg) alone or in combination with castration-induced androgen-deprivation therapy (ADT) for 14-days. EMT, apoptosis, cell proliferation, and differentiation were evaluated. *In vivo* cabazitaxel treatment induced MET in both LNCaP and 22Rv1 prostate xenografts (increased E-cadherin and decreased N-cadherin). In addition to reversion to epithelial phenotype compared to vehicle controls, treatment resulted in increased apoptosis and decreased cell proliferation among prostate tumor cells. RNAseq analysis demonstrated that therapeutically resistant and sensitive C4-2B cells have unique gene signatures and differential response to cabazitaxel. Pathway association analysis of these gene changes revealed that treatment sensitive (naïve) cells exhibit expression of genes involved in DNA damage, while resistant cells express genes involved in protein regulation in response to cabazitaxel. Tumor specimens from PDX models from patients with advanced prostate cancer that received treatment with cabazitaxel following ADT showed high expression of E-cadherin and low expression of vimentin and HSET and re-differentiation of the tumor towards epithelial phenotype. These studies provide new insights into re-programming the prostate tumors into an acquisition of epithelial phenotype, therefore re-sensitizing the tumor cells to therapeutic targeting. Sequential treatment with ADT, antiandrogens and then cabazitaxel leads to phenotypic reversion, kinesin loss and transporter gain towards overcoming therapeutic resistance in mCRPC. Our findings have a translational significance in therapeutic sequencing (cabazitaxel and ADT), an avenue towards improved therapeutic response, to overcome lethal prostate cancer CRPC.

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FOXA1 regulates hypoxia and macrophage infiltration in prostate cancer

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Abstract

Background: Androgen-deprivation therapies are the mainstay treatment for metastatic prostate cancer (PCa). Yet, many patients relapse with castration-resistant PCa (CRPC). We have recently shown that FOXA1, an epithelial transcription factor, is down-regulated in CRPC. Its loss contributes to aberrant signaling by the androgen receptor (AR) and induces epithelial-mesenchymal transition and cell motility by regulating cytokines such as TGF-β and IL-8. In addition to such tumor-intrinsic factors, hypoxia in the tumor microenvironment has been associated with CRPC. Hypoxia Inducible Factor 1 (HIF-1) is a heterodimeric transcription factor composed of HIF-1 alpha (HIF1A) and HIF-1 beta (HIF1B). Notably, HIF-1 signaling has been shown to promote tumor infiltration by immunosuppressive cells, such as tumor-promoting M2-like macrophages. However, how hypoxia may be regulated by tumor-intrinsic factors is incompletely understood.

Methods: We performed RNA-seq, ChIP-seq, qPCR, western blot, and ELISA analyses to evaluate gene regulation and HIF1A cistrome. We utilized an *in vitro* macrophage infiltration transwell assay, in which M2-like macrophages were added to the upper chamber, and PCa cells were plated in the lower chamber to examine how perturbations to PCa cells affect macrophage migration. Finally, we performed bioinformatic analysis of PCa patient datasets to confirm the clinical relevance of our findings.

Results: By integrating RNA-seq and ChIP-seq data, we showed that FOXA1 proteins bound an intragenic enhancer of the HIF1A gene to repress its expression directly, such that FOXA1 depletion induced HIF1A expression and increased HIF1A occupancy at hypoxia gene loci. We further showed that Monocyte Chemoattractant Protein-1 (MCP-1/CCL2) became upregulated upon FOXA1 depletion in a HIF1A-dependent manner. Moreover, loss of FOXA1 or gain of CCL2 promoted macrophage migration toward PCa cells and increased PCa cell motility, which was abolished by genetic or pharmacological inhibition of the HIF1A-CCL2 axis. In accordance, bioinformatics analysis of human PCa patient datasets demonstrated that FOXA1 level is negatively correlated with CCL2 and macrophage infiltration. Future studies are needed to further elucidate the effects of FOXA1 on tumor hypoxia and metastasis *in vivo* and evaluate its therapeutic targeting in preclinical models.

Conclusion: This study proposes a novel role for FOXA1 loss in promoting hypoxia and macrophage infiltration in PCa and suggests HIF-1 targeting as a promising approach for CRPC treatment.

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RNAseq Profiling with Deep Learning in Prostate Cancer Tissue Images

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Abstract

Background

Prostate cancer (PCa) diagnosis starts with PSA level detection, which if above the normal range, the patient is subjected to genomic tests like- 4K scores, PCA3 or PHI test. After confirmation, MRI is used to identify potential areas of PCa. The biopsy is clinically extracted, inspected by a pathologist and is sometimes subjected to genomic testing. Typically, the worst area is selected for the clinical test leaving a large area from consideration. In case of false outcome, patients are subjected to the same steps making it financially cumbersome for the patients and increasing time/accuracy for treatment. We propose a multi-tiered approach using machine learning (ML) to analyze the entire section and integrate gene signatures in real time. This allows us to A) identify/characterize the areas of cancer vs normal tissue, B) monitor the areas more precisely enabling us to study the therapy response in a longer observation period.

Methods

Prostate slide tissues from 500 individuals in the PRAD study contained in TCGA were downloaded and evaluated. We selected 107 random images across all Gleason scores (GS) that were then scored by 3 board certified pathologists as a basis for training images to create the network based on the Xception algorithm to automate GS grading. This automation identified cancer regions of interest on test slides with minimal parameter manipulation. Additionally, gene expression profiles by obtaining RNAseq data grouped by GS. To reduce variance, these profiles were then put through a rigorous analysis pipeline to identify and remove variability and potential confounding factors such as age, and therapy options. Finally, these profiles were analyzed for differential expression to obtain genes that are uniquely different at a given Gleason grade.

Results

Extreme heterogeneity plays a key role when evaluating all 107 images and requiring all 3 pathologists, the AI as well as TCGA agree with only 25% accuracy. When requiring only 2 of 3 pathologists, the AI, and TCGA to agree this rises to 53%. However, using more generic classification system focused on normal tissue, low grade tumor (GS6 or GS7), and high grade tumor (GS8 or GS9) this accuracy improves to 71%. However, integration with the transcriptome classifier this further enhanced to 81%. Classification on it's own is on average responsible for a 12% increase in accuracy in the GS6 and GS7 categories.

Conclusions

ML can be applied to determine severity of tissue images, but enhanced with transcriptome integration. We are hopeful that future modifications will improve the accuracy of our current model and will aid in enhancing treatment decisions.

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Toxoplasma gondii infection induces LUTS in mice and correlates to BPH-LUTS incidence and epithelial nodule formation in men

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Abstract

Background: Benign prostatic hyperplasia (BPH) is associated with lower urinary tract symptoms (LUTS) characterized by increased urinary frequency, urgency, incomplete emptying, and pelvic pain. Common histological features of highly-symptomatic BPH include inflammation and epithelial nodule formations harboring microglandular hyperplasia. Our recent data indicate that systemic infection with the common parasite *Toxoplasma gondii* induces both inflammation and epithelial nodule formations with pronounced microglandular hyperplasia in mice. *T. gondii* is found in approximately 20% of the US population. The purpose of this study was to characterize the LUTS profile of *T. gondii*-induced prostatic hyperplasia in mice and to determine if *T. gondii* presence correlates to BPH-LUTS in men.

Methods: To assess void patterning in mice, we infected three strains (CBA/j, CD-1, and C57Bk6/j) i.p. with active *T. gondii* tachyzoites. Mice were analyzed weekly from 14 days post infection (d.p.i), until sacrifice at 60 d.p.i. using Void Spot Analysis. Mice were isolated in empty cages lined with chromatography paper for two, two-hour increments, and the paper was imaged using UV transillumination. Void Whizzard Software was utilized to analyze the void patterns by counting the number and area of each void spot. To assess human BPH with T. gondii seropositivity, we assessed human blood samples from patients treated for BPH and age-matched donor controls (age range 45-70; average 60 and 56) by ELISA for anti-*T. gondii* IgG.

Results:

Mice infected with *T. gondii* were significantly more likely to exhibit increased number of urine spots and substantially-increased smaller spots (less than 1 cm area) than control mice. These mice exhibit prostatic inflammation, reactive hyperplasia, and the formation of epithelial nodular formations with microglandular hyperplasia. Men with BPH had a *T. gondii* positivity rate of 38% versus 9% in the donor controls. Within the BPH group, 9/9 prostates from seropositive men exhibited large epithelial nodules with microglandular hyperplasia versus 7/21 in the seronegative samples.

Conclusions:

Systemic *T. gondii* infection induces inflammation and epithelial nodule formation in mouse prostates that is associated with an increased voiding phenotype. Seropositivity to *T. gondii* is associated with increased BPH-LUTS in men and is associated with inflammation and the formation of epithelial nodules with microglandular hyperplasia.

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Inferring treatment emergent prostate cancer phenotypic subtypes from cell-free DNA analysis

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Abstract

Background: With the adoption of potent AR signaling inhibitors, there has been a prominent rise in the frequency of treatment-resistant tumor phenotypes. Highly aggressive transdifferentiated tumors may emerge as AR low or AR null prostate cancers with or without neuroendocrine like signatures. An accurate determination of these subtypes has direct clinical implications, including choice of therapy. Current approaches for performing disease subtype determination often require tissue biopsies, which are limiting due to high-risk of comorbidity. For an evolving cancer, a minimally invasive molecular assay is urgently needed to accurately determine treatment-emergent disease subtypes and guide appropriate clinical decisions and therapy.

Methods & Results: Cell free DNA (cfDNA) primarily consists of DNA protected by nucleosomes and DNA binding regulatory elements and are shed into the bloodstream by cells that have undergone apoptosis. We hypothesized that differential nucleosome protection, transcription factor binding, and gene activity predictor profiles of circulating tumor DNA (ctDNA) lead to coverage features that accurately classify advanced prostate cancer into phenotypic and molecular subclasses.

We generated H3K27ac, H3K27me1, H3K27me3 chromatin profiles from tumors resected from 33 patient derived xenograft (PDX) models and classified each according to treatment-emergent subtype-specific epigenetic regulatory states (ARPC, NEPC, ARlowPC; PAM50 Basal & Luminal). We also performed whole-genome sequencing on plasma cfDNA from 30 PDX lines to achieve deep coverage (>10X) of “pure” human ctDNA sequence data. We applied *Griffin* to determine transcription factor binding site (TFBS) and transcription start site (TSS) nucleosome protection signatures predictive of phenotypic subtypes of prostate cancer. Guided by differential nucleosome profiles, we derived novel gene body and genome locus-specific sequence coverage and fragment size entropy signatures in ctDNA. Our robust prediction model accurately classified phenotypic and molecular subtypes of prostate cancer. The machine learning based integrative classification model achieved 93% AUC in cross-validation analysis and accurately predicts basal-luminal molecular subtypes.

Conclusion: The ctDNA analysis method we have developed can accurately classify phenotypes of advanced prostate cancer that span AR active, neuroendocrine and luminal-basal subtypes. In addition to single-point diagnostics, the approach is also suitable for longitudinal molecular monitoring and the prediction of treatment-emergent prostate cancer transdifferentiation.

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CXCR7 drives prostate cancer cell proliferation through AURKA activation

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Abstract

Background: Prostate cancer (PC) is the most commonly diagnosed cancer among males in the US. The mainstay treatment for metastatic PC patients is androgen-deprivation therapies including the use of high-affinity androgen receptor (AR) antagonists, such as enzalutamide. Unfortunately, many patients relapse with castration-resistant PC. We have previously reported up-regulation of CXCR7, an atypical chemokine receptor, in enzalutamide-resistant PC. CXCR7 interacts with ARRB2, a cytoplasmic scaffold protein. The CXCR7-ARRB2 complex then translocate to the cytoplasm in clathrin-coated vesicles to induce downstream signal transduction. However, little is known regarding CXCR7 target genes and its role in prostate cancer.

Methods: RNA-seq was used to profile gene expression and comprehensive phosphoproteomics analysis used to study changes in kinases activity regulated by CXCR7. Cell cycle progression and proliferation was assayed by flow cytometry and WST1 respectively. We used Co-IP to identify protein interaction between CXCR7, ARRB2 and AURKA. We truncated ARRB2 and AURKA to map the exact domains in protein complex. To visualize intracellular distribution of CXCR7 we performed IF confocal imaging.

Results: Gene Ontology analyses of CXCR7-regulated genes revealed remarkable enrichment of genes involved in G2/M cell cycle progression. Through comprehensive phospho-proteomics analysis of CXCR7-knockdown PC cells we found greatly reduced phosphorylation of AURKA, a mitotic serine/threonine kinase that regulates mitosis and G2/M progression and has been implicated in aggressive PC. Western blotting confirmed that CXCR7 depletion reduces phosphorylation of AURKA and TACC3, another AURKA substrate. Co-IP demonstrated that CXCR7 binds to AURKA. Further analyses revealed that ARRB2 C-terminal domain is critical for mediating CXCR7-AURKA interaction. On the other hand, AURKA binds to ARRB2 through kinase-domain. Confocal imaging showed that CXCR7 localizes to perinuclear zone, at proximity with AURKA and ARRB2 that are localized at centrosomes. Functionally, PC cells with CXCR7 knockdown or ARRB2 deletion undergo cell cycle arrest and cell death.

Conclusions: Our results describe for the first time that CXCR7-ARRB2 complex interacts with AURKA to induce its activation, thereby enhancing cell cycle progression and PC growth. CXCR7-ARRB2-AURKA complex may be a promising target for therapeutic intervention in PC.

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Cooperation between NME2 and MYC drives resistance to Enzalutamide treatment in CRPC patients

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Abstract

Background: Second-generation androgen receptor inhibitor Enzalutamide is becoming a mainstay of treatment for patients with advanced prostate cancer. Yet, therapeutic resistance to Enzalutamide is causing a significant clinical challenge with research in this area being hampered by the complexity and heterogeneity of the mechanisms involved.

Methods: To address this problem, we have developed and validated a novel systems biology network-based algorithm, which defines regulatory relationships between transcription factors and molecular pathways, establishing their potential hierarchical top-down regulation. We have applied this algorithm to SU2C East Coast CRPC cohort and queried the reconstructed network using phenotypic transitions that lead to Enzalutamide resistance in prostate cancer.

Results: Our analysis nominated a cooperation of transcription factor NME2 and MYC pathway as a marker of Enzalutamide failure, demonstrating the concordant activation of NME2 and MYC in patient samples that rapidly failed Enzalutamide treatment. To experimentally validate this finding, we utilized hormone sensitive LNCaP cells and subjected them to short term (up to 15 days) and long term (up to 2 months) Enzalutamide treatment, obtaining Enza-sensitive and Enza-resistant model systems, respectively. We have observed concordant downregulation of NME2 and MYC in sensitive cells and upregulation in resistant cells, similar to patient samples. We are currently validating the functional role of NME2 and MYC in acquired Enzalutamide resistance by a combination a genetic knockdown of NME2 (via specific siRNAs) and novel small molecule MYC inhibitor (Myci 975).

We further clinically validated a cooperation of NME2 and MYC in single-cell samples (He et al, Welch t-test $p < 2.26e-16$) and bulk-sequenced patient cohorts (Abida et al, log rank p-value = 0.004, hazard ratio = 2.4 and SU2C West Coast, log rank p-value = 0.001, hazard ratio = 3.04). Finally, comparison to other known markers of disease aggressiveness and Enzalutamide resistance demonstrated unique ability of NME2-MYC cooperation to accurately and precisely identify patients at risk of Enzalutamide resistance.

Conclusions: We propose that NME2-MYC cooperation can be utilized as a marker to assist in clinical decision-making for optimal therapeutic strategy and prioritizing patients for future clinical trials.

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Bladder smooth muscle cells migrate during scratch wound and ex vivo bladder subtotal cystectomy.

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Abstract

Rodents have the capacity for spontaneous bladder regeneration following a subtotal cystectomy (STC), however the capacity of SMC to migrate and participate in this process is unclear. Here we tested the whether bladder smooth muscle cells (SMC) migrate in response to scratch wound stimuli and tested the role of the developmental pathway of Yap/Taz in this process. We also examined how bladder SMC migrate in an *ex vivo* model of STC. Methods: low passage number human bladder SMC (Sciencell) were plated on 12 well plates to create monolayers. SMC were serum-starved prior to creating scratch wounds using 10 uL pipette tips. Migration into the scratch wound was recorded by live cell microscopy over 16 hours. Inhibitor of Yap/Taz signaling, Verteporfin, was utilized at 0.1 uM after cells adhered to plates. For *ex vivo* STC, bladders were harvested from C57bl/6 adult mice, and the domes removed with the bases ligated. Controls included whole bladders. AAV-serotypes were tested for binding to SMC *ex vivo*, and AAV6-GFP was shown to produce strong GFP signal in SMC. STC and control *ex vivo* bladders were injected with 109 AAV6-GFP for 2 hours then washed and incubated in DMEM growth media for 3 days and then monitored by live cell microscopy for GFP-positive cell or tissue movement. Results: Migration of bladder SMC was seen both *in vitro* and *ex vivo*. Cell migration in the *ex vivo* bladder included movement of the entire groups of cells, with some contraction seen, as well as migration of individual cells, especially near the wound edge (videos to be provided). Verteporfin inhibited scratch wound closure from 68 +/-2 to 49+/-5 % closure at 7 hours, $p<0.05$, but not at later timepoints. Conclusions: Bladder SMC migrate both *ex vivo* and *in vitro* proximal to the wound environments. While scratch wound models *in vitro* show a dependency on Yap/Taz, further work to uncover the pathways and the role of ECM remodelling in regulating bladder SMC migration in tissue, as well as the direction of the migration is pending.

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In-vivo Effect of Nitric Oxide Donor on Neuroendocrine Prostate Cancer in Mice: Preliminary Results.

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Abstract

Background: Neuroendocrine prostate cancer (NEPC) is an aggressive variant of prostate cancer that may arise de novo or in patients previously treated with hormonal therapies for prostate adenocarcinoma as a mechanism of resistance. An active nitric oxide donor, s-nitrosoglutathione (GSNO), is known to reduce castration resistant prostate cancer tumor burden by targeting the tumor microenvironment. In the present study, we tested the hypothesis that GSNO will similarly reduce tumor burden of NEPC.

Method: An in-vivo experiment was conducted in castrated SCID mice in compliance with the Institutional Animal Care and Use Committee of our institution. Total of 10 mice were grafted with H660 cells to generate NEPC murine models. Once the tumors became palpable, the animals were divided into two equal groups. Group 1 received vehicle control and group 2 received GSNO treatment at the dosage of 10mg/kg/day intraperitoneally (IP) for two weeks. The tumor growth was measured twice per week for a month after completion of treatments. Tumors were extracted from the mice at one-month post-treatment. Proteins were harvested from the tumors to study expression of neuroendocrine markers and markers of cancer progression. GraphPad Prism (GraphPad Software) was used for statistical analysis. The data were presented as the means ± SEM. The statistical significance between two groups was estimated by unpaired two-tailed t test.

Results: Total of 3 mice from group 1 and 4 mice from group 2 survived till the completion of the study. Although we did not find any significant difference in tumor weight between the two groups, treatment with GSNO resulted in significant decrease in tumor volume as compared with control ($p<0.05$). Molecular analysis revealed significant reduction in chromogranin A and synaptophysin in tumors treated with GSNO as compared to control.

Conclusion: Our preliminary study showed that treatment with NO donor, GSNO, reduces NEPC tumor burden in mice models. This will provide framework for further larger studies to investigate possible mechanism of effect of nitric oxide on NEPC through targeting of the tumor microenvironment.

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The cell free DNA methylome captures distinctions between localized and metastatic prostate tumors

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Abstract

Background: Metastatic prostate cancer remains a major clinical challenge and metastatic lesions are highly heterogeneous and difficult to biopsy. Liquid biopsy is emerging as a promising tool in disease management and provides valuable opportunities to gain insights into the underlying biology. Of interest, in mCRPC, ctDNA has shown to reflect the genomic profiles of tumor or metastatic lesions, and epigenetic characteristics such as methylation status can reflect tumor burden and subtypes. Despite these advantages, cell-free DNA methylome profiles of mCRPC have yet to be investigated on a genome-wide scale.

Methods: The recent development of cell-free methylated DNA immunoprecipitation coupled with next generation sequencing (cfMeDIP-seq) presents an effective approach for the analysis of cfDNA methylomes. Using this technology, we analyzed the DNA methylome in 30 and 175 plasma samples from patients with localized and metastatic prostate cancer, respectively. The plasma samples for localized tumors were collected as part of the Canadian Prostate Cancer Genome Network (CPC-GENE) project, while the mCRPC samples were assembled from three ongoing studies on metastatic prostate cancer, of which 52 have matched multi-omics profiling in the tumors and 139 with DNA sequencing of the cfDNA.

Results: The cell-free DNA methylomes capture variations of and beyond the DNA fraction directly originating from the tumor. A global hypermethylation in metastatic samples was observed, coupled with hypomethylation in the pericentromeric repeat regions. Hypermethylation at a site located in the promoter region of the glucocorticoid receptor gene NR3C1 is associated with increased immune signature in both localized and metastatic tumors. The cell-free DNA methylome was able to distinguish localized and metastatic samples and machine learning predictors were built to achieve over 0.8 prediction accuracy. Finally, we showed the ability of predicting copy number alterations (CNA) from the cfMeDIP-seq data, providing opportunities for joint genetic and epigenetic analysis on limited biological samples.

Conclusions: We compiled a collection of the genome wide cell-free DNA methylation profiles for prostate cancer. The samples were collected from four independent sources and encompassed localized and metastatic diseases. With this we identified consistent changes and created classifiers that can distinguish between localized and metastatic samples with high accuracy. The results presented multiple new insights into the disease biology and demonstrated the biomarker potential for detecting metastatic lesions with a minimally invasive and cost-effective strategy.

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CKB inhibits epithelial-mesenchymal transition and prostate cancer progression by sequestering and inhibiting AKT activation

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Abstract

Background: In tumors of epithelial origin, aberrant induction of epithelial-mesenchymal transition (EMT) contributes to tumor invasion, metastasis and drug resistance. AKT is a key regulator for EMT, cell survival and proliferation, migration, metabolism, angiogenesis, and self-renewal of stem cells. While many positive regulators for either EMT or AKT activation have been reported, few negative regulators are established.

Methods: We performed a kinome cDNA screen and discovered a number of potential regulators of EMT. We then employed prostate cancer patient samples and tumor xenografts, as well as molecular, cellular and biochemical approaches to characterize novel EMT regulators and to investigate their mechanisms. In this study, we established brain-type creatine kinase (CKB or BCK) as a potent negative regulator for both EMT and AKT activation.

Results: As a ubiquitously expressed kinase in normal tissues, CKB is significantly downregulated in several solid cancer types. Lower CKB expression is significantly associated with worse prognosis. Phenotypically, CKB overexpression suppresses, while its silencing promotes, EMT and cell migration, xenograft tumor growth and metastasis of prostate cancer cells. AKT activation is one of the most prominent signaling events upon CKB silencing in prostate cancer cells, which is in line with prostate cancer TCGA data. EMT enhanced by CKB silencing is abolished by AKT inhibition. Mechanistically, CKB interacts with AKT and sequestrates it from activation by mTOR. We further elucidated that an 84aa fragment at C-terminus of CKB protein interacts with AKT's PH domain. Ectopic expression of the 84aa CKB fragment inhibits AKT activation, EMT and cell proliferation. Interestingly, molecular dynamics simulation on crystal structures of AKT and CKB independently demonstrates that AKT's PH domain and CKB's 84aa fragment establish their major interaction interface.

Conclusion: We have discovered a new mode of regulation in AKT activation and CKB as a negative regulator of EMT and AKT activation, and have revealed that CKB downregulation is a poor prognosticator and is sufficient to promote prostate cancer progression.

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Role of Lgr5^{+} stromal cells in the periurethral prostatic ducts in prostate homeostasis

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Abstract

Background: Anatomy-associated heterogeneity of tissue-resident stromal cells has been widely appreciated. Previous studies from our laboratory and others demonstrated that the stromal cells in different anatomic prostatic regions exhibit distinct gene expression profiles, including the Wnt-related genes. Although the spatially distributed stromal cell sublineages have been implicated in the regulation of prostate epithelial stem/progenitor cell activity, little is known regarding the prostate anatomy-associated stromal lineage heterogeneity and its biological implication in tissue homeostasis due to the lack of cell lineage markers and research models. By identifying Lgr5 as a periurethral stromal cell marker and specifically ablating these Lgr5-expressing cells in mouse prostates, our study will provide novel insights into how prostate tissue homeostasis is maintained.

Methods: We generated the *Lgr5-CreER^{T2};R26-LSL-eYFP* bigenic reporter mice and employed a novel open-top light-sheet (OTLS) 3D microscopy and flow cytometry analysis to illuminate the localization and percentage of Lgr5^{+} stromal cells in the prostate. After ablating the Lgr5^{+} stromal cells with diphtheria toxin (DT) treatment in the *Lgr5-DTR-eGFP* mouse model, we performed H&E staining and immunofluorescence staining of Ki67, Cleaved Caspase 3 (CC3), and epithelial cell markers Krt5 and Krt8 for the analysis of prostate tissue structure, epithelial proliferation, apoptotic index and cell lineage plasticity, respectively.

Results: Lgr5^{+} stromal cells are abundantly identified in the periurethral ducts of all mouse prostate lobes but are extremely rare in the epithelial lineages. The prostate weight decreased post the ablation of Lgr5^{+} stromal cells. There is a transient increase in cell apoptosis and proliferation and an increase in the transit-amplifying cells (TAC) that express both the basal cell marker Krt5 and luminal cell marker Krt8.

Conclusions: Collectively, these results demonstrate that Lgr5 is the specific marker for a stromal cell subpopulation in the periurethral prostatic ducts. Ablating the Lgr5^{+} stromal cells disrupts prostate tissue homeostasis. Our study supports that the Lgr5^{+} cells in the stromal cell lineage rather than in the epithelial lineage are critical in maintaining prostate tissue homeostasis.

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ASCL1 activates neuronal stem cell-like lineage programing through remodeling of the chromatin landscape in prostate cancer

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Abstract

Background: Second generation AR pathway inhibitors (ARPIs) such as Enzalutamide (ENZ) are highly effective in castration resistant prostate cancer (CRPC). However, they play a role in emergence of a more aggressive, AR-independent phenotypes including treatment induced neuroendocrine prostate cancer (t-NEPC). With the exception of genomic alterations to RB1 and TP53, few other genetic differences are observed between CRPC and NEPC; suggesting an epigenetic dysregulation underlining this conversion.

Method: In order to capture the evolution of CRPC to NEPC, we measured changes in chromatin accessibility of CRPC cells upon exposure to ENZ and in NEPC cell lines via ATAC-seq. We performed RNA-seq from matched treatment. In addition, we successfully conducted ASCL1, H3k27me3 and EZH2 ChIP-seq.

Result: Using ATAC-seq we identified ASCL1 motif becoming accessible as early as 3 days, with continued enrichment at 10 days post ENZ-treatment as well as in NEPC cell-line models. ASCL1 expression and activity is significantly upregulated in NEPC cell lines and patient tumors. Using our unique model of tNEPC, we showed that knockdown of ASCL1 causes extensive chromatin reorganization leading to reduced expression of neuronal and plasticity markers and overall abolishment of the NEPC program. Combining ASCL1 and H3k27me3 ChIP-seq discovered that in NEPC models close to 40% of ASCL1 binding sites overlap with H3k27me3. Loss of ASCL1 function dysregulated polycomb repressor complex 2 (PRC2) activity (loss of H3k27 methylation). This results was captured across multi-omic analysis integrating RNAseq, ChIPseq and protein expression. Interestingly this global loss of H3K27me3 occurred without any affect on the expression of PRC2 complex members. Cell fractionation, confocal microscopy and EZH2 ChIP-seq identified loss of EZH2 binding to the chromatin as the likely cause of the H3k27 demethylation. Pheno-copying EZH2 inhibition, this loss of H3K27me3 reactivated luminal programing, potentially re-sensitizing these cells to further treatments. Altogether, our result suggesting that ASCL1 may drive early transcriptional and epigenetic reprogramming through the PRC2 complex, therefore, facilitating the emergence and maintenance of NEPC.

Conclusion: We report a novel role for pro-neuronal transcription factor ASCL1 in modulating the chromatin dynamics to support a plastic lineage by orchestrating early chromatin events and regulatory networks that determine a neuronal stem cell-like lineage commitment. In the treatment-resistant, high plasticity state inhibition of ASCL1 reverses the lineage switch to epithelial-luminal, providing a potential for targeting these highly aggressive tumors. Similar to NEPC, a subset of glioblastoma and small cell lung cancers are defined by elevated expression of ASCL1. This work provides much-needed insight into ASCL1 function and dependency that together nominates ASCL1 as a bona fide clinical target.

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THE ROLE OF RECEPTOR TYROSINE KINASE ROR2 IN STEMNESS AND IMMUNE EVASION

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Abstract

Prostate cancer is characterized as an immunologically “cold” tumor with loss of effector CD8+ T-cells and expansion of M2 macrophages and Tregs. These features explain, at least in part, the poor efficacy of immunotherapy in prostate cancer. An increasing number of genomic, epigenomic, transcriptomic profiles, and experimental analysis have suggested negative associations between the cancer stem cell (CSC) phenotype and cytotoxic T-cell responses and anticancer immunity in different cancers. However, the molecular mechanisms responsible for immunomodulating features of CSCs remain poorly understood.

Interrogating different sequencing datasets from metastatic prostate cancer patients revealed an inverse correlation between CSC score and AR activity. Patients were then segregated based on AR expression/activity and were screened for receptor tyrosine kinases (RTK). We identified ROR2 as the most common upregulated RTK in AR low/indifferent patients with increased CSCs markers. Combined transcriptional profiling and functional studies reveled that ROR2/JNK signaling as a powerful inducer of CSC phenotype via up-regulation of the reprogramming transcription factors Oct4 and Sox2. Moreover, depletion of ROR2 decrease immunosuppressive checkpoints (i.e., PD-L1, CD70, Vista, TDO) and dysregulation of cytokines and chemokines that are associated with an immunosuppressive tumor microenvironment. Notably, we found that ROR2 loss of function significantly induce CD8+ T-cell-meditated tumor cell-killing along with a decrease of expression of CCL2 and BMP6 known to recruit M2 macrophages on tumor site.

Our findings identify targetable mechanisms by which ROR2/JNK signaling regulate the reprogramming transcription factors Oct4 and Sox2 expression that may drive the immunomodulatory gene expression and may contribute to the immunosuppressive microenvironment in AR-indifferent prostate cancers.

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The epigenetic reprogramming in NEPCa

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Abstract

Background: Neuroendocrine (NE) phenotype contributes to 25 to 30% androgen deprivation resistant prostate cancer cases. Until now, this aggressive phenotype is untreatable. Therefore, it is urgent to study the molecular mechanism of NE development to reveal therapeutic strategies. Based on our previous results, we proposed several milestone events during NE development that can be represented by different PCa cell lines.

Methods: Public transcriptome datasets were acquired from CCLE, cBioPortal, and GEO. All data manning were performed by custom R codes and visualized by “ggplot2” and “pHeatmap” packages. RT-qPCR, western blot (WB), Immunohistochemistry (IHC), and Immunofluorescence (IF) were used to assess gene expression.

Results: Our multi-datasets bioinformatics analysis revealed the transcriptome changes during NE development. We found the differential expressed genes fell into 3 categories: neurogenesis, cell cycle, and androgen receptor signaling. Further, we established a prostatic HOX code (39 HOX genes expression) that can accurately identify prostate adenocarcinoma (AdPCa) samples from other cancer types. We found NEPCa tumors have a distinct HOX code than prostatic HOX code, reflecting a switch from prostatic lineage to NE. This research highlighted HOXB13 expression is lost in NEPCa compared with AdPCa and it has been confirmed in both RNA and protein levels. Decreased HOXB13 expression was seen in the NEPCa cell line (NCI-H660) and an AdPCa cell line (DU145), suggesting that the loss of HOXB13 or prostatic HOX code is an early event during NE development and DU145 could be the most similar AdPCa cell line to NEPCa. We also found the YAP1 gene expression is lost in both RNA and protein levels in NEPCa. The exclusive loss of YAP1 expression in NEPCa samples suggests that loss of YAP1 expression can be used as a diagnostic marker for NEPCa. In PCa cell lines, NCI-H660 cells have decreased YAP1 expression but not other AdPCa cell lines. This suggests that the loss of YAP1 expression is a relatively late event during NE development.

Conclusion: Androgen-sensitive cell lines, LNCaP for example, represent the naïve stage of AdPCa. The decreased HOXB13 expression/changed prostatic HOX code in DU145 represents an early event during PCa progression, which is followed by the loss of YAP1 expression and the gain of neuronal gene expression that is represented by NCI-H660 cell line.

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Preclinical evaluation of Wee1-targeted synthetic lethal combination in mouse model of neuroendocrine prostate cancer

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Abstract

The mitotic regulator Wee1 is a critical regulator of G2/M cell cycle checkpoint and DNA damage response pathways. Wee1-targeted agents are under active clinical investigation in multiple cancer types. However, Wee1 and its inhibitors have not been well studied in prostate cancer, particularly neuroendocrine prostate cancer (NEPC). In this study, we investigated the therapeutic potential of Wee1 inhibitor MK-1775 and its combinations in the context of lethal NEPC. Our data demonstrated increased Wee1 expression in NEPC cells and tumors. Treatment of NEPC cells with MK-1775 resulted in sustained cell growth inhibition at concentrations that did not affect the growth of other prostate cancer cells, indicating greater sensitivity of NEPC cells to MK-1775. Using the autochthonous transgenic adenocarcinoma mouse prostate (TRAMP) model, we assessed the efficacy of MK-1775 and its combinations on progression of NEPC tumors in castrated TRAMP mice. Our data showed that MK-1775 and the Chk1 inhibitor CCT245737 alone or in combination significantly suppressed tumor growth and improved overall survival of mice with NEPC tumors. We further identified synthetic lethality between MK-1775 and additional kinase inhibitors including Akt inhibitors. Mechanistically, treatment of MK-1775 resulted in increased DNA-double-strand breaks and abrogation of G2/M cell cycle checkpoint, leading to mitotic catastrophe and cancer cell apoptosis. The combination of Chk1 and Wee1 inhibitors led to greater reduction of Cdc25c phosphorylation at S216 and Cdc2 phosphorylation at Y15, supporting abrogation of G2/M checkpoint and consequent premature mitotic entry as the primary mechanism of synergy.